HANDBOOK OF BIOFERTILIZERS AND BIOPESTICIDES

A.M.DESHMUKH
R.M.KHOBRAGADE
P.P.DIXIT

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Biofertilizers and Biopesticides

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Edited by

A.M. Deshmukh
R.M. Khobragade
P.P. Dixit

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Preface

Advance agricultural practices are using more input of water, soil and plant protection chemicals in the soil. Continuous use of chemical fertilizers and pesticides make the soil less fertile as beneficial and useful microogranism are destroyed by them. Moreover, they create many serious problems like health hazards, air and water pollution, killing many beneficial insects.

These facts indicate that there is an urgent need to develop newer approach to increase the soil fertility and to manage the pests. Use of biofertilizers and biopesticides is one of the most suitable and widely accepted approach to chemical control.

The research articles in the book provide information on different types of biofertilizers and biopesticides and their applications for different crops.

We hope that book will be helpful to students, teachers and researchers in the field of agricultural microbiology.

We would like to acknowledge the efforts taken by ABD publishers to publish this book in time.

A.M. Deshmukh
R.M. Khobragade
P.P. Dixit

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List of Contributors

A. Venkatesh
Forest College and Research Institute Mettupalayam-641 301, T.N., India

A.M. Deshmukh
P.G. Department of Microbiology Y.C. College of Science Karad-415 124, Maharashtra, India

A.M. Thopatel
Department of Agriculture Microbiology

A.R. Madhav Rao
Department of Agricultural Microbiology Department of Agricultural Sciences G.K.V.K. Compus Bangalore-560 065, Karnataka, India

A.S. Ponnuswamy
Department of Seed Technology Tamil Nadu Agricultural University Coimbatore-641 003, Tamil Nadu, India

A.U. Narayana
Department of Plant Pathology Dr. Panjabrao Deshmukh Krish Vidyapeeth Akola-444 104, Maharashtra, India

Ajay Singh
Department of Plant Pathology, C.P. College of Agriculture Sardarkrushinagar Dantiwada Agricultural University Sardarkrushinagar–385 506

Almas Zaidi
Department of Agricultural Microbiology Institute Muslim University Aligarh–202 002, U.P., India

Anu Kalia
Department of Microbiology Punjab Agricultural University Ludhiana, Punjab-141 004

B.D. Shinde
Central Sugarcane Research Station Padegaon, P.O. Nira – 412 102 Dist. Pune, Maharashtra, India

B.N. Prasad
Biotechnology Unit, Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal

B.V. Prasada Reddy
Biotechnology Cenre for Tree Improvement Tirupati-517 507, Andhra Pradesh

Bharose, A.A.
MGM, Department of Bioinformatics & Bioftechnology, Aurangabad (M.S.)

Birhare, S.C.
Dept. of Biotechnology, Govt. Institute of Science, Aurangabad 431 004.

C. Narayana Reddy
Department of Botany Plant Pathology and Mycorrhiza Laboratory Gulbarga University Gulbarga–585 106, Karnataka, India

C. Udayassorian
Department of Environment Sciences Tamil Nadu Agricultural University Coimbatore-641003, Tamil Nadu, India.

C.A. Agasimani
University of Agricultural Sciences Dharwal-580 005, Karnataka, India

D. Anusuya
Department of Botany Bangalore University Bangalore–560 056, Karnataka

D.B. Shinde
Asstt. Microbiologist Central Sugarcane Research Station Padegaon, P.O. Nira, Dist. Pune Maharrashtra – 412 102, India

Deshmukh, L.S.
National Agril. Research Station, Aurangabad, MAU, Parbhani (M.S.)

Deshpande, V.D.
MGM, Department of Bioinformatics & Bioftechnology, Aurangabad (M.S.)

Dharmesh Shah
Institute of Biotechnology, Chudasama Main Road, Near Amrapali Crossing, Rajkot-360 002, Gujarat

Dr. Masood Ahmad
Department of Biochemistry, Faculty of Life Sciences, A.M.U., Alighrh, U.P., India

Eshwarlal Sedamkar
Department of Botany Plant Pathology and Mycorrhiza Laboratory Gulbarga University Gulbarga–585 106, Karnataka, India

Foroutan, Abdolreza
Agricultural & Natural Resources Research Center of Mazandaran, Sary, Iran.

Foroutan, Aidin
Faculty of Agricultural Sciences of Mazandaran University, Sary, Iran.

G. Arunachalam
Professor and Head, Dept. of Agricultural Chemistry Kllikulam–625 272, Tamil Nadu

G. Mani
Department of Seed Technology Tamil Nadu Agricultural University Coimbatore-641 003, Tamil Nadu, India

G. Naresh Kumar
Department of Biochemistry Faculty of Science, M.S. University Vadodara-390 002, Gujarat, India

G. Rajannan
Department of Environment Sciences, TNAU, Coimbatore-641 003, Tamil Nadu.

G.D. Radder
University of Agricultural Sciences Dharwal-580 005, Karnataka, India

G.D. Saratale
Department of Biotechnology Engineering, Tatyasaheb Kore Institute of Engineering and Technology, Warananagar. Tal: Panhala, Dist: Kolhapur-416 113 (M.S.) India.

G.R. Pathade
P.G. Department of Microbiology Y.C. College of Science Vidyanagar, Karad-415 124, Maharashtra

H.C. Lakshman
P.G. Department. of Botany (Microbiology Lab.) Karnatak University, Dharwad-580 003, India.

J. Prabakaran
Department of Environmental Sciences Tamil Nadu Agricultural University Coimbatore-641 003, Tamil Nadu, India

Jaydeep Joshi
Institute of Biotechnology, Chudasama Main Road, Near Amrapali Crossing, Rajkot-360 002, Gujarat.

K. Govindan
Faculty of Agriculture and Animal Husbandry Gandhigram Rural Institute Gandhigram-624 302, Dist. Dindigal Tamil Nadu

K. P. Sreenath
Department of Botany Bangalore University Banglore-560 056, Karnataka, India

K. Srinivasan
Department of Environmental Sciences Tamil Nadu Agricultural University Coimbatore–641 003, Tamil Nadu, India

K. Vanangamudi
Department of Seed Technology Tamil Nadu Agricultural University Coimbatore-641 003, Tamil Nadu, India

K.D. Thakur
Department of Plant Pathology Dr. Panjabrao Deshmukh Krish Vidyapeeth Akola-444 104, Maharashtra, India

K.G. Thakare
Department of Plant Pathology Dr. Panjabrao Deshmukh Krishi Vidyapeeth Akola–444 104, Maharashtra, India

K.K. Khode
Sugarcane Specialist Central Sugarcane Research Station Padegaon, P.O. Nira, Dist. Pune Maharashtra – 412 102.

K.T. Parthiban and Ravichandran
Forest College and Research Institute Mettupalayam-641 301, T.N., India

Kamal Prasad
Department of Post-Graduate Studies and Research in Biological Science Rani Gurgavati University Jabalpur–482 001 (M.P.), India

Kamlesh Pansheria
Institute of Biotechnology, Chudasama Main Road, Near Amrapali Crossing, Rajkot-360 002, Gujarat

Kamlesh S. Pansheria
Institute of Biotechnology, Raiya Road, Rajkot-360 002, M.G. Science Institute, Ahmedabad. (Gujarat)

Kavita Sharma
Department of Plant Pathology, C.P. College of Agriculture Sardarkrushinagar Dantiwada Agricultural University Sardarkrushinagar–385 506

Khapre
Agril. Research Station, Badnapur, MAU, Parbhani

Kohire, O.D
Agril. Research Station, Badnapur, MAU, Parbhani

Kohirepatil, V.O
Agril. Research Station, Badnapur, MAU, Parbhani

Kulkarni, A.M
Agril. Research Station, Badnapur, MAU, Parbhani

I.J. Parekh
Department of Biochemistry Faculty of Science, M.S. University Vadodara-390 002, Gujarat, India

L.S. Deshmukh
National Agril. Research Station, Aurangabad, MAU, Parbhani

Laxman B. Kadam
P.G. Department. of Botany (Microbiology Lab.) Karnatak University, Dharwad-580 003, India.

M.D. Shukla
Institute of Biotechnology, Raiya Road, Rajkot-360 002, M.G. Science Institute, Ahmedabad. (Gujarat)

M.G. Bodhankar
Department of Microbiology Bhartiya Vidyapeeth's A.S.C. College Sangli-416 416, Maharashtra, India

M.N. Sreenivasa
Deptt. of Agricultural Microbiology University of Agricultural Sciences Dharwad-580 005. Karnataka

M.V. Kulkarni
Maharashtra Pollution Control Board, Sangli, Maharashtra, India

Mallika Vanangamudi
Forest College and Research Institute Mettupalayam-641 301, T.N., India

Md. Saghir Khan
Department of Agricultural Microbiology Institute Muslim University Aligarh–202 002, U.P., India

Mehra, V.P.
Dept. of Biotechnology, Govt. Institute of Science, Aurangabad 431 004.

Mohd. Amil
Department of Agricultural Microbiology Institute Muslim University Aligarh–202 002, U.P., India

Mrunalini A. Gupte
Department of Plant Pathology Dr. Panjabrao Deshmukh Vidyapeeth Akola-444 104, Maharashtra, India

Mudalagiriyappa
University of Agricultural Sciences Dharwal-580 005, Karnataka, India

N. Venugopal
Department of Botany Bangalore University Banglore-560 056, Karnataka, India

N.A. Patel
Department of Plant Pathology, C.P. College of Agriculture Sardarkrushinagar Dantiwada Agricultural University Sardarkrushinagar–385 506

N.A. Sahasrabudhe
Scientist, Plant Molecular Biology Unit Division of Biochemical Sciences National Chemical Laboratory Pune—411 008, Maharashtra, India

N.M. Vanitha
Department of Botany Bangalore University Banglore-560 056, Karnatka, India

N.P. Patil
College of Agricultural Engineering, MAU, Parbhani (M.S.)

N.S. Kulkarni
Department of Microbiology R.A. College Washim, Maharashtra, India

Nikunj Chhag
Institute of Biotechnology, Chudasama Main Road, Near Amrapali Crossing, Rajkot-360 002, Gujarat

Nikunj H. Chhag
Institute of Biotechnology, Raiya Road, Rajkot-360 002, M.G. Science Institute, Ahmedabad. (Gujarat)

Nirali J. Joshi
Institute of Biotechnology, Raiya Road, Rajkot-360 002, M.G. Science Institute, Ahmedabad. (Gujarat)

O.D. Kohire
Plant Pathology, Agril. Research Station, Badnapur MAU, Parbhani (M.S.)

P. Arjunan
Faculty of Agriculture and Animal Husbandry Gandhigram Rural Institute Gandhigram-624 302, Dist. Dindigal Tamil Nadu

P. Gyaneshwar
Department of Biochemistry Faculty of Science, M.S. University Vadodara-390 002, Gujarat, India

P. Jothimani
Department of Environmental Sciences Tamil Nadu Agricultural University Coimbatore-641 003, Tamil Nadu, India.

P.A. Hatwalne
P.G. Department of Plant Pathology Panjabrao Deshmukh Krishi Vidyapeeth Akola–444 104, Maharashtra, India

P.B. Sandipan
Department of Plant Pathology, C.P College of Agriculture Sardarkrushinagar Dantiwada Agricultural University Sardarkrushinagar–385 506

P.G. Lavanya
Krishi Vigyan Kendra, Kattupakkam–603 203

P.L. Patil
Ex. Associate Dean and Principal of College of Agriculture Dhule-424 004, Maharashtra.

P.V. Hatwalne
Department of Plant Pathology Dr. Panjabrao Deshmukh Krishi Vidyapeeth Akola–444 104, Maharashtra, India

Palak R. Jadeja
Institute of Biotechnology, Raiya Road, Rajkot-360 002, M.G. Science Institute, Ahmedabad. (Gujarat)

Pande, A.K.; Kshirsagar, A.B; Harke S.N; Puthan, P.N.
Dept. of Biotechnology, Govt. Institute of Science, Aurangabad 431 004.

R. Saravanan
Department of Environment Sciences, TNAU, Coimbatore-641 003, Tamil Nadu.

R. Sowmya
Department of Botany Bangalore University Bangalore–560 056, Karnataka

R.B. Somani
P.G. Department of Plant Pathology Panjabrao Deshmukh Krishi Vidyapeeth Akola–444 104, Maharashtra, India

R.L. Patel
Department of Plant Pathology, C.P. College of Agriculture Sardarkrushinagar Dantiwada Agricultural University Sardarkrushinagar–385 506

R.M. Kothari
School of Life Sciences North Maharashtra University Jalgaon–425 001, Maharashtra, India

R.P. Gupta
Department of Microbiology Punjab Agricultural University Ludhiana, Punjab-141 004

R.R. More
Department of Agriculture Microbiology

R.W. Ingle
Department of Plant Pathology Dr. Panjabrao Deshmukh Krishi Vidyapeeth Akola–444 104, Maharashtra, India

Rana Athar
Institute of Agriculture, A.M.U., Aligarh, U.P. India

Roshan Sharma Poudyal
Biotechnology Unit, Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal

S. Kannaiyan
Department of Agricuture Microbiology Tamil Nadu Agricultural University Coimbatore-641 003, T.N., India.

S. Mariappan
Department of Environmental Sciences Tamil Nadu Agricultural University Coimbatore–641 003, Tamil Nadu, India

S. Sundaravarathan
Department of Agricuture Microbiology Tamil Nadu Agricultural University Coimbatore-641 003, T.N., India.

S.A. Patil
Department of Biotechnology Engineering, Tatyasaheb Kore Institute of Engineering and Technology, Warananagar. Tal: Panhala, Dist: Kolhapur-416 113 (M.S.) India

S.B. Chincholkar
School of Life Sciences North Maharashtra University Jalgaon–425 001, Maharashtra, India

S.B. Jadhav
Department of Agriculture Science and Technology Vasantdada Sugar Institute Manjri (BK.), Dist. Pune, Maharashtra, India

S.C. Kale
P.G. Department of Microbiology Y.C. College of Science Vidyanagar, Karad-415 124, Maharashtra

S.D. Khambe
Department of Microbiology, Miraj Mahavidyalay, Miraj-416 410, Maharashtra, India

S.K. Talegaonkar
School of Life Sciences North Maharashtra University Jalgaon–425 001, Maharashtra, India

S.O. Kolte
Department of Plant Pathology Dr. Panjabrao Deshmukh Krish Vidyapeeth Akola-444 104, Maharashtra, India

S.R.S. Dange
Department of Plant Pathology, C.P. College of Agriculture Sardarkrushinagar Dantiwada Agricultural University Sardarkrushinagar–385 506

S.W. Kulkarni
Department of Microbiology S.B. Zadbuke College Barshi-413 401, Dist. Salapur Maharashtra, India

Sandeep Garg
Department of Microbiology University of Delhi South Campus Benito Juarez Road New Delhi - 110 021, India

Satwekar, A.A.
Dept. of Biotechnology, Govt. Institute of Science, Aurangabad 431 004.

Shams Tabrez Khan
Institute of Agriculture, A.M.U., Aligarh, U.P. India

T. Satyanarayana
Department of Microbiology University of Delhi South Campus Benito Juarez Road New Delhi - 110 021, India

T.H. Shankarappa
Department of Agricultural Microbiology Department of Agricultural Sciences G.K.V.K. Compus Bangalore-560 065, Karnataka, India

T.Vijaya
Presently at Department of Biotechnology S.V. University, Tirupati-517 502, Andhra Pradesh

V.S. Shinde
Department of the Agronomy, M.A.U. Parbhani (M.S.)

V.W. Tarsekar
Department of Plant Pathology Dr. Panjabrao Deshmukh Krish Vidyapeeth Akola-444 104, Maharashtra, India

Vandana Gupta
Department of Microbiology University of Delhi South Campus Benito Juarez Road New Delhi - 110 021, India

Yasari, Esmaeil
Agricultural Organization of Mazandaran Province. Sary, Iran.

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Introduction

The success of green revolution depends upon the availability of fertilizers, high yielding variety of seeds, improved agronomical practices and timely availability of water. The demand for nitrogenous fertilizers has been increasing out its production has always fallen short. In spite of unlimited supply of N_2 in the air, manufacturing the fertilizer for today's needs required 544 $\times$ 10^9 MJ of fossil fuel energy which is equivalent to about 13 million tons of oil—a non-renewable source. We need about 230-240 m.t. of foodgrains by 2000 A.D. On the other hand the demand for the fertilizer nitrogen produced by using non-renewable fossil fuels cannot be met through domestic production. In such a scenario, the use of microbes who do not need fossil energy is of immense value for increasing soil productivity in India, where most of agriculture is low input subsistence farming through biological nitrogen fixation or increased efficiency of fertilizers applied.

The part of increasing deficit of nitrogenous fertilizers can be made up if one can tap the vast reservoir of atmospheric nitrogen in a simpler way—in the nodules of roots legumed plants *e.g.* soybean, groundnut, chickpea etc. These are called nature's minifertilizer factories. Some specific bacteria or micro-organisms in the soil convert this nitrogen into ammonia and amino acids. These amino acids can be used by the plants to build up proteins. This process world wide is known as "biological nitrogen fixation". The nitrogen fixation is done by two ways:

1. Symbiotically by *Rhizobium, Azolla* and *Frankia*
2. Non-symbiotically by *Azotobacter, Azospirillum, Acetobacter, Beijerinckia*, and Blue green algae.

Biofertilizers are the preparations containing cells of microorganisms which may be nitrogen fixers, phosphorus solubilizers, sulphur oxidisers or organic matter decomposers. In short,

they are called as bioinoculants which on supply to plants improve their growth and yield. In recent years a need has arisen for organic fertilizers including biofertilizers to minimise our dependence on fertilizer nitrogen. The experiments conducted in India and abroad on biofertilizers revealed that legumes *viz*, beans, soybean, chickpea, pigeonpea can fix 50-500 kg atmospheric nitrogen per hectare under ideal conditions. In recent years a use of *Rhizobium*, culture has been routinely recommended as an input in pulse cultivation. In India about 30 million hectares of land is under pulses. Therefore use of *Rhizobium* is made when India can stop the import of nitrogenous fertilizers.

The association between non-legumes and N_2 fixing bacteria as shown by increased nitrogenase activity is now well established *Azotobacter* and *Azospirillum* have been widely tested to increase yields of cereals. Of late, attention has been shifted from *Azotobacter* to *Azospirillum* due its wide distribution in soils and is relatively more efficient in utilisation of carbon to support N_2 fixation. In an evaluation of the reported worldwide success of *Azotobacter* and *Azospirillum* increase inoculation. Statistically significant in yield has been obtained. In general inoculation the C_4 plants corn, sorghum, *Paricum* and *Setaria* shoed greater yield increases than C_3 plants wheat. Most striking effects of these organisms is their ability to stimulate the germination of seeds, improve plant stand, augment root biomass and accelerate the initial vigour and plant stand, so also synthesis of chlorophyll. There is a conclusive evidence that these organisms secrete IAA, Kinetin, Gibberelins and B-vitamins. These factors are partly responsible for enhanced growth of plants and the ability of organisms to fix atmospheric nitrogen.

It has also been proved beyond doubt that *Azotobacter* synthesises antifungal antibiotics which prevent the seedling mortality caused due to fungal pathogens. Therefore, the organism is considered for production of biopesticides. It is also known that the organism secretes gum or polysaccharides which contribute to the soil improvement particularly soil structure. *Acetobacter* is a non-symbiotic (micro-symbiont) bacteria which is mostly associated with sugarcane crop. It grows along with one inside the root as well as stems to some extent, and fixes the atmospheric nitrogen and benefits the crop. It is rather difficult to isolate the organism and grow artificially on a large scale. It is for the scientists gathered here to find out suitable techniques in order to make the organism to grow faster in the laboratory for the purpose of biofertilizers. If this is done successfully we may save huge quantity of chemical nitrogenous fertilizers used to grow sugarcane crop.

Blue green algae are another potential input for low lying rice crop, which are also called a cyanobacteria. Cyanobacteria multiply through vegetative and sexual methods. They are widely distributed and responsible to increase rice yield as reported from places including Pune. This type of biofertilizers could contribute about 30-50 kg nitrogen per ha. thereby saving input cost of chemical fertilizers. They are very simple to multiply even farmers can adopt techniques to carry out production of BGA on their farms.

Maharashtra has a large acreage of saline-alkali soils which are unsuitable for growing any crop. Various methods are applied to reclaim them including providing proper drainage, adoption of suitable cropping systems, and using proper irrigation methods. However, these systems

need serious efforts at various levels. There are scanty reports that BGA could reclaim such soils and reduce the salinity to 25-50%, pH, electrical conductivity and exchangeable sodium. It was further reported that it increases soil aggregation, hydraulic conductivity, soil nitrogen and permeability. However, the mechanism is yet to be investigated. The commonly known species of BGA are *Anabaena, Nostoc, Calothrx* and *Scytonema*. I therefore, appeal to this august gathering to conduct more research to solve soil problems affecting crop yield mostly salinity and alkalinity.

Azolla is an aquatic fern that assimilates atmospheric nitrogen in association with the nitrogen fixing symbiotic blue green algae. *Anabaena azollae*. The agronomic potential of *Azolla* is quite significant particularly for rice crop and it may be applied as biofertilizer increasing rice yield. It is reported that *Azolla* also suppresses the weeds in wetland rice besides providing additional nitrogen to rice. Temperature is the most important factor responsible for growing *Azolla* in tropics. This is considered as limiting factor for cultivation of *Azolla* and I request the scientists gathered here to do the research for obtaining the strains tolerance to high temperature.

Secondly, *Azolla* need to be multiplied and kept on water throughout its growth and fresh *Azolla* is used for inoculation. This is one of the constraints to store the *Azolla*. Besides, the viability of *Azolla* forming seeds is very poor and hence it will be prime responsibility of research workers to breed *Azolla* forming seeds having longer viability.

Some non-leguminous shrubs and trees are modulated by nitrogen fixing actinorrhizae, *Frankia* and actiomycete. There are no species of *Frankia* so fare created. Nitrogen fixation occurs in terminal swellings of the actiomycetes hyphae called vesicles. The nodule size can range from a few mm to several cm diameter. They are not pink in colour but a leg haemoglobin like molecules have recently been detected in the nodules. The genus *Frankia* has been studied in detail by a student of Dr. P.L. Patil who reported several new species from Pune for the first time.

This study includes growing of *Azolla* in pure culture. *Frankia* is a micro-aerophillic. Nitrogen fixation in pure culture appears to be relatively insensitive to oxygen. The *Frankia* fixes atmospheric nitrogen up to 150 kg per hectare. However, its commercial utilisation as biofertilizer has not been studied which needs to be investigated by the scientists actively involved in microbiological research.

Amongst the most widespread, yet least recognised, relationship between microorganisms and higher plants are symbiosis known as mycorrhizae (fungus + plant root). In this system plant roots and certain species of fungi form an intimate association one which is beneficial to each other. Three general types of mycorrhizae are known such as ecto, endo (also-vasicular arbuscular mycorrihzae) and ectoendomycorrhizae. VAM are obligate parasites and hence cannot be cultured on artificial media. VAM increases efficiently mineral nutrient from soil resulting in enhanced growth. It was also reported that VAM increases water uptake or alter plants physiology to reduce stress response to soil drought. There are several other benefits derived from VAM including withstand to high temperature, help to reduce toxicity etc.

Though VAM are beneficial to shrubs or herbs their use has been limited because they are obligate and hence they cannot be cultured on a large scale. I will therefore appreciate if

anybody works out a method to culture the VAM which can break through the research of mycorrhizae.

For degradation of any agricultural waste organic material of any source needs microorganisms although in some cases chemical degradation is possible but not economical. In nature, organic matter is degraded by different groups of micro-organism *viz* bateria, fungi, Actinomycetes and Protozoa. Bacteria are beginners who take active part in degradation. Secondary flora of fungi are developed which also take active part in degradation of agricultural and city wastes.

Agricultural College, Pune is pioneer in developing bio-inoculant for degradation of organic wastes and preparation of decomposing cultures. These micro-organisms include *Aspergillus, Penicillium, Trichoderama, Trichurus,* and *Paecelomyces*. These organisms form different types of acids resulting into decomposition of organic matter. Use of compost/FYM is essential in order to increase the efficiency of chemical fertilizers. Under this situation the degradation of organic wastes is essentially a biological process. By using these efficient cultures period of degradation of agricultural wastes can be minimised by 30-40%. A group of microbiologists headed by Dr. P.L. Patil, at Pune has developed a unique process of production of decomposing cultures which was subsequently followed by others. Now most of the sugarcane factories use decomposing cultures for the process of spentwash-pressmud degradation. This has resulted into utilisation of spent was and pressmud thereby increasing the availability of organic matter for agricultural production. The role of 'P' solubilising micro-organisms has been extensively studied by different workers. Next to nitrogen 'P' is important nutrient required by plants for their growth. Phosphorous applied to soil in one or other way is fixed and not easily available to crop plants resulting into depraving of plants from using the phosphorus. It is very necessary to solubilise this phosphorus so that plants can obtain as and when required by them. The micro-organisms *viz*, bacteria and fungi play a significant role in solubilising the applied as well as native phosphorus. Some bacteria mobilise insoluble forms of phosphate into soluble forms. Pre-treatment of seeds of cereals with phosphobacteria has been reported to help in reducing fertilizer phosphate requirements of the crop and increasing its grain yield. The bacteria or fungi are known to dissolve native phosphate through enzymatio action and made it available for plat growth. Crop rotation in which cereal crops follow legumes are known to derive double benefits by way of added organic nitrogen and soluble phosphate due to actions of micro-organisms in and around the legume root system.

Although, an extensive research has been made on utilisation of phosphorus solubilising micro-organisms in order to provide soluble form of phosphate to plants its large scale implementation has not been seen. Hence it is necessary to extent the use of micro-organism in enhancing the solubility of native as well as applied phosphate more efficient strains need to be isolated and used for preparation of biofertilizers.

In order to reach the estimated target of 240 Mt of foodgrains by 2000 A.D. we must harvest the benefits from microbes particularly BNF. We have large area under pulses as well as cereals and hence we need biofertilizers in million tons. Besides we have to provide good

quality biofertilizers. I was told that at present there is no law for defaulters those who don't produce good quality biofertilizers. I therefore, alert the scientists to press for such law in every state.

Why there is specificity in certain bacteria to selected plants? The genus *Rhizobium* is selective in choosing roots of particular legumes. Similar is the case of other species of microbes. We should find out reasons why this is so.

Secondly search for organisms which will have modulating capacity on cereals not to be done although false nodules have been reported on cereals. Can we evolve nitrogen-fixing plants? So that question of adding chemical fertilizers will not arise. These are some of the constraints in biological nitrogen fixation. You may ponder during next two clays over these areas so also other important aspects of microbiology.

There are not may micro-organisms which can be used as biopesticides except *Bacillus thuringiensis* and *Trichoderma*. Besides, there are no proper methods to use them effectively in control of plant diseases. These are some of the future research in microbiology.

Authors

Chief Editor: Dr. A.M. Deshmukh, *Reader & Head, Dept. of Microbiology, Dr. Babasaheb Ambedkar Marathwara University, Subcentre Osmanabad-413 501 (M.S.)*

Co-editors: Shri R.M. Khobragade & Shri P.P. Dixit, *Lecturers, Dr. Babasaheb Ambedkar Marathwara University, Subcentre Osmanabad-413 501 (M.S.)*

1

Effect of Biofertilizers on Seedling Growth, Physiology, Nodule Production and VAM Colonization in Pungam, *Pongamia pinnata* (L.) Pierre

Introduction

Pungam is extensively used for afforestation of watersheds in the drier parts of the country. It is drought resistant, moderately frost hardy ad highly tolerant to salinity. It is being propagated by direct sowing or by transplanting one year old seedlings raised in nursery (Troup, 1983).

Micro-organisms that are sued as biofertilizers stimulate plant growth by providing necessary nutrients as a result of their colonisation at the rhizosphere (*Azotobacter, Azospirillum, Pseudomonas*, phosphate-solubilising bacteria, and Cyanobacteria) or by symbiotic association (*Rhizobium, mycorrhizae* and *Frankia*). The role of biofertilizers has already been proved extensively in annual crops, but its exploitation in perennial trees in India is scanty. In forestry, few research reports are available to demonstrate that biofertilizers stimulate the growth (Niranjan *et al.*, 1990), biomass (Sekar *et al.*, 1995), nodulation and VAM-colonization, (Reena nd Bagyaraj, 1990). Keeping this in view, the present study was designed to elicit information on the effect of biofertilizers on the growth and development of pungam (*Pongamia pinnata*) (L.) Pierre.

Material and Methods

The study was carried out at Forest College and Research Institute (11°19 'N, 76°56 'E, 300 above MSL). Six month old uniform sized seedlings were chosen for biofertilizer inoculation. The seedlings were transplanted in 30 × 45 cm polybags filled with nursery mixtures of red soil, sand and FYM (2:1:1). The experiment was set up in a completely randomised design and replicated thrice. The nursery soil mixture of the polybags were inoculated with biofertilizers, *viz.*, (i) *Rhizobium*, (ii) phosphobacteria, (iii) Vesicular-Arbuscular Mycorrhizae (VAM), (iv) *Rhizobium* + phosphobacteria, (v) phosphobacteria + VAM, (vi) Rhizobium + VAM,

(vii) *Rhizobium* + phosphobacteria + VAM. Uninoculated seedlings were maintained as control.

Biofertilizer inoculation was prepared with a base of peat soil. Two hundred grams of *Rhizobium* and phospobacteria were weighed and mixed with 3 kg of well decomposed and powdered FYM separately. Fifty grams of this inoculum mixture and twenty grams of VAM inoculum were applied to each polybag at 5 cm depth near the root zone.

Six seedlings in each treatment were selected at random and observed initially, 2 and 4 months after inoculation for number of leaves, leaf area (LICOR Model LI 3000 Leaf Area Meter), root volume, total dry matter, DGR, NAR (Williams, 1946), CGR (Watson, 1958), number of nodules and VAM colonisation (Phillips and Hayman, 1970). The results were subjected to analysis of variance and tested for significant differences ($P < 0.05$) after Panse and Sukhatme (1967).

Results and Discussion

A significant increase in number of leaves (47.7%) (Table 1), total leaf are (44.4%) (Table 1) and root volume (47.3%) (Table 2) was achieved over control by inoculating the seedlings with *Rhizobium*, phosphobacteria and VAM conjointly. The results corroborated the findings of Sekar *et al.*, (1995) in Shola species. The microorganisms that are used as biofertilizers colonise in the rhizosphere and stimulate the plant growth by providing necessary nutrients, thanks to their symbiotic association (Varma and Schuepp, 1995). The association may also regulate the physiological process in the ecosystems by involving in the decomposition of organic matter, fixation of atmospheric nitrogen, secretion of growth promoting substances, increased availability of mineral nutrients and protection of plants from pathogens besides increasing the availability of nutrient elements at the root zone (Newman, *et al.*, 1992).

An increase of 54.5% in total dry weight over the control was evident in seedlings inoculated combined with *Rhizobium*, phosphobacteria and VAM (Table 1). Similar increase in biomass production due to VA-mycorrhizal inoculation was documented in *Acacia* spp. (Reena and Bagyaraj *et al.*, 1990); and *Albizia* spp. (Jamaluddin *et al.*, 1995). In the present investigation, individual inoculation VAM also increased biomass relative to the control though not to the tune of combined biofertilizer inoculation. The increase in seedling biomass production may be strongly correlated with increased accumulation of N due to *Rhizobium* (Jamaluddin *et al.*, 1995) and P due to VAM and phosphobacteria (Reddy *et al.*, 1996).

Tripartite inoculation of *Rhizobium*, phosphobacteria and VAM registered higher number of nodule production (69.9 %) than uninoculated control (Table 2). Dual inoculation with *Rhizobium* and *G. fasciculatum* improved the nodulation status in *L. leucocephala* compared to the single inoculation (Manjunath *et al.*, 1984). In the present experiment, the combined inoculation of *Rhizobium*, phosphobacteria and VAM registered higher VAM-colonisation (65.6%) than the uninoculated seedlings (56.1 %) (Table 2). Such an increase in root colonisation due to VAM and *Rhizobium* inoculation was reported in *L. leucocephala* (Manjunath *et al.*, 1984).

The relative growth rate (RGR) of *P. pinnata* seedlings inoculated with *Rhizobium*, phosphobacteria and VAM was higher (0.0071 $g.g^{-1}.day^{-1}$) compated to the control seedlings

Table 1

Effect of biofertilizers onnumber of leaves, total leaf area and total dry weight of pungam seedlings.

Biofertilizers (B)	*Number of leaves Months after inoculation (M)*				*Total leaf are (cm^2 $plant^{-1}$) Months after inoculation*				*Total dry weight (g. $plant^{-1}$) Months after inoculation*			
	0	*2*	*4*	*Mean*	*0*	*2*	*4*	*Mean*	*0*	*2*	*4*	*Mean*
Rhizobium (R)	18.7	43.7	48.7	37.0	312.6	431.1	569.0	437.5	9.14	27.56	36.13	24.27
Phosphobacteria (P)	18.3	45.3	47.7	37.1	305.4	437.9	569.0	464.0	9.45	32.35	41.01	24.60
VAM	19.0	46.3	53.3	39.6	302.0	462.5	648.6	491.6	9.08	27.61	39.80	25.50
R + P	20.0	48.7	54.3	41.0	305.0	498.9	710.2	504.3	9.25	31.15	40.12	26.84
P + VAM	19.0	47.7	57.3	41.3	286.4	499.6	651.3	479.1	9.44	29.88	40.60	26.64
R + VAM	19.3	52.3	62.0	44.6	297.6	556.1	739.0	531.0	9.11	32.22	42.39	27.91
R + P + VAM	19.7	58.7	68.3	48.9	303.0	641.2	850.0	589.0	0.01	43.18	46.94	33.04
Control	19.7	37.3	42.3	33.1	291.8	408.0	524.1	408.0	9.11	23.15	31.88	21.38
Mean	19.2	47.5	54.3		300.4	488.6	675.1		9.20	30.89	39.86	

	SEd	CD (P=0.05)	SEd	CD (P=0.05)	SEd	CD (P=0.05)
B	1.04	2.10	24.76	49.76	0.839	1.686
M	0.64	1.29	15.15	30.47	0.514	1.033
B × M	1.81	3.64	42.86	86.19	1.453	2.921

Table 2
Effect of biofertilizers on root volume, number of nodules and vesicular-arbuscular mycorrhizae colonisation of pungam seedlings.

Biofertilizers (B)	*Root value (mm^3) Months after inoculation (M)*				*Number of nodules Months after inoculation*				*VAM colonisation % Months after inoculation*			
	0	*2*	*4*	*Mean*	*0*	*2*	*4*	*Mean*	*0*	*2*	*4*	*Mean*
Rhizobium (R)	13.0	25.0	29.0	22.3	9.0	19.0	21.3	16.4	51.0 (45.6)	58.3 (49.8)	66.0 (54.3)	58.4 (50.0)
Phosphobacteria (P)	13.3	26.3	29.0	22.9	9.3	12.3	23.3	15.0	51.3 (45.8)	57.7 (49.4)	65.7 (54.1)	58.2 (49.8)
VAM	13.5	25.0	30.0	22.8	10.0	11.3	17.3	12.9	51.7 (45.9)	61.7 (51.8)	70.0 (56.8)	61.1 (51.5)
R + P	12.8	26.0	30.7	23.2	10.0	15.7	18.3	14.7	51.0 (45.6)	59.0 (50.2)	66.0 (54.3)	58.7 (50.0)
P + VAM	13.4	31.3	34.7	26.5	9.0	13.0	17.0	13.0	51.3 (45.8)	63.3 (52.7)	71.0 (57.4)	61.9 (52.0)
R + VAM	12.9	36.7	42.0	30.5	9.0	18.0	20.0	15.7	51.3 (45.8)	64.0 (53.1)	72.7 (58.5)	62.7 (62.7)
R + P + VAM	13.6	43.3	49.7	35.5	8.3	20.0	29.3	19.2	52.0 (46.1)	66.7 (54.7)	78.0 (62.0)	65.6 (54.3)
Control	13.3	21.7	27.0	20.7	9.0	10.3	14.7	11.3	51.3 (45.8)	55.0 (47.9)	62.0 (52.0)	56.1 (48.5)
Mean	13.2	29.4	34.0		9.2	14.9	20.2		51.4 (45.8)	60.7 (51.2)	69.0 (56.2)	

	SEd	CD (P = 0.05)	SEd	CD(P=0.05)	SEd	CD (P 0.05)
B	1.19	2.40	0.88	1.77	0.44	0.89
M	0.73	1.47	0.54	1.09	0.27	0.55
L . M	2.07	4.16	1.53	0.37	0.77	1.55

Table 3
Effect of biofertilizers on relative growth rate, crop rate and net assimilation rate of pungam seedlings.

Biofertilizers (B)	*RGR (g. g $^{-1}$. day $^{-1}$) Months after inoculation (M)*			*CGR (g.m^{-2}.day $^{-1}$) Months after inoculation*			*NAR (g.m^{-2}.day^{-1}) Months after inoculation*		
	0-2	*2-4*	*Mean*	*0-2*	*2-4*	*Mean*	*0-2*	*2-4*	*Mean*
Rhizobium (M)	0.0080	0.0020	0.0050	0.307	0.144	0.225	3.60	1.48	2.54
Phosphobacteria (P)	0.0089	0.0017	0.0053	0.382	0.145	0.263	4.54	1.44	2.99
VAM	0.0080	0.0052	0.0052	0.304	0.203	3.256	3.56	1.47	2.52
R + P	0.0089	0.0018	0.0053	0.365	0.149	0.257	4.04	1.49	2.77
P + VAM	0.0083	0.0023	0.0053	0.341	0.179	0.260	3.85	1.43	2.64
R + VAM	0.0091	0.0020	0.0056	0.385	0.169	0.277	4.05	1.53	2.79
R + P + VAM	0.011	0.0028	0.0071	0.569	0.145	0.357	5.62	1.83	3.73
Control	0.0067	0.0024	0.0045	0.234	0.062	0.148	2.92	1.38	2.15
Mean	0.0086	0.0022		0.361	0.149		4.02	1.51	

	SEd	CD(P = 0.05)	SEd	CD (P = 0.05)	SEd	CD (P = 0.05)
B	0.00049	0.00100	0.0287	0.0584	2.734	NS
M	0.00024	0.00050	0.0143	0.0292	1.146	2.341
B × M	0.00069	NS	0.0406	0.0826	1.596	3.257

(0.0045 $g.g^{-1}.day^{-1}$) (Table 3). Similarly, increase was also evident in crop growth rate (CGR), the quantum of increase being 141.12 per cent over the control. However, the net as simulation rate (NAR) was not significantly influenced due to the treatments. The CGR is function of dry matter production (Milthrope, 1967) hence, the increase in CGR and RGR in the present investigation might be due to the increased dry matter production in the seedlings inoculated with *Rhizobium* + phosphobacteria + VAM.

The results of the present study are in strong support of combined inoculation of the *P. pinnata* seedlings with *Rhizobium* phosphobacteria and VAM. It is hence recommended that seedlings produced for largescale afforestation should be subjected to such an inoculation treatment to ensure better plant growth and survival.

Summary

An experiment was conducted to study the response of pungam to biofertilizer inoculation viz., *Rhizobium*, phosphobacteria and VAM individually and conjointly under nursery conditions. The uninoculated seedling formed the control. The results indicated an enhancement in number of leaves, total leaf area, total dry matter, root volume, number of nodules and VAM colonisation due to triple inoculation with *Rhizobium*, phosphobacteria and VAM. This also recorded increased RGR and CGR, relative to the control.

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Authors

A. Venkatesh, Mallika Vanangamudi, K. Vanangamudi,
K.T. Parthiban and Ravichandran
Forest College and Research Institute
Mettupalayam-641 301, T.N., India

2

Biofertilizer: A Supplementary Nutrient Source for Sugarcane

Introduction

Biofertilizer have an important role to play in improving nutrient supplies and their crop availability in the years to come. They are of environment friendly non-bulky and low cost agricultural inputs. A biofertilizer is an organic product containing a specific micro-organisms in concentrated form which is derived either from the plant roots or from the soil of root zone (*Rhizozsphere*). High productivity in irrigated areas like sugarcane leads to higher nutrient turnover and a larger gap between nutrient supply and removal. Therefore, there is strong need to have complementary use of available source to plant nutrient including biofertilizer along with mineral fertilizers for maintenance of soil productivity.

The microorganisms responsible for fixing atmospheric nitrogen has been recognised since time immemorial, however, commercialisation of their use is a relatively new innovation. In view of their apparent low-cost and risk-free application leading to yield advantage both for current as well as for subsequent crop growth or residual fertility. Their large scale application in intensive farming call for systematic efforts. Their use especially important in view of the fact that in the country to be self sufficient in the production of nitrogenous and phosphatic fertilizers. Among the biofertilizers *Azotobacter*, *Azospirillum*, *Acetobacter* are the important for nitrogen fixation, *Bacillus* sp. and *Aspergillus* sp. are important for phosphate solubilisation and other soil mineral nutrients.

Experiment and Results

Azotobacter

This is a group of bacteria, which are free-living nitrogen fixer. The mechanism by which the plants, inoculated with *Azotobacter*, derive possible benefits in terms of increased grain,

plant biomass and nitrogen uptake which in turn are attributed to small increase in nitrogen input from biological nitrogen fixation, development and branching of roots, production of plant growth hormones, vitamins, enhancement in uptake as NO_3, NH_4, H_2PO_4K and Fe, improved water status of the plant, increased nitrate reductase activity and antifungal compounds.

Table 1
Effect of *Azotobacter* culture on sugarcane yield (T/ha).

Nitrogen levels	*Inoculated*	*Uninoculated*	*Mean*
250 kg/ha	146.43	132.97	139.70
300 kg/ha	152.77	145.70	149.23
350 kg/ha	156.73	149.07	152.90
400 kg/ha (control)	154.67	149.98	152.32
Mean	152.65	144.43	

SE ± 1.83; C.D. (5%) 5.52

Fixation of nitrogen to the soil by use of *Azotobacter* culture is a natural phenomenon and sufficient research work has been carried out on the role of *Azotobacter* culture in sugarcane cultivation at Sugarcane Research Station, Padegaon. The results, in general, indicate that application of *Azotobacter* at the rate of 5 kg/ha helps in reducing nitrogen dose by 50 kg/ha with increase in cane yield by 5 to 10 per cent (Anonymous, 1992, Patil & Hapase, 1981). However, it can help in reducing nitrogen dose even up to the tune of 100 kg/ha without loss in yield.

The *Azotobacter* plays an important role in improving cane germination, establishing of seedlings, survival of tillers, increase the population of millable cane and improve the soil fertility.

Azospirillum

It is an associative micro-aerophilic nitrogen fixer. It colonises the root mass and fixes nitrogen in close association with plant. It fixes nitrogen in an environment of low oxygen tension. These bacteria induce the plant roots to secrete a mucilage which creates low oxygen environment and helps to fix atmospheric nitrogen. High nitrogen fixation capacity, low energy requirement and abundant establishment in the roots of sugarcane and tolerance to high soil temperature makes these most suitable for tropical conditions. Use of *Azospirillum* inoculum under saline-alkali conditions is also possible because their strains are known to maintain high nitrogen activities under such stress conditions (Hegade & Dwivedi, 1994; Marwaha, 1995).

The field experiment revealed that *Azospirillum* inoculatin increase the cane yield up to 4.47 tonnes/hectare and could save 25% nitrogen dose in medium black soil under Adsali planting (Shinde *et al.*, 1991).

Table 2
Effect of *Azospirillum* culture on sugarcane yield

Treatment	*Yield (T/ha)*	*Available N in soils (kg/ha)*
100% N (control)	122.19	179.55
75% N + *Azospirillum*	126.44	169.95
50% N + *Azospirillum*	117.10	150.60

SE ± 1.37; C.D. (5%) 4.10

Table 3
Effect of *Azotobacter* and *Azospirillum* inoculation under graded levels of nitrogen on yield and CCS of sugarcane (average of 3 years).

Cultures	*Nitrogen levels kg/ha*				
	175	*200*	*225*	*250*	*mean*
Control	103.50 (12.25)	105.73 (12.71)	107.03 (13.07)	110.47 (13.23)	106.68 (12.81)
Azotobacter	106.70 (12.58)	108.20 (12.83)	11.97 (13.42)	112.67 (13.50)	109.88 (13.08)
Azospirillum	108.23 (12.59)	109.87 (12.93)	112.63 (13.55)	113.43 (13.69)	111.04 (13.19)
AZT + AZP	108.53 (12.59)	110.73 (13.11)	114.33 (13.85)	115.00 (13.95)	112.14 (13.37)
Mean	106.74 (12.50)	108.63 (12.89)	111.49 (13.47)	112.89 (13.59)	

AZT-*Azotobacter*, AZP-*Azospirillum*
*Figures in parentheses denote the CCS in MT/ha. (commercied cane sugar)

1. Yield	C.D. (P-0.05)	CCS C.D. (P-0.5)
2. Culture	0.32	0.032
3. N levels	1.78	0.102
4. Interaction	3.79	0.33

Phosphate Solubilising Microoganisms

Phosphorous is the most important micro-nutrient required by sugarcane and micro-organisms for their normal growth and development. However, Indian soil test, generally low to medium

in available phosphate and not more than 30% of applied phosphate is available to the current crop, remaining part gets converted into relatively unavailable forms.

Many common heterotrophic bacteria like *Bacillus megaterium* and *B. circulans* and fungi like *Aspergillus awanori* and *Penicilium striata* posses the ability to bring sparingly soluble/ insoluble inorganic and organic phosphorus into soluble forms by secreting organic acids. These organic acids lower soil pH and in turn brings about dissolution of unavailable forms of soil phosphorous. Some of the hydroxy acids may chelate Ca, Al, Fe and Mg resulting in effective availability of soil and hence its higher utilisation by sugarcane plants. The field experiments showed that the inoculation of phosphorus solubilising cultures to sugarcane sets at the time of planting increased cane and sugar yield significantly over uninoculated control (Shinde *et al.*, 1987). It could be reduced the phosphate dose by 50% and could be applied in the form of rock phosphate which is the cheaper source of phosphorus.

Table 4

Effect of phosphate solubilising culture on cane and sugar yield

Treatment	*Cane yield*	*CCS (T/ha)*
Control (N + K)	145.28	21.71
N + K_2O + P.S. Culture	152.48	22.63
Rock phosphate + N + K_2O	164.07	25.65
Rock phosphate + N + K_2O + P.S. Culture	166.81	22.49
Superphosphate + N + K_2O + Culture	194.81	28.67
Rock phosphate + 50% + N + K_2O	149.35	20.95
Rock phosphate 50% + N + K_2O + P.S. Culture	158.70	23.61
Superphosphate 50% + N + K_2O	171.48	24.29
Superphosphate 50 + N + K_2O + P.S.C.	174.86	25.68
Rock-P Super-P. 50% + N + K_2O	182.79	26.00
Rock-P + Super-P 50% + N + L_2O + P.S.C.	191.87	27.43

P.S.C. = Phosphate Solubilising Culture
SE = ±3.20
C.D. 5% = 9.40

The other groups of micro-organisms like *Acetobacter dizotrophicus* a nitrogen fixer. *Thiobacillus thiooxidans* sulphur and iron oxidizers and Vasicular Arbuscular Mycorrhiza (VAM) a phosphate solubilisers are found to be effective but needs more experimentations at multiplications and soil type for confirmation and recommendation.

Summary

Biofertilizers are low cost agricultural input playing a significant role in improving nutrient availability to the crop plants. The present paper examines potential of different bio-inoculants in sugarcane crop for increasing cane and sugar yield as well as saving of chemical fertilizers. The recommendations made to sugarcane growers based on research work done at Central Sugarcane Research Station, Padegaon are highlighted. The field experiments revealed that *Azospirillum* and/or *Azotobacter* inoculation at the rate of 5kg/ha increased the cane yield ranged from 4.57 to 11.50 t/ha with saving of 25% nitrogenous fertilizer. However, the beneficial effect was more pronounced in dual inoculation of *Azotobacter* plus *Azospirillum* culture.

The phosphate solubilising bacteria *Bacillus megaterium* could increase the availabilityof phosphatic fertilizer and resulted into increased cane andsugar yield upto 15 per cent. The costly single super phosphate could be replaced up to 50 per cent by using cheaper rock phosphate with phosphate solubilising biofertilizer. The study on *Acetobacter dizotrophicus* a endophytic nitrogen fixer in sugarcane has also been reviewed in the paper.

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Authors

B.D. Shinde and K.K. Khade
Central Sugarcane Research Station
Padegaon, P.O. Nira – 412 102
Dist. Pune, Maharashtra, India

3

Effect of Composite Bioinoculations and Fertilizer Levels on Growth of *Sorghum* (CCH-14)

Introduction

India, due to increase in population, may face the problems of food. So contribution towards the agricultural productivity improvement is necessary. *Sorghum* is the staple food crop of Maharashtra State. The main principle of obtaining maximum yield from *Sorghum* hybrid is judicious use of chemical fertilizers which at present is proving a set back in increasing the yield as the chemical fertilizers are not only in short supply but also are expensive. Use of chemical fertilizers is limited to rich farmers and they show certain drawbacks like their manufacture depends on energy derived from fossil fuels which are getting depleted at faster rate. Many crops suffer from potassium deficiency because of excessive use of nitrogen based fertilizers, excessive potassium treatment decrease available nutritive food such as ascorbic acid etc., continuous use of ammonium fertilizers increase soil acidity and radioactivity, lastly chemical fertilizers have less effect on succeeding crops. On the other hand biofertilizers are advantageous over chemical fertilizers due to low cost, simple methodology of production, no any hazard to agroecosystem and considerable residual effect on succeeding crop. Tilak *et al.*, 1982 reported that microbial inoculants application gives equivalent output as derived from the application of 30 to 40 kg/ha. N. Biofertilizer makes a significant contribution towards the development of strategies for productivity improvement and may help for economising the production cost. Since long effect of nitrogen fixers, phosphate solublisers singly has been studied by many workers on many crops. But little work on combined effect of inoculant has been reported.

Keeping this view in mind an attempt has been made to see the effect of bioinoculants on growth parameters of *Sorghum* using *Azotobacter, Azospirillum*, VAM and their different combinations. The same experiment is compared with recommended dose of fertilizer treatment and with no any treatment as a control.

Material and Methods

Pure cultures of *Azotobacer chrocooccum, Azospirillum brasilense* and VAM fungus, *Glomous fasiculatum*, wher obtained from Sorghum Research Unit, Akola and ICRISAT, Hyderabad. Keeping all the recommended packages of practice the experiment was laid out in split plot design with 3 replication, using 24 treatment combinations. The main plot treatments were recommended full dose of fertilizer 80N : 40P : 40K kg/ha, three-forth dose of fertilizer 60N : 30P : 30K and no fertilizer. While the subplot treatment were seed treatment with *Azotobacter, Azospirillum*, VAM, Nitrogen Fixers + VAM and no seed treatment.

Seed treatment was carried out using jaggery solution as an adhesive which was prepared by boiling 100 gm of jaggery in 1 litre of water. The seeds were treated in a pan by sprinkling the adhesive on seed cultures 10 gm of bioinoculant was used for treating 250 gms of seed. The seed material was eventually coated with cultures, seeds were treated two hours prior to sowing. Sowing was done by dibbling seeds. Germination studies were carried out in pot cultures. 10 seeds were treated with bioinoculants singly and combination. Germination count was taken 10 days after sowing. Simultaneously germination studies in laboratory was also carried out using Rad dolls paper towel method (ISTA 1976) 100 seeds were plated on two well moistened blotters (48 × 48 cms) and then covered with another well moistened blotters of the same size and then roll was prepared. For each treatment 100 seeds were tested. The rolls were then kept in upright position in a germination chamber at 30°C. To provide continuous moisture to the germinating seed, one end of the roll is kept in small amount of water. Germination count was recorded on 10^{th} day after seeding. Simultaneously field trials was undertaken at various fertilizer levels and bioinoculant seed treatment. Observation regarding growth parameters like germination count, root and shoot length, biomass production and seedling vigour index were recorded by selecting 4 plants randomly at an interval of 15, 30, 45, 60 days. The data recorded for the growth parameters was analysed by standard statistical methods and all the treatments were tested for their significance at 5% probability levels.

Results and Discussion

Effect of different treatments on germination count in pot cultures (Table 1) indicates that with increase dose of fertilizers germination percentage also increased but the increase was not significant, seed germination using single and composite culture inoculation in the pot studies revealed that maximum percentage of seed germination was observed when seeds were treated with *Azotobacter* + *Azospirillum*, followed by *Azotobacter* + *Azospirillum* + VAM. Germination percentage with treatment VAM culture was better than in combined treatment with *Azotobacter* + VAM and *Azospirillum* + VAM and single inoculation of *Azotobacter* and *Azospirillum*. Seed germination percentage observed in paper towel test in laboratory conditions also showed similar effects those in pot studies. From the literature referred there are few reports on the effect of the combined culture inoculation especially on the germination in *Sorghum*. However, present studies revealed that seed inoculation with *Azotobacter* and *Azospirillum* along with VAM increased the seed germination of *Sorghum* compared to single culture inoculation. Seed

Table 1

Effect of different treatments on germination count, root length and shoot length (cm) in pot after 10 days.

No.	*Particulars*	*Germination %*	*Root length (cm)*	*Shoot length (cm)*	*SVI Seeding vigor Index*
1.	**Fertilizer treatments**				
	T_1-full dose of fertiliser	84.16	12.16	10.7	1940.72
	T_2-three-fourth dose of fertilizer	76.66	9.87	9.6	1492.57
	T_3—no dose of fertilizer	75.41	8.70	8.6	1304.59
	'F' test	NS	Sig	Sig	
	SE (M) ±	2.01	0.16	0.12	
	CD at 5%	–	0.66	0.49	
2.	**Seed treatments**				
S_1	Seed treatment with *Azotobacter*	77.77	9.6	8.9	1438.74
S_2	Seed treatment with *Azospirillum*	77.77	9.8	9.2	1477.63
S_3	Seed treatment with VAM	78.88	10.0	9.3	1522.38
S_4	Seed treatment *Azotobacter* + *Azospirillum*	84.44	10.5	10.2	1747.90
S_5	Seed treatment *Azotobacter* + VAM	75.55	10.4	10.1	1548.77
S_6	Seed treatment *Azospirillum* + VAM	76.66	11.4	10.2	1655.85
S_7	Seed treatment *Azotobacter* + *Azospirillum* + VAM	82.22	10.9	10.0	1718.39
S_8	No seed treatment	76.66	9.1	8.3	1333.88
	''F test	NS	Sig	Sig	
	SE (M) ±	3.81	0.18	0.41	
	CD at 5%	–	0.51	1.17	

Bar notation	T_1	T_2	T_3
Root length (cm)	12.36	9.87	8.70
Shoot length (cm)	10.7	9.6	8.6

Bar notation	S_6	S_7	S_4	S_5	S_3	S_2	S_1	S_8
Root length	11.4	10.9	10.5	10.4	10	9.8	9.6	9.1
Bar notation	S_6	S_4	S_5	S_7	S_3	S_2	S_1	S_8
Shoot length	10.2	10.2	10.1	10	9.3	9.2	8.9	8.3

SE (M) ± = Standard error of mean; Cd = Critical Difference; Sig = Significant

Table 1a.

Effect of seed treatment on *Sorghum* with biofertilizer in paper towel test.

Particulars	*Germination %*
Azotobacter	80
Azospirillum	80
VAM	81
Azotobacter + *Azospirillum*	87
Azotobacter + VAM	80
Azotobacter + VAM	82
Azotobacter + *Azospirillum* + VAM	85
No seed treatment	78

bacterization with either *Azotobacter* or *Azospirillum* had improved the seed germination in Sorghum and the present results are in acquiescence with those reported by Lazarov *et al.*, (1964).

Lazarov *et al.*, in 1994, Mallikarjunaiah *et al.* in 1982 and Gaskins *et al.*, in 1979 reported increase in root and shoot length of *Sorghum* when treated with *Azotobacter* and *Azospirillum* respectively. From the literature referred no citation on root and shoot length in *Sorghum* with combined culture inoculation could be traced. As well as no reports with effect of VAM on root and shoot length in *Sorghum* in pot studies are available. In present studies it was observed that under laboratory conditions *Azotobacter* and *Azospirillum* seed treatment increases root and shoot length, *Azospirillum* + VAM had significant effect on root and shoot length and hence confirms the findings of above workers. Further it was also noticed that full dose of recommended fertilizers contributed in increasing root and shoot length in pot. (Table 2).

Lazarov *et al.*, (1964) reported more root and shoot length of *Sorghum* due to *Azotobacter* inoculation whereas Umali (1980) reported increased root and shoot development by *Azospirillum* inoculation under field conditions, present results of mean root and shoot length was found to be maximum in even full dose of fertilizers whereas minimum in the treatment T-3 control. In case of seed treatment it was found that treatment with nitrogen fixers + VAM shows maximum root and shoot length which was significantly superior over control (Table 2). Hence, present studies confirm the findings of above workers. Result of pot studies regarding increase in root and shoot length were also confirmed under field conditions.

Kapulnik *et al.* in 1981 reported increase in root and shoot weight of Sorghum with *Azospirillum* treatment, Nagarjun in 1989 reported that combined effect of VAM + *Azospirillum* increases biomass of Sorghum whereas Sarig and Kapulnik in 1981 noticed more dry matter produced with *Azospirillum* inoculation as compared to control. Pocovasky and Fuller (1985) reported that *Azospirillum* + VAM in combination results more dry matter in Sorghum as compared to uninoculated control.

Table 2
Effect of different treatment on root and short length of Sorghum (CSH-14) in field trials.

No.	Particulars	MeanRoot and Shoot length (cm) at various intervals in days after sowing									
		15		*30*		*45*		*60*		*Mean*	
		RL	**SL**	**RL**	**SL**	**RL**	**SL**	**RL**	**SL**	**RL**	**SL**
1.	**Fertiliser treatment**										
	T_1 full dose of fertilizer	21.19	7.58	100.41	28.29	103.95	68.74	106.16	97.40	82.92	50.50
	T_2 three-fourth dose of fertilizer	17.36	7.24	97.12	24.80	98.66	63.78	100.29	90.43	78.35	46.56
	T_3 no dose of fertilizer	15.47	6.39	90.41	22.11	92.29	60.11	97.08	83.05	73.81	42.92
	'F' test	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
	SE (M) ±	00.689	0.12	00.602	00.34	1.45	00.63	1.45	0.36	1.04	0.36
	CD at 5%	02.706	0.48	02.362	01.34	5.60	2.49	5.72	1.41	4.07	1.43
2.	**Seed treatments**										
	S_1 (*Azotobcter*)	17.52	7.33	94.11	24.76	96.66	61.26	98.55	91.07	76.71	46.10
	S_2 (*Azospirillum*)	18.20	6.96	95.11	26.15	95.66	64.07	101.11	90.26	77.52	46.86
	S_3 (VAM)	19.11	7.24	98.00	26.10	99.22	63.95	101.66	91.06	79.49	47.08
	S_4 (S_1 + S_2)	17.18	7.18	95.55	25.48	96.88	68.02	102.22	92.44	77.95	48.28
	S_5 (S_1 + S_3)	17.76	7.05	97.22	24.06	98.77	65.28	101.22	90.20	78.74	46.65
	S_6 (S_2 + S_3)	18.20	6.87	98.90	24.91	99.22	64.78	102.66	93.17	79.74	45.72
	S_7 (S_1 + S_2 + S_3)	19.40	7.41	101.44	26.24	105.11	69.51	106.44	94.24	83.09	49.35
	S_8 (no treatment)	16.62	6.50	89.33	22.84	95.55	56.80	96.00	81.88	74.37	42.01
	'F' test	Sig	NS	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
	SE (M) ±	0.576	0.21	1.36	0.66	2.01	0.96	1.35	1.02	1.32	0.71
	CD at 5%	1.644	–	3.90	1.88	5.71	2.76	3.85	2.90	3.75	2.51
	Bar notation for RL	T_1	T_2	T_3							
		82.92	78.35	73.81							
	Bar notation for SL	50.50	46.56	42.52							
	Bar notation for SL	S_7	S_6	S_3	S_5	S_4	S_2	S_1	S_8		
		83.09	79.74	79.49	78.74	77.95	77.52	76.71	74.37		
	Bar notation for SL	S_7	S_4	S_3	S_2	S_5	S_1	S_6	S_8		
		49.35	48.28	47.08	46.86	46.65	46.10	45.72	42.35		

RL = Root length; SL = Shoot length; SE (M) ± = Standard error of mean; CD = Critical difference; Sig = Significant

Table 3
Effect of different treatment on biomass of *Sorghum* in gm at various intervals.

No.	Particulars	Biomass of plant in days									
		15		30		45		60		Mean	
		F	D	F	D	F	D	F	D	F	D
1.	**Fertilizer treatment**										
	T_1 full dose of fertiliser	5.2	1.61	129.80	26.32	247.16	64.45	461.45	122	210.90	53.60
	T_2 three-fourth dose of fertilizer	3.4	1.42	124.00	22.81	215.00	58.9	392.58	113.16	183.74	49.09
	T_3 no dose of fertiliser	2.8	1.38	101.50	20.22	179.91	52.37	333.16	104.37	154.34	44.59
	'F' test	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
	SE (M) ±	0.137	0.07	3.60	0.57	6.61	0.59	7.3	0.85	4.41	0.52
	CD at 5%	0.53	0.61	14.13	2.23	25.96	2.32	28.70	3.30	17.33	
2.	**Seed treatments**										
	S_1 (*Azotobacter*)	3.8	1.36	116.1	22.80	219.22	55.77	339	108.77	169.95	47.17
	S_2 (*Azospirillum*)	3.8	1.34	122.3	20.85	222.55	58.22	391.5	108.88	185.00	47.32
	S_3 (VAM)	4.0	1.38	21.4	23.80	239.77	61.00	411.4	115.66	194.14	50.46
	S_4 ($S_1 + S_2$)	3.9	1.63	121.2	23.52	233.88	58.33	426.11	121.22	196.27	51.18
	S_5 ($S_1 + S_3$)	3.7	1.65	111.2	23.13	212.55	55.22	414.00	115.00	185.3	48.75
	S_6 ($S_2 + S_3$)	3.8	1.70	122.00	22.30	232.00	64.22	424.40	118.88	195.50	51.78
	S_7 ($S_1 + S_2 + S_3$)	4.3	1.80	129.30	26.18	241.11	66.77	489.11	137.77	215.95	58.13
	S_8 (no treatment)	3.3	0.90	104.00	19.30	183.11	50.22	270.22	95.11	140.15	41.38
	'F' test	Sig	NS	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
	SE (M) ±	0.25	0.09	3.90	0.83	7.6	0.92	12.58	1.86	6.08	0.925
	CD at 5%	0.72	0.28	11.13	2.37	21.84	2.62	35.90	5.33	17.39	
	Bar notation for (F)	T_1 210.90	T_2 183.74	T_3 154.34							
	Bar notation for (D)	53.60	49.09	44.59							
	Bar notation for (F)	S_7 215.95	S_4 196.14	S_6 195.50	S_3 194.14	S_5 185.3	S_2 185.00	S_1 169.95	S_8 140.15		
	Bar notation for (D)	S_7 58.13	S_6 51.78	S_4 51.18	S_3 50.46	S_5 48.75	S_2 47.32	S_1 47.17	S_8 41.38		

F = Fresh Biomass; D = Dry Biomass; SE (M) ± = Standard error of mean; CD = Critical difference; Sig = Significant

Biomass production in Sorghum as affected by different treatments in field (Table 3) clearly indicates that increase in the level of fertilizers biomass of the Sorghum also increases linearly. Maximum green weight was recorded at full dose of fertilizer followed by ¾ dose and was significantly superior over control. Treatment with *Azotobacter* + *Azospirillum* + VAM gave significantly highest biomass over all the treatments followed by *Azotobacter* + *Azospirillum* and *Azotobacter* + VAM. Lowest fresh biomass was recorded in control treatment.

Similar trend was noticed with dry matter production as in fresh biomass with various treatments.

From the studies, it was concluded that *Azospirillum* was more effective as compared to *Azotobacter* specially in some characters like root and shoot length and biomass production etc. The results are encouraging in most parameters studied with combined application of *Azotobacter* + *Azospirillum* + VAM followed by other combinations of biofertilizer over single culture inoculation.

Summary

It is well known that bioinoculants have number of advantages over chemical fertilizers. An attempt has been made to study the effect of bioinoculants singly and in combination on growth parameters of *Sorghum* CSH-14. Experiments were conducted using *Azotobacter chrococcum*, *Azospirillum brasilense* and VAM fungus *Glomous fasiculatum* alone and in combination at three fertilizer levels on growth or *Sorghum* (SCH-14) under rainfed farming system on vertisols.

The study revealed that *Azospirillum* is more effective than *Azotobacter*. As well as findings are encouraging in most of the parameters studied with combined application of *Azotobacter* + *Azospirillum* + VAM by other combinations of biofertilizers over single culture inoculation.

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Authors

R.B. Somani and P.A. Hatwalne
P.G. Department of Plant Pathology
Panjabrao Deshmukh Krishi Vidyapeeth
Akola–444 104, Maharashtra, India

N.S. Kulkarni
Department of Microbiology
R.A. College
Washim, Maharashtra, India

4

Significance of *Azospirillum brassilense* and *Pseudomonas striata* on Growth and Yield of Ragi (*Elucine crocana*) in Alfisol

Introduction

Under subtropical climatic condition with low N and P in alfisol will never be provided the effective crop productivity and its establishment. To achieve with sustainable crop productivity the use of certain beneficial microbes can be considered. Biofertilizer have recently gained with momentum for effecting the substantial increase in crop yield under various ago climatic condition. Role of Diazotrophs on the crop yield was documented by Okon and Kapulnik, (1986) and Ramasamy *et al.* (1992) and Srinivasan and Prabakaran (1992). To mobilise the impounded P in alfisol the use of phosphobacteria is useful (Prabakaran, 1992 and 1994). The present investigation has been taken up to assess the significance of nitrogen fixer and P mobilises on the improvement of growth and yield of finger millet in alfisol.

Material and Methods

A field experiment was conducted during Kharif 1995 season under alfisol (pH 5.6; EC: 0.34 mmho/cm^2) to record the performance of two cultivars of fingermillet (Elucine crocana) *viz.* CO-11 and CO-12 with biooinoculation of nitrogen fixing *Azospirillum brasilense* and P solubilising *Pseudomonas striata* and were inoculated alone and in combination. These inoculants were applied as seed, seedling and soil application at the rate of 2, 3 and 10 packets in which each packet consisting of 200 g of active inoculants at 10^7 cells/g in a peat based carrier system. To compare the efficacy of these microbial inoculants N and P controls were also maintained. Each treatment was replicated five times and conducted in RBD. Seedlings were transplanted at 20 × 10 cm spacing in 3 × 5 m^2 plots. During vegetative phase of the crop vigour index (20 days after planting), plant biomass, root and shoot growth at 30 and 45 days and tillers initiation at 45 days were recorded at harvest the yield attributes *viz.*, finger counts

earhead length, 1000 grain weight, haulms and grain yield were recorded and harvest index was calibrated.

Results and Discussion

The results on the effect of nitrogen fixer and P mobilises on the growth and yield attributes of two cultivars of finger millet (CO-11 and CO-12) in alfisol is presented in tables 1 to 4.

Table 1

Influence of *Azospirillum* and *Pseudomonas* on growth attributes of CO-11 ragi.

Treatments	*At 20 days Vigour index*	*At 30 and 45 days after sowing*						
		Plant Biomass (g/plant)		*Root Growth (cm/plant)*		*Shoot Growth (cm/plant)*		*Tillers Production (number/plant)*
		30	*45*	*30*	*45*	*30*	*45*	*45*
Control	585	2.61	4.02	8.1	12.3	15.1	32.2	2.9
N-Control	790	4.92	7.39	13.2	17.2	26.1	48.2	4.3
P-Control	620	3.72	5.93	11.2	15.2	22.4	42.3	3.8
Azospirillum	720	4.26	6.12	14.5	19.6	30.7	52.1	4.6
Pseudomonas	660	3.98	5.87	12.4	16.4	25.1	48.3	3.9
Azos + Pseudo	740	5.23	8.41	16.6	21.6	33.7	54.6	4.8
CD (p = 0.05)		0.32	0.61	0.08	1.2	3.3	4.1	NS

Table 2

Influence of *Azospirillum* and *Pseudomonas* on yield attributes of CO-11 ragi.

Treatment	*Finger (no./plant)*	*Ear head length (cm/plant)*	*1000 grain (t/ha) (g)*	*Haulms yield (t/ha)*	*Grain yield (t/ha)*	*Per cent increase over control*	*Harvest Index*
Control	4.0	5.7	2.3	5.214	3.160	–	60.6
N-Control	6.6	7.9	2.7	5.880	3.765	18.6	63.2
P-Control	6.0	6.9	2.6	5.440	3.634	15.0	66.8
Azospirillum	7.2	7.5	2.7	5.885	4.075	29.0	69.5
Pseudomonas	6.6	6.9	2.6	5.695	3.730	18.0	66.0
Azos + Pseudo	7.3	7.9	2.9	6.290	4.315	36.6	68.5
CD (p = 0.05)	NS	0.23	NS	0.34	0.21	–	

Growth Attributes

Under alfisol bioinoculation of nitrogen fixing *Azospirillum* and P mobilising *Pseudomonas* as seed, seedling and soil broadcasting affected enhanced establishment and vigour indexes in two cultivars of finger millet. Similarly the various growth biometrics *viz.*, plant biomass, root and shoot growth at 30 and 60 days after transplanting in main field was found significantly increased over untreated control. The effect being registered with the dual inoculation of both the bio inoculants which might be due to the provision of nitrogen and growth promoting substances (IAA, GA) by *Azospirillum* and the possible solublisation of fixed P (as alumina and iron phosphate) by *Pseudomonas* created with sustainable growth of the crop in alfisol. Apparao podle (1995) reported seedling of *Bacillus subtilis* found to increases the yield of pigeonpea. Similar to the present investigation, Upadhyaya *et al.* evinced the role of nitrogen fixing microbes in the rhizosphere of finger millet found to record high nitrogenase activity and it was altered by the varieties, age and nature of the soil. Gopal (1991) critically reviewed the role of biofertilizers on the sustainable crop productivity.

Table 3

Influence of *Azospirillum* and *Pseudomonas* on growth attributes of CO-12 ragi.

Treatments	*At 20 days Vigour index*	*At 30 and 45 days after sowing*						
		Plant Biomass (g/plant)		*Root Growth (cm/plant)*		*Shoot Growth (cm/plant)*		*Tillers Production (number/plant)*
		30	*45*	*30*	*45*	*30*	*45*	*45*
Control	560	3.01	5.11	7.3	1.9	16.2	31.1	3.6
N-Control	820	4.32	9.36	11.2	16.3	23.2	43.1	6.2
P-Control	670	4.29	7.93	10.1	13.8	22.1	40.2	4.6
Azospirillum	790	4.96	7.20	12.1	16.1	28.2	46.1	5.8
Pseudomonas	660	4.72	7.21	10.2	17.3	23.6	42.2	4.8
Azos + Pseudo	820	6.21	9.95	15.2	21.4	31.4	32.6	4.7
CD (p = 0.05)		0.31	0.43	1.1	1.7	2.3	2.9	NS

Yield Attributes

At harvest the coinoculation of *Azospirillum* and *Pseudomonas* significantly enhanced the straw yield and grain yield in two cultivars of finger millet with reference to grain productivity bioinoculants cumulatively recorded 38.0 and 36.6 per cent increased yield over their respective control. Kundu and Gaur (1982) reported the yield of wheat was increased due to the inoculation of *Azotobacter* and phosphobacteria. Saxena and Tilak (1994) critically analysed the role of biofertilizers on the crop productivity.

Table 4

Influence of *Azospirillum* and *Pseudomonas* on yield attributes of CO-12 ragi.

Treatments	*Finger (cm/pl)*	*Ear head weight (g/pl)*	*Haulms yield (t/ha)*	*Grain yield (t/ha)*	*Per cent increase over control*	*1000 grain (t/ha)*	*Harvest Index*
Control	4.0	6.1	5.31	3.26	–	2.2	60.7
N-Control	6.6	8.0	5.90	3.77	16.8	2.8	63.2
P-Control	6.0	6.9	5.43	3.64	15.0	2.6	66.8
Azospirillum	7.0	7.2	5.86	4.08	29.0	2.7	69.6
Pseudomonas	6.6	6.9	5.69	3.73	18.0	2.6	66.1
Azos + Pseudo	7.4	7.9	6.29	4.32	36.6	2.9	68.6
CD (p = 0.05)	NS	0.12	0.3	NS	–	–	–

Among the two cultivars of finger millet CO-12 performed better than CO-11 in alfisol Tilak and Singh (1988) reported the response of pearl millet to be inoculation of P mobilises and nitrogen fixer. Prabakaran *et al.* (1994) also indicated the significance of *Azospirillum* and P solubiliser on growth and establishment of *Tectona grandis* in alfisol.

Summary and Conclusion

A field study was conducted to assess the significance of nitrogen fixing *Azospirillum brassilense* and P mobilising *Pseudomonas striata* on the growth and yield of two cultivars of finger millet (*Elusine crocana*) in alfisol. Both the inoculants were applied at rate of 15 packets (2 for seed treatment; 3 packet for seedling dipping and 10 packets for soil application). These inoculants were tried at as a single and dual inoculation and comprised with N and P controls. The results revealed that the single inoculation of *Azospirillum* and *Pseudomonas* recorded 34 and 21 per cent increased grain yield in CO-11 ragi whereas their combination yielded 38.9 per cent over control. In CO-12 cultivar the single inoculation had affected 27 and 18 per cent increased yield over their control and in combination the effect was at 36.6 per cent.

The possible synergistic effect of enhancing the biogrowth as well as the yield productivity in finger millet by the seed, seedling and soil inoculation of nitrogen fixing *Azospirillum brasilense* and P-solublising *Pseudomonas striata* in alfisol might be due to the following reasons.

1. Inherent production of auxins by both microbes (IAA, GA) will bring out with better germinability of seedling and fast establishment after transplantation in the main field can bring out required number of plant population and its better stand in alfisol.
2. By the associative symbiosis the *Azospirillum* can able to supply the need based nitrogen for the crop during its carious growth phases will bring out with better plant growth and yield enhancement.

3. The possible role of *Pseudomonas* in solublising impounded P (iron and alumina P) to available form ways of organic acid production (acetic formic, propionic acids) oxidation of sulphur to sulphur dioxide and the convert as sulphuric acid might solubilise the fixed P in soil or by means of chelation effect the P mobility can be improved for crop used.

Over all influences by these two microbes played a vital role in supplying N and P to the finger millet and found enhanced the growth yield over the untreated conrol.

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Authors

J. Prabakaram C. Udayassorian and K. Srinivasan
Department of Environment Sciences
Tamil Nadu Agricultural University
Coimbatore- 641003, Tamil Nadu, India.

5

Application of Vesicular Arbuscular Mycorrhizae (*Glomus fasciculatum*) and *Rhizobium* on Biomass Production of *Acacia nilotica* in Saline and Forest Soils

Introduction

The depletion of forest cover leading to the scarcity of fuelweed and animal coupled with increasing wasteland formation is a major concern of the developing nations. Leguminous tress and shrubs have been suggested as a renewable source of fuel and wood products (National Academy of Science, 1979, 1980) and are known to have symbiotic association with VAM Fungi and *Rhizobium* (Rose and Youngberg, 1981). VAM Fungi which constitute a group of important soil micro-organisms are ubiquitous throughout the world are known to improve the plant growth through better uptake of nutrients. They also improve the activity of N fixing organisms in the root zone (Mosse *et al.*, 1975). VAM Fungi can increase the drought resistance. Their extra-radical hyphae can influence rhizosphere architecture and improve host water dynamics (Hordie, 1985). Shortage in the availability of the woody biomass do not only to cover exploitation but also to poor productivity as a result of growth limiting factors in the environment. VAM Fungi have been reported to occur naturally to Saline/Alkaline environments (Pond *et al.*, 1984, Sidhu and Behl, 1990) and have been linked with increased plant biomass and development in saline soils (Poss *et al.*, 1985). Application of biofertilizers as VAM Fungi and *Rhizobium* can facilitate higher establishment and increase biomass of trees species in saline soil sites. The present paper deals with the impact of interaction between VAM Fungi and *Rhizobium* on the biomass of *Acacia nilotica* (highly promising multipurpose tree species) in saline and forest soils during different phases of plant growth.

Material and Methods

The pots (40 × 30 × 30 cm) filled with thoroughly sieved sterilised saline and forest soil form Muzaffarpur and forest range Bettiah respectively. The saline soil was amended with forest

soil in 1:1, 2:1 and 3:1 ratios. Surface sterilise seeds of *Acacia nilotica* were soaked in water for 48 hours at room temperature to break the dormancy and also to soften hard seed coat. Seed were sown about 1.5 cm deep in the soil and regularly watered.

Rhizobium inoculum (3×10^9 cells/seed) was mixed in a cooled solution of sugar and Arabic gum. Such mixing forms a slurry to which the experimental seeds were added. Inoculated seeds were placed on the paper and dried in shade. These seeds were sown to demonstrate the impact of *Rhizobium* in relation to production of biomass yield. Inoculum of *Glomus fasciculatum* (Thaxter sensum Geredemann) were obtained from the Bhartiya Agro Industry Foundation, Pune. Inoculation (300 VAM Spores/seed) was done by placing the inoculum 2 cm below the seed in pot filled with soil. The experiment was laid out as completely randomised designed and comprised of 20 treatments. They were provided normal condition for growth. 5 replicates were taken for each set of experiments. The plants were harvested after third, sixth and ninth month of growth. Spores were isolated by wet sieving and decanting method (Gerdemann and Nicolson, 1963). Root infection was evoluted by a microscopic examination of cleared and stained root samples following Phillips and Hayman (1970). The percentage of root infection was recorded by the Grid line intersection method of Giovanetti and Mosse (1980). Data on physical growth parameters shoot dry weight, number of leaves, number of nodules and survival percentage was recorded for each plant and statistically analysed by ANOVA.

Results and Discussion

The results of the study are presented in Tables 1 and 2. The plant biomass, *i.e.* shoot height, root length, collar diameter, shoot and root dry weight, number of leaves and number of nodules increased with the increase of plant age in different treated plants of *Acacia nilotica*. However, the increase was more prominent in VAM and *Rhizobium* inoculated plant. Inoculation of saline soil with VAM fungi significantly increase plant height. The maximum plant height (226.5 cm) with an increase of 30.5% over control was observed in plant growing in saline soil mixed with forest soil (1:1) and 24.5% increase in case of forest soil. Inoculation with *Rhizobium* did not significantly improve growth in any soil treatment in respect of VAM treatment.

Maximum dry matter yield was observed in plant growing in saline soil and mixed with forest soil (1:1) and inoculated with VAM Fungi in different Plant growth *i.e.* 3 months, 6 months and 9 months.

Collar diameter of plants growing in saline soil amended with forest soil (1:1) and colonised with VAM Fungi and *Rhizobium* was highest.

The maximum number of leaves (13-1155) was observed in plants grown in saline soil mixed with forest soil (1:1) and inoculated with VAM fungi and *Rhizobium* and minimum in control (8.226). Maximum number of nodules were observed in plants grown in forest soil treated with VAM fungi with an increase of 95.4% over control. Similar trend was also recorded in all soil groups treated with either VAM or with VAM fungi and *Rhizobium*. In general plants grown in forest soil had more number of nodules than that of those grown in saline soil. Maximum

number of VAM spore in soil and percentage infection in root were recorded in saline and forest soil (1:1) treated with VAM and *Rhizobium*.

Table 1

Effect of VAM and *Rhizobium* on growth response, nodulation and biomass production of *Acacia nilotica* after 9 months.

Treatments	*Shoot Height (cm)*	*Root length (cm)*	*Collar diameter (cm)*	*Shoot dry wt (g)*	*Root dry wt. (g)*	*No. of leaves per plant*	*No. of nodules per plant*
T_1	130.5	15.2	12.5	29.96	4.5	220	5
T_2	182.0	16.2	12.7	30.50	4.6	226	16
T_3	173.5	19.9	12.9	31.60	4.4	326	10
T_4	139.6	17.3	12.7	30.41	4.7	270	7
T_5	142.3	17.6	12.8	30.49	4.4	280	20
T_6	149.4	18.9	12.8	31.21	4.1	390	22
T_7	150.4	19.4	12.9	31.49	4.2	440	18
T_8	162.6	18.9	31.1	32.00	4.1	445	24
T_9	161.6	19.6	13.6	32.09	4.6	490	25
T_{10}	162.8	19.9	31.7	32.99	4.7	501	21
T_{11}	165.9	20.5	13.8	33.00	4.8	530	29
T_{12}	174.6	22.4	14.9	33.15	5.1	930	23
T_{13}	195.8	23.3	15.1	34.10	5.6	1060	31
T_{14}	226.5	24.0	16.2	35.40	7.7	1155	65
T_{15}	171.3	21.3	14.3	40.60	7.9	835	49
T_{16}	193.2	22.1	14.5	33.01	8.5	890	34
T_{17}	204.1	23.1	14.6	34.11	6.9	970	50
T_{18}	175.2	21.4	14.4	34.91	7.0	844	51
T_{19}	195.5	22.4	14.6	33.90	7.5	895	41
T_{20}	206.4	23.1	14.7	34.35	7.9	901	52

T_1-Saline soil; T_2-Forest soil; T_3-Saline soil + forest soil (1:1); T_3-Saline soil + Forest soil (2:1); T_5-Saline soil + Forest soil (3:1); T_6-Saline soil + *Rhizobium*; T_7-Saline soil + VAM; T_8-Saline soil + *Rhizobium* + VAM; T_9-Forest soil + *Rhizobium*; T_{10}-Forest soil + VAM; T_{11}-Forest soil + *Rhizobium* + VAM; T_{12}-Saline soil + Forest soil (1:1) + *Rhizobium*; T_{13}-Saline soil + Forest soil (1:1) + VAM; T_{14}-Saline soil + Forest soil (1:1) + *Rhizobium* + VAM; T_{15}-Saline soil + Forest soil (2:1) + *Rhizobium*; T_{16}-Saline soil + Forest soil (2:1) + VAM; T_{17}-Saline soil + Forest soil (2:1) *Rhizobium* + VAM; T_{18}-Saline soil + Forest soil (3:1) + *Rhizobium*; T_{19}-Saline soil + Forest soil (3:1) + VAM; T_{20}-Saline soil + Forest soil (3:1) + *Rhizobium* + VAM.

Table 2

Effect of VAM and *Rhizobium* on percentage VAM colonisation, spore population and phosphorus content of *Acacia nilotica* after nine months of plant growth.

Treatments	*Root infection (%)*	*VAM spores per 100g soil*	*P content in shoot (%)*	*Seedling viability*
T_1	–	–	0.03	30.7
T_2	13.2	65	0.04	65.0
T_3	12.6	61	0.05	65.4
T_4	14.0	76	0.06	63.3
T_5	15.2	86	0.05	64.4
T_6	–	–	0.04	35.5
T_7	39.1	110	0.05	35.4
T_8	46.3	115	0.07	46.5
T_9	–	–	0.08	95.1
T_{10}	56.4	156	0.07	94.1
T_{11}	65.4	186	0.08	95.4
T_{12}	–	–	0.08	66.3
T_{13}	66.4	196	0.09	65.4
T_{14}	85.6	210	0.11	66.9
T_{15}	29.1	76	0.07	96.7
T_{16}	64.1	176	0.08	63.4
T_{17}	69.4	186	0.09	64.2
T_{18}	28.4	105	0.06	65.1
T_{19}	65.3	176	0.08	63.6
T_{20}	71.2	187	0.09	64.3

Treatment T_1 to T_{20} as per Table 1.

The pH value varied from 7.5 to 9.5 indifferent combination but maximum biomass of plant and VAM spore were recorded in pH 8.5 amended with saline soil and forest soil (1:1) treated with VAM and *Rhizobium*.

Seedling survivability percentage was 95% in forest soil and 30.7% in saline soil. Inoculation of with VAM fungi and *Rhizobium* individually improved seedling survival percentage in saline soil than that of either forest soil or saline soil amended with different proportion of forest soil.

This study indicates that none of without VAM seedling grew as fast as those with VAM fungi in saline soil. The impact of VAM fungi on biomass of *A. nilotica* was positive but this trend was not notice with *Rhizobium* alone or with dual inoculation. This could perhaps be due

to incompatibility by *Rhizobium* strain with this plant species or its ineffectiveness in soils. The biomass of plant was significantly more in combination of VAM and *Rhizobium* as compared to VAM and *Rhizobium* alone and also with control treatment. Similar result was recorded by Bagyaraj *et al.* (1979), Verma (1979), Roskoshi *et al.* (1986), Mukerji & Jagpal (1987) and Kaushik & Kashik (1995).

In other studies also conducted by authors, VAMF and *Rhizobium* have been found effective in plant establishment, nutrient uptake, leaf area, photosynthesis efficiency and biomass in drought affected plants. However, there was a tendency towards drought evidence rather than drought tolerance. Soil salinity may influence the growth and activity of VAM fungi and *Rhizobium* through several mechanisms, either discreetly or interactively. The exact mechanism by which VAM fungi tolerate unbalanced, ionic conditions as in salty soils is not known. Since it has not so far been possible to maintain VAM fungi in auxanic culture, it is extremely difficult to distinguish between direct and plant mediated effects on their biomass. It may be concluded that a suitable combination of VAM fungi and *Rhizobium* may be useful in ensuring higher biomass production and establishment of *A. nilotica* in saline soil.

We are grateful to Bhartiya Agro Industry Foundation (BAIF), Pune for providing VAM cultures.

Summary

Impact of VAM (*Glomus fasciculatum*) enhance the biomass yield of *Acacia nilotica* in terms of shoot height, root length, collar diameter, shoot and root dry weight, number of leaves and number of nodules. The biomass of *Acacia nilotica* increased significantly in the combination of saline soil amended with forest soil (1:1) and inoculated with VAM fungi and *Rhizobium*. The maximum biomass of shoot height increase 24.5%, root length 20.5%, collar diameter 24.9% over the control in combination of VAM fungi and *Rhizobium* amended with saline and forest soil (1:1). The maximum number of nodules were observed in plants growing in forest soil inoculated with VAM Fungi and increase 95.4% over control. Survivability of seedling was 95% in forest soil and 30.9% in saline soil. Studies on the mechanism of VAM and *Rhizobium* effectivity and selection of isolates suitable for stress sites can be exploited to utilise these microbes as biofertilizers in order to reclaim and ameliorate degraded soil site for increased productivity.

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Authors

Kamal Prasad
Department of Post-Graduate Studies and
Research in Biological Science,
Rani Gurgavati University
Jabalpur–482 001 (M.P.), India

6

Effect of VAM Fungi and PSB on Growth and Chemical Composition of Micropropagated Banana (*Musa paradisiaca L.*) Plants

Introduction

Banana is one of the important commercial fruit crops which is commonly grown throughout the humid tropics and subtropics. The potential value of plant tissue culture (PTC) technology is being commercially exploited by arious organisations all over the world. This technique provides as existing tool to multiply banana plants vegetatively in large numbers within a short period of time.

The potential of arbuscular mycorrhizal fungi and phosphate solubilising microoganisms as biofertilizers and bioprotectors to enhance micropropagated plantlets is recognised, but not well exploited with because of the current agronomic practices with their implications for environment (Bagyaraj and Verma 1995).

In the present study an attempt has been made to discuss the response of micropropagated banana plants to dual inoculation with PSB and VAM.

Material and Methods

The experiments were conducted in the department of Botany, Bangalore. The methodology followed for the experiments is detailed below.

Phosphate Solubilising Microogranism

Bacillus megatherium was procured from the Department of Agricultural Microbiology U.A.S., Bangalore.

Mycorrhizal Inoculum

Glomus mosseae and *Glomus fasciculatum* inoculum were procured form U.A.S. Bangalore.

Plant Maerial

Micropropagated banana plantlets variety Nanjanagudu Rasbale were procured from Tissue Culture Laboratory. Lalbagh, Bangalore.

Phosphate solubilising microorganism and vesicular arbuscular mycorrhizal fungi were added to 4 weeks old micro propagated banana plants. In addition to 4 weeks (acclimatization stage) a further 13 and 16 weeks of growth in green house was allowed before harvest.

Treatment

T_1-Un-inocualted control.

T_2-Phosphate solubilising bacteria, (PSB), (*Bacillus megatherium*) (*B.m*).

T_3-*Glomus mosseae* alone (*G.m*).

T_4-*Glomus fasciculatum* alone (*G.f*).

T_5-G.m + PSB.

T_6-G.f. + PSB.

The following observations were made:

1. **Mycorrhizal percent infection:** The percent infection of banana plant roots were estimated according to Philips and Hayman (1970).
2. **Estimation of mycorrhizal spores:** Extra metrical chlamydospores produced by the mycorrhizal fungus was estimated by wet sieving and decanting methods outlined by Gerdman and Nicolson (1963).
3. **Plant growth parameters:** Root length and shoot length were recorded for each treatment.
4. **P-Content:** Phosphorus content was estimated by Vanadomolybdate phosphoric yellow colour method (Hackson 1971).
5. **Ca and Mg content:** Ca and Mg contents were estimated by the Versanate titration method (Dewis and Frietas 1970).
6. **Na and K content:** Na and K contents were estimated by flame photometric method (Jackson, 1967).

Results and Discussion

Banana plant growth, biochemical composition and nutrient uptake of mineral elements were greatly improved by simultaneous inoculation with VAM and PSB.

The development variation found in inoculated and uninoculated plants resulted in significant increase in shoot length and root length.

Combined inoculation with *G. mosseae* and *B. megatherium* substantially increased the survival of banana plants (*Musa paradisiacal* L.). The bacterium *Bacillus megatherium* in combination with *G. mosseae* gave a significant increase in plant height and highest frequency of root infection. Dual inoculation considerably increased mineral elements than single inoculated plants (Table-1 and Figs. 1 & 2).

Dual inoculation with *G. mosseae* and *B. megatherium* resulted in significant increase in Ca concentration than *Glomus fasciculatum* treated with *B. megatherium* alone.

Table 1.

Response of micropropagated banana plants (Musa paradisiacal L.) to dual inoculation with PSB and VAM.

Treatment	*% Infection*		*Spores/ 100ml*		*Shoot length (cm)*		*Root length (cm)*		*Total-P (µg/g)*	
	Weeks After Incubation									
	13	*16*	*13*	*16*	*13*	*16*	*13*	*16*	*13*	*16*
Unionculated Control	30	45	69	112	10.23	11.43	7.31	9.58	7	10
B. megatherium alone	45	54	128	180	12.45	16.12	9.68	12.58	13	21
G. fasciculatum alone	64	81	212	228	11.89	15.31	7.69	11.81	12	22.5
B.m. + G.f.	73	91	224	348	13.28	16.28	9.25	12.92	15	26
Uninoculated Control	36	40	68	109	10.31	11.38	7.1	9.72	8	10
B. megatherium alone	40	50	132	179	12.68	16.00	9.82	12.70	12	21
G. mosseae alone	64	91	262	336	12.47	15.23	9.01	11.38	16	25
B.m. + G.m.	81	100	268	338	13.40	16.40	9.93	13.34	18	27.5

The response due to mixed culture inoculation was more than single inoculation showing the synergistic effect of the two types of organisms. The response may be due to supply of two major plant nutrients and other factors. The results indicated that the mixed culture inoculation can be safely used for better growth and survival of micropropagated banana plants in green house.

The present research showed that the mycorrhizal fungi colonising the micropropagated banana plants showed differences in growth rate. This is in confirmation with results respond (Declerck *et al.* 1995) on seven banana cultivars.

The exact mechanism by which VAM and PSB contribute to enhanced growth and survival of micropropagated plants were not fully understood.

Summary

Simultaneous inoculations with VAM fungi and PSB in micropropagated banana plants during transition period from *in vitro* condition to glasshouse environment were studied. The results

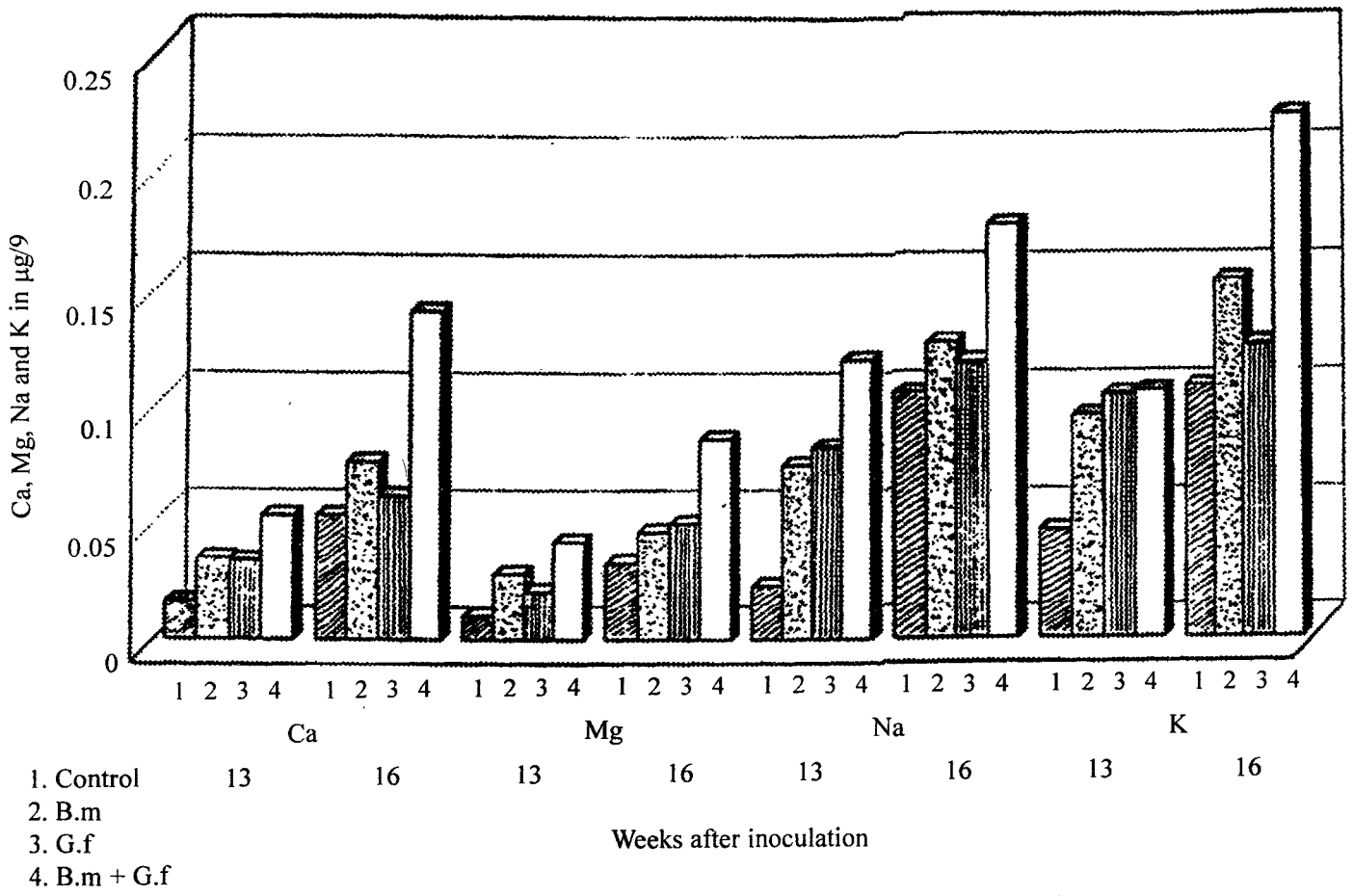

Fig. 1 Effect of dual inoculation on mineral nutrients (G.f. + B.m.)

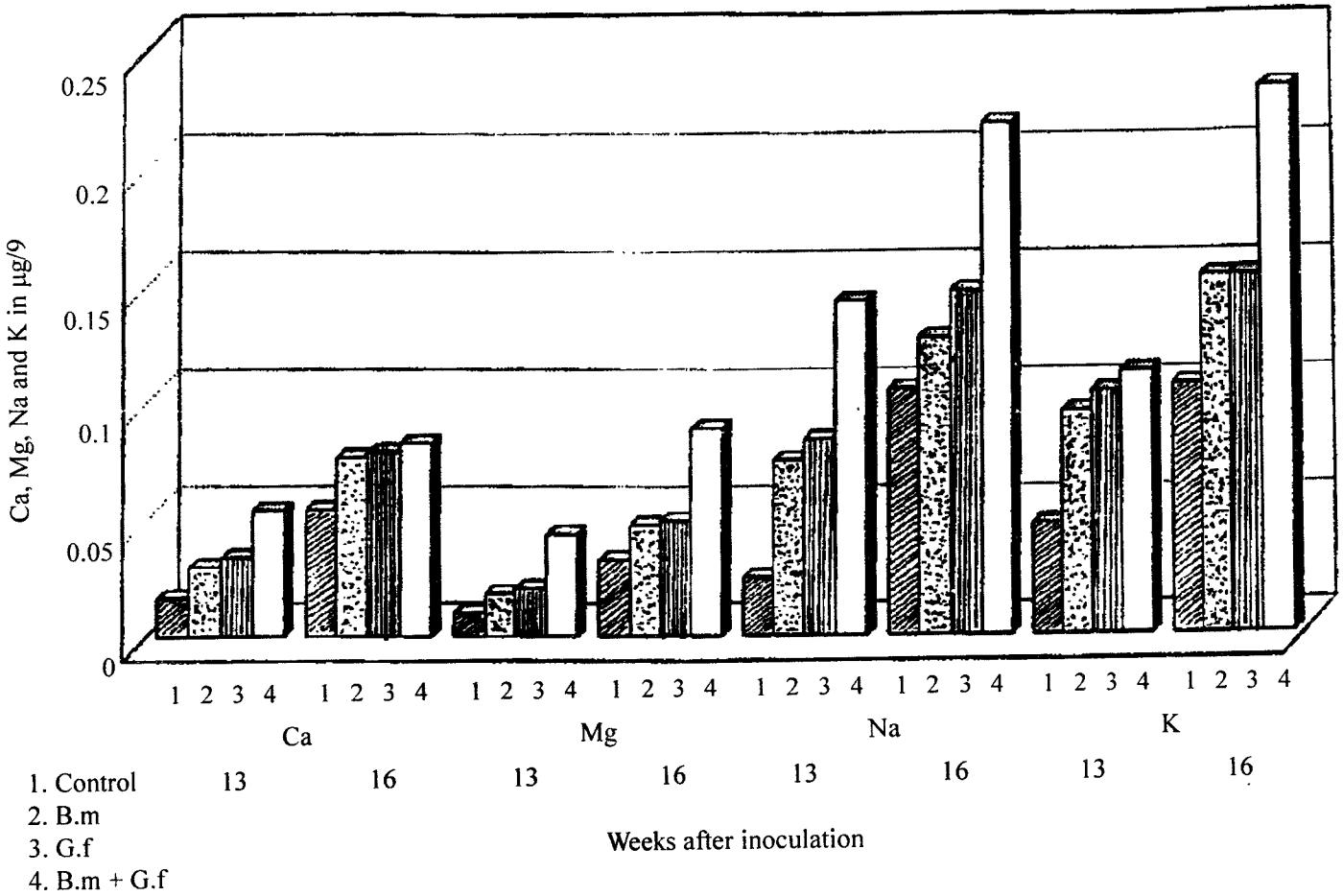

Fig. 2 Effect of dual inoculation on mineral nutrients (G.m. + B.m.)

showed that percent root infection, spore content in the soil and the plant growth was significantly increased. Mineral nutrient contents were appreciably increased by simultaneous inoculation with VAM fungi and PSB.

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Authors

R. Sowmya and D. Anusuya
Department of Botany
Bangalore University
Bangalore–560 056, Karnataka

7

Mungbean [*Vigna radiata* (L.) Wilczek] Response to Inoculation with N-fixing and phosphate Solubilizing Bacteria

Introduction

The introduction of high yielding varieties, better irrigation potential, plant protection measures and judicious use of chemical fertilizer have contributed to a break through in Agriculture. But the fast depleting fossil fuel resources, escalating cost of chemical fertilizers and environmental hazards caused by their use are some of the constraints for better crop yield. These constraints have forced researchers to think of better and economic alternatives. The use microbes have however, drawn the attention which are not only economic source of fertilizers, yet increase the availability of nutrients to the plants.

Among the major plant nutrients, phosphorus (P) is next to nitrogen required for better crop yields. It is estimated that 98% of Indian soil contain insufficient amounts of available phosphate to support maximum plant growth (Ghosh and Hasan, 1979). Application of phosphatic fertilizers is therefore essential for optimum crop yield. But the main problem concerning phosphatic fertilizer is its fixation with soil complexes within a very short period of application rendering more than two-thirds unavailable (Mandal and Khan, 1972). Thus, the non-availability of soluble forms of phosphate limits the growth of plants. However, some microbes solubilise insoluble (Fe-P, Al-P etc.) phosphate and make it available to the plants resulting in higher crop yields. The beneficial effect of phosphate dissolving microorganisms (PDMOS) is mainly due to the production of organic acids (Taha *et al.* 1986; Venkateshwarlu *et al.*, 1984), enzymes (Bottcher, 1962) and growth promoting substances (Barea *et al.*, 1976; Azcon *et al.*, 1978).

Significant increase in crop yield and N_2-fixation was observed when plants were inoculated with mixed cultures of associative N_2-fixers (Maudinas *et al.*, Fayez, 1989), mixtures of *Azospirillum* and AM fungi (Sreeramula *et al.*, 1988) and *Azotobacter* spp. and phosphate

solubilizing bacteria (Kundu and Gaur, 1980, 1984; Monib *et al.*, 1984). Furthermore, favourable effect of phosphate solubilising bacteria on survival of N_2-fixers have also been found encouraging (Ocampo *et al.*, 1975; Sarojini and Mathur, 1989). But sufficient work has not been conducted on interactive effect of *Bradyrhizobium* sp. (vigna) with phosphate solubilising bacteria in Indian conditions. As both set of microorganism has a specific role in plant growth, the present study was aimed to investigate the interaction of *Bradyrhizobium* sp. (vigna) with phosphate solubilising bacteria in *in vitro* condition and their interactive effect on the performance of mungbean crop under pot conditions.

Material and Methods

Bacterial strains:

Bradyrhizobium sp. (vigna) strain A-4 was obtained from the Department of soil Science, G.B. Pant University of Agriculture and Technology, Pantnagar, whereas phosphate solubilisers, *Pseudomonas striata* and *Bacillus polymyxa* were obtained from the culture collection centre, Division of Microbiology, IARI, New Delhi. *Bradyrhizobium* sp. (vigna) strain A-4 was grown and maintained on yeast extract mannitol agar medium whereas phosphate solubilisers on Pikovskaya's medium (Pikovokaya, 1948).

In vitro interaction study:

The interaction study between N_2 fixing and phophate solubilising bacteria was made using yeast extract mannitol and Pikovskaya's broth. Solubilisation of tri-calcium phosphate (TCP) in Pikovskaya's broth with single and mixed culture was studied. In each case, one ml thick suspension of bacteria (OD 1.0) was inoculated. The two organisms were added in equal proportion in the combined treatment. The flasks were incubated at 30 ± 2°C and observation on growth of the organisms were made at regular intervals. The amount of phosphate solubilised by the organism was determined colorimetrically by stannous blue colour method as outlined by Jackson, 1967.

Pot experiment:

A pot experiment on mungbean *Vigna radiata (L.) Wilczek* var. T-44 was conducted on unsterile soil of sandy clay loam texture with pH 8.5 and WHC, 40%. The soil was uniformly packed in pots (20 cm high and 20 cm internal diameter) at the rate of 4 kg per pot. The treatment consisted of phosphorus application at the rate of 198.36 mg/pot through Mussoorie rock phosphate together with 20.5 mg N. kg^{-1} soil as urea in the presence and absence (control) of *Bradyrhizobium* sp. (vigna) strain A-4 and phosphate solubilisers. A total of eight treatments were maintained and each treatment was replicated three times. Ten surface sterilized seeds soaked in liquid culture (Approx. 10^9 cells/ml as determined by the plate technique method) were sown in each pot. The plants were thinned to 4 uniform plants after ten days of germination. The pots were watered regularly to maintain optimum moisture level. The plants were harvested after 45 days and data was analysed statistically.

Nodulation Pattern and Nodule Rating:

The nodulation pattern was determined as outlined by International Network of legume inoculation trial under NifTAL project of University of Hawaii, College of Tropical Agriculture and Human Resources, Honolulu, Hawaii, USA. The nodules were rated using the formula of Burton and Curleyl (1965).

$$\text{Nodule rating} = \frac{\begin{array}{c}10 \times \text{no. of plants with tap root} + 5 \times \text{no. of plants} \\ \text{with nodulation close to tap root} + 1 \times \text{no. of plants} \\ \text{with scattered nodulation}\end{array}}{\text{Total no. of plants}}$$

Results and Discussion

The results on interaction between *Bradyrhizobium* sp. (vigna) strain A-4 and phosphate solubilisers (*P. striaa* and *B. polymyx*a) in liquid culture medium on their growth pattern indicated that N_2-fixer and phosphate solubilisrs can grow together and no antagonistic behaviour of one organism towards another was noticed. This finding suggested the feasibility of using them together as microbial inoculants. The population of *Bradyrhizobium* sp. (vigna) A-4, however was low when grown alone in the modified Pikovskaya's liquid medium as compared to their growth in YEM broth (Table 1). The decrease in N_2-fixer population may be due to non-solubilising properties of insoluble phosphorus by the organism. Similar findings have been reported by Epsito and Wilson (1956) with *Azotobacter* sp. which showed a decreased growth and N_2 fixation in culture medium devoid of phosphorus.

Both bacteria (*P. striata* and *B. polymyxa*) were found to solubilise tri-calcium phosphate (TCP) to varying degree (Table 2). Maximum solubilisation of TCP was observed at 6th day of incubation than 13th day and 20th day. The solubilization of TCP by *P. striata* and *B. polymyxa* was however greater when they were grown in combination with *Bradyrhizobium* sp. (vigna) (37% increase over *P. striata alone in P. striata* + *Bradyrhizobium*, 32.1% increase over *B. polymyxa* alone in *B. polymaxa* + *Bradyrhizobium*). Increase in solubilisation could be attributed to the organic acids secreted extracellularly by *Bradyrhizobium* in the medium (Halder *et al.*, 1990). The result also indicated an inverse correlation between phosphate solubilisation and pH of the medium (Table 2). Phosphate solubilisation, however, decreased with increase in pH. In all probability, the decrease in pH may possibly be due to production of organic acid and hence increased phosphate solubilisation (Pareek and Gaur, 1973; Arora and Gaur, 1978; Illmer and Schinner, 1992).

The results obtained on the associative effect of N_2-fixer and phosphate solubilizers in culture medium promoted us to assess their performance on mungbean crop in natural soil under pot culture conditions. The combination treatment of *B. polymyxa* + *P. striat* + *Bradyrhizobium* sp. (vigna) A-4 was found to be statistically superior of all the treatments (Table 3). The increase in dry matter of plants, grain yield and nodule number and nodule weight over uninoculated plant or plant receiving only rhizobial inoculation can be due to the synergistic effect of plant genotype and the mciroorganisms application, as all other factors remained identical for both sets of plant (*i.e.*, inoculated and uninoculated plants). Among the

Table 1

Population of phosphate solubiliser and nitrogen fixer (× 10^6/ml) in modified liquid medium and YEM broth.

Treatment	*Period of incubation (hours)*						
	0	*24*	*48*	*72*	*96*	*120*	*144*
In YEM broth							
Bradyrhizobium sp. A-4	0.020	0.182	8.4	350	62	226	10
In modified medium							
Bradyrhizobium sp. A-4	0.020	0.011	0.029	0.53	0.55	0.78	0.12
B. polymyxa	0.04	4.9	425	1800	3800	1100	250.20
P. striata	0.030	2.89	230	992.0	2410	420	1.5
P. striata +	0.30	3.5	41	560+	220	4.1	0.97
Bradyrhizobium sp. A-4	0.020	0.041	0.211	16.8	27.6	44.0	41.0
B. polymyxa +	0.06	6.2	600.0	1300	3600	490	140.0
Bradyrhizobium sp. A-4	0.02	0.031	0.91	10.9	4.0	245.0	30.0

Table 2

Solubilisation of tricalcium phosphate by phosphate solubilising bacteria in modified liquid medium as affected by *Bradyrhizobium* sp.

Treatment	*Period of incubation (days)*					
	6		*13*		*20*	
	pH of medium	*Soluble P (mg/ 50ml)*	*pH of medium*	*Soluble P (mg/ 50ml)*	*pH of medium*	*Soluble P (mg/ 50ml)*
P. striata	5.00	13.00	5.59	11.40	5.88	7.40
B polymyxa	5.10	12.80	5.89	10.91	5.89	9.82
P striata +	5.02	17.81	5.23	12.92	5.76	10.92
Bradyrhizobium		(37)*				
Sp.-(vigna)		(53)+				
B. polymyxa	5.01	16.91	5.30	13.20	6.01	10.10
Bradyrhizobium sp.		(32.10)**				

* = % increase over *P. striata*
** = % increase over *B polymyxa*
+ = % increase over *B polymyxa* + *Rhizobium* sp.

Table 3

Effect of N_2-fixer and phosphate solubilisers on dry matter, yield and nodulation of mungbean (T-44).

Treatment	*Root dry wt. per plant (g)*	*Shoot dry wt. per plant (g)*	*Grain yield per plant (g)*	*No. of nodules per plant*	*Nodule dry wt per plant (g)*	*Nodulation pattern*	*Colour of pigment*	*Nodulating rating*
Control	0.16	1.00	0.35	15.00	3.20	8	White	2.25
Bradyrhizobium sp. A-4	0.31	1.56	0.75	26.00	5.46	6	PR	8.75
	(93.75)*	(56.00)*	(114.30)*	(73.30)*	(70.62)*			
Pseudomonas striata	0.41	1.50	0.52	18.00	3.78	8	White	2.75
Bacillus polymyxa	0.34	1.50	0.57	16.00	3.36	9	White	2.00
B. polymyxa + *P. striata*	0.30	1.52	0.58	17.00	3.57	8	White	2.75
B. polymyxa + *Bradyrhizobium* A-4	0.366	1.57	1.06	28.00	6.30	6	PR	7.75
B. striata + *Bradyrhizobium* A-4	0.40	1.85	1.11	38.0 (86.6)*	5.88	6	PR	7.75
B. polymyxa + *P. striata* + *Bradyrhizobium* sp.	0.48 (200.0)*	1.91 (91.0)*	1.50 (228.50)* (53.3) +	36.00 (140)* (38.40) +	7.56 (136.25)* (38.46) +	5	PR	7.75
CD at 5%	0.004	0.030	0.005	0.154	0.003			

* = % increase over control.
+ = % increase over Rhizobium inoculation alone.

dual inoculation, maximum number of nodules (30/plant) was however, produced on plant inoculated with *P. striata* + *Bradyrhizobium* sp. (vigna) A-4 as compared to *B. polymyxa* +*Bradyrhizobium* sp. (vigna) treatment. The increase innodulation can be due to increased supply of phosphorus to the host plant due to the production of plant hormones by the organisms (Madhok, 1961). But the nodules were smaller in size (5.88/plant) as compared to the treatment receiving *B. polymyxa* + *Bradyrhizobium* sp. A-4 and hence, low dry weight. The production of low number of nodules in general, on mungbean in all the treatments under identical set of condition could be due to inefficient release of bacteria from infection threads or to limitation in bacterial proliferation within the infected cells.

The presence of indigenous rhizobia that might be delaying the nodulation cannot be ignored, as the soil taken for study was unsterile. Similarly root dry weight, shoot dry weight and grain yield was recorded maximum (200%, 228.6% and 91% respectively increase over control) with triple inoculation (Table 3). However, the effect of dual inoculation *P. striata* + *Bradyrhizobium* sp. *(vigna)* resulted in higher dry matter production and yield than *B. polymyxa* + *Bradyrhizobium* sp. *(vigna)*. Substantially increased yield in soybean, pigeonpea and French bean due to inoculation with *Rhizobium* spp. has also been reported by Khan (1996), Kothari and Saraf (1988) and Gosh (1989) in mungbean, Algawadi *et al.* (1995) using N_2-fixing and PSB in barley and Mallik and Sanoria (1980) in lentil crop.

Colour of the uninocolated nodule pigments as well as the treatment devoid of *Bradyrhizobium* sp. (vigna) inoculation were found to be white suggesting the presence of ineffective species of rhizobia. On the other hand, inoculated legumes showed pink to red colour pigmentation indicating an effective association between legumes and rhizobia. The nodulation pattern of mungbean varied between 5-9. The nodulation pattern of *Bradyrhizobium* inoculation indicated a root system with larger nodules concentrated on tap root whereas interaction on *Bradyrhizobium* sp. (vigna) with *B. polymyxa* and *P. striata* exhibited a nodulation located mostly on secondary roots. As compared to nodulation pattern, nodulation rating seemed to be a better qualitative procedure for differentiating *Rhizobium* sp. and phosphate solubilisers varying in degree of effectiveness. Single inoculation with *Bradyrhizobium* as well as *Bradyrhizobium* inoculated with phosphate solubilisers exhibited a high degree of nodule rating (Table 3). Inoculation with *Bradyrhizobium* sp. (vigna) A-4 (8.75), *Bradyrhizobium* sp. A-4 + *B. polymyxa* (7.75) and *Bradyrhizobium* sp. A-4 + *P. striata* + *B. polymyxa* (7.75) were found to be highly efficient in pot trials. Since the nodulation rating is based on the classification of plants on the basis of nodule in tap root, close to tap root and scattered nodulation, a high nodulation rating indicated tap root nodulation in most of the plants. It is the tap root that develop earliest and hence supply N_2 to the planet for a longer period of time resulting in better crop yield. The synergistic interaction between *P. striata* or *B. polymyxa* with *Bradyrhizobium* and increased phosphate solubilisation thus, may prove beneficial for developing a mixed inoculant for increasing crop productivity.

Summary

The present experiment was carried out to investigated the interaction of N_2-fixing and phosphate solubilising microorganism in liquid culture medium and their effect on mungbean (vigna)

radiata crop under unsterile pot conditions. No antagonistic behaviour of one organism towards other was noticed in *in vitro* condition. Maximum tricalcium phosphate (TCP) solubilisation was recorded when *Pseudomonas striata* and *Bacillus polymyxa* were grown in association with *Bradyrhizobium* sp. (vigna) strain A-4, specific to mungbean in liquid culture medium. A significant positive effect on dry matter production and nodulation efficiency of the crop was observed following inoculation with mixture of *P. striata* + *B. polymyxa* + *Bradyrhizobium* sp. (vigna) strain A-4 supplemented with Mussoorie rock phosphate (MRP). The combined inoculation of P. *striata* + *Bradyrhizobium* sp. (vigna) was found superior over dual inoculation with *B. polymyxa* + *Bradyrhizobium* sp. (vigna).

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Authors

Md. Saghir Khan, Mohd. Amil and Almas Zaidi
Department of Agricultural Microbiology
Institute Muslim University
Aligarh–202 002, U.P., India

8

Field Performace of Asymbiotic Biofertilizers on Grain Yield of Rain-fed Kharif Sorghum CSH-14

Introduction

Nowadays advance agriculture as applications of chemical nitrogenous fertilizer is not economical because the applied fertilizer, only 50% of the nitrogen is utilised by the plants and the remaining is being lost through denitrification or leaching. In contrast, biological system fix atmospheric nitrogen at no cost and at constant rate which permit immediate incorporation into plant proteins. Recent findings revealed the association of tropical grasses with dinitrogen fixing bacteria which under favourable conditions, contribute significance to the nitrogen economy of plant.

The increasing demand for nitrogen in agriculture could therefore, be solved by the exploitation of biologically fixed nitrogen, *Azotobacter* and *Azospirillum* are known to be responsible for dinitrogen fixation but also secret growth promoting substance like auxins, gibberalins, cytokinins and some fungistatic substances in grasses and grain crop, like sorghum, bajra, maize, rice and wheat, etc. (Dobereiner and Day, 1976). The VAM are known for solubilisation of insoluble phosphorus and they play significant role in phosphorus uptake and translocation, beside uptake of other nutrient elements, such as zink, sulphur and copper (Saif and Khan, 1977 and Hayman, 1982).

The present paper therefore deals with the study of individual and combined effect of VA-mycorriza (*Glomus fusiculatum*), *Asotobacter chroococcum* and *Azospirillum hrasilense* at different doses of chemical fertilizer on yield, dry matter weight, plant uptake of N and P and residual of N and P of sorghum hybrid CSH-14, so as to minimise the dose of chemical fertilizer.

Material and Methods

A field trial was conducted at Department of Plant Pathology Field, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, during kharif 1992-93. The soil of the experiment site was medium black, having pH 7.55, total nitrogen 150.64 kg/ha organic carbon 0.42% available P_2O_5 20.75 kg/ha and available K_2O_4 16.00 kg/ha.

Soil of experimental plot was low in nitrogen and very poor in phosphorus content and rich in potassium. Split plot design block design with three main treatment and eight subtreatments, replicated thrice. The culture of VAM (*Glomus fasiculatun*) was obtained from Dr. Jalali, Haryana Agricultural. University, Hissar and those *Azotobacter chrococcum* and *Azospirillum hrasilense* from ICRISAT Hyderabad.

The three doses of fertilizer *viz.*, full dose of fertilizer (80:40:40) T_1, ¾ dose of fertilizer (60:30:30) T_2 and no fertilizer application T_3. The dose of fertilizers were applied of which half dose of nitrogen and full dose of phosphorus and potash at the time of sowing and remaining nitrogen dose was applied month after sowing as top dose. The biofertilizer seed treatment were *Azotobacter* (S_1), *Azotobacter* (S_2) + *VAM* (S_3), *Azotobacter* (S_4), *Azospirillum* + VAM (S_5), *Azospirillum* + VAM (S_7) and No seed treatment (S_8).

The healthy seeds of *Sorghum* cv. CSH-14 were inoculated with the culture at the rate of 25 g/kg seed by following slurry method. The inoculated seeds were then dried in shade and dibbled (at the rate of 8 kg/ha) at spacing of 45cm between row and 15cm between plants. The observations on root length, shoot length, and green matter weight were recorded at 15-day interval taking randomly selected plants.

For estimation of the dry matter weight, five plants were randomly selected from each plot at an interval of 15 days and were air dried followed by oven drying at 60°C till constant weight were obtained. Grain yield data and fodder yield data were recorded after harvest of the crops.

For the soil analysis, the soil samples were collected from 20cm sol depth from each subplot before rowing and after harvesting. The samples were ground to fine powder to estimate residual nitrogen and phosphorus by Kjeldahl's Method (Jackson, 1967) and Olsen method (Jackson, 1967) respectively.

For the plant analysis, the plant samples were cut into pieces, dried and converted into fine powder which was used to estimate uptake of nitrogen by Kjeldahl method and phosphorus by Olsen method.

Results and Discussion

It is revealed from the data (Table 1) That the application of recommended dose of fertilizer along with *Azotobacter* + *Azospirillum* + VAM gave more grain yield (47.86 q/ha) followed by VAM (46.37 q/ha) and *Azospirillum* + VAM (46.19 q/ha). Fodder yield also obtained same trained. In the present investigation significant results were obtained for grain yield and fodder yield over uninoculated control, positive results were obtained in combined inoculation rather than single inoculation (Table 2), there were seen to be synergistic effect in imposing the grain and fodder yield Natarajan and Oblisami (1980) and Tilak *et al.* (1980)]. The effect of

Table 1

Effect of different interaction on grain yield (q/ha), fodder yield (q/ha) plant uptake of N and P (q/ha), and residual N and P in soil (kg/ha).

Observations	*Treat-ment*	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	
Grain yield	T_1	43.22	43.67	46.37	46.08	45.58	46.19	47.86	42.51	N.S.
	T_2	36.24	36.24	37.37	38.60	39.44	38.31	41.66	35.83	
	T_3	25.91	26.83	26.13	26.58	27.88	27.90	29.98	24.10	
Fodder yield	T_1	96.08	96.32	98.23	97.70	99.89	99.88	106.03	94.19	Sig.
	T_2	88.88	90.10	90.30	92.55	93.40	93.60	95.97	82.42	SE(m)±0.69
	T_3	73.30	73.62	77.33	77.91	78.22	78.42	81.07	74.93	CD%1.89
Plant uptake of N	T_1	218.11	218.52	219.00	220.81	223.28	222.65	225.5	216.15	Sig.
	T_2	209.99	211.19	213.45	215.33	217.51	216.35	218.80	204.82	SE(m)±0.68
	T_3	182.99	184.44	185.68	188.36	190.18	189.43	192.34	176.21	CD%1.87
Plant uptake of P	T_1	28.57	29.09	29.66	30.1	31.46	30.81	34.04	28.25	Sig.
	T_2	24.43	25.64	26.39	26.81	28.40	27.81	31.58	24.18	SE(m)±0.26
	T_3	23.85	23.92	24.65	24.98	25.94	25.77	26.79	20.61	CD%0.71
Residual N in soil	T_1	153.89	154.94	156.09	160.12	162.04	161.14	163.14	151.90	Sig.
	T_2	145.46	147.32	148.33	152.05	154.22	153.32	156.22	141.9	SE(m)±0.30
	T_3	136.47	137.88	138.85	143.27	146.81	145.54	148.42	131.76	CD%0.82
Residual P in soil	T_1	28.57	29.09	29.66	30.10	31.46	30.81	34.4	28.25	Sig.
	T_2	25.43	25.64	26.39	26.81	28.40	27.88	31.58	24.18	SE(m)±0.26
	T_3	23.85	23.92	24.65	24.98	25.94	25.77	26.79	20.61	CD%0.71

Residual of N and P (20.75 kg/ha) Before sowing (150.64 kg/ha)

Seed treatment = S_1 = *Azotobacter*, S_2 = *Azospirillum*, S_3 = VAM

S_4 = *Azotobacter* + *Azospirillum*, S_5 = *Azotobacter* + VAM

S_6 = *Azospirillum* + VAM, S_7 = *Azotobacter* + *Azospirillum* + VAM

S_8 = No treatment

T_1 = Full dose of NPK (80:40:40), T_2 = ¾ dose of NPK (60.30:30), T_3 = No fertilizer

Table 2

Effect of main and subtreatments on grain yield (q/ha), fodder yield (q/ha), N, P uptake and N, P (kg/ha) residue in soil.

Treatments	*Grain yield*	*Fodder yield*	*Plant uptake*		*Residue*	
			N	*P*	*N*	*P*
T_1 Full dose of fertilizer	46.18	98.52	220.50	61.11	167.97	30.84
T_2 ¾dose of fertilizer	38.05	90.93	213.44	54.52	159.85	27.68
T_3 No fertiliser	26.79	77.55	186.26	50.82	151.12	24.06
SE(m)±	0.18	0.47	0.25	0.24	0.12	0.41
CD 5%	0.73	1.85	2.5	2.35	1.22	3.6
Sub Treatments						
S_1 *Asotobacter*	35.32	86.75	203.70	53.50	155.27	25.95
S_2 *Azospirillum*	35.69	87.68	204.75	53.79	156.71	26.21
S_3 VAM	36.62	88.88	206.04	55.57	151.76	26.90
S_4 *Azotobacter* + *Azospirillum*	37.08	89.38	208.16	54.62	161.81	27.29
S_5 *Azotobacter* + + VAM	37.26	90.40	209.48	56.30	163.33	28.15
S_6 *Azotobacter* + VAM	39.83	90.69	210.32	57.29	164.35	28.60
S_7 *Azotobacter* + *Azospirillum*	39.83	94.35	212.21	59.43	165.93	30.80
S_8 No seed treatment	34.15	83.86	199.06	51.23	151.85	24.34
S.E.(m)±	0.39	0.40	0.39	0.24	0.17	0.15
CD at 5%	1.13	1.14	1.25	0.63	0.56	0.47

inoculant and fertilizer showed significant results, however interaction effect were found non-significant.

Among the two non-symbiotic nitrogen fixer *Azospirillum* was noticed to be superior over *Azotobacter*. Similar results were also reported by Tilak *et al.* (1982) and Pandey and Kumar (1984).

In present investigation it was observed that grain and fodder yield was more due to VAM inoculation as compared to *Azotobacter* or *Azospirillum* inoculation. This increase can be attributed to antimicrobial activity. secretion of growth promoting substances, fixing of atmospheric nitrogen and increased P availability to plant. Maximum uptake of P_2O_5 was noticed in combined inoculation and inoculation of VAM compared to *Azotobacter* + *Azospirillum*. Raju *et al.* (1987) had reported that along N and P_2O is also increased in VAM inoculated plant compared to non-inoculated plant.

The present paper showed that soil poor in N and P but application of biofertilizer inoculation with fertilizer minimise the cost of production also increased the grain yield but also save nitrogen and phosphorous some extent without affecting normal yield of sorghum.

Summary

Field trial was conducted during Kharif 1992-93 to study the effect of VAM (*Glomus fasiculatum*), *Azotobacter chroococcum* and *Azospirillum hrasilense* singly and in combination as seed inoculant at different doses of fertilizer on plant height dry matter weight, fortnightly interval, grain yield and fodder yield of CSH-14 recorded and also estimated the N and P_2O_5 uptake by plant and residue in the soil.

Bioinoculant and fertilizer application significantly increased shoot and root length, dry matter weight as well as grain yield over control treatment. The recommended dose of fertilizer and seed treatment with *Azotobacter* + *Azospirillum* + VAM resulted in highest rain yield of 47.8% q/ha as against 42.51 q/ha in control. *Azospirillum* + VAM was next best. Fodder yield and plant uptake of N and P recorded similar promising trends at a negligible investment.

The present studies revealed that bioinoculant along with fertilizer not only increases the yield but also saves the 50% of costly chemical fertilizers.

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P.V. Hatwalne, R.W. Ingle, K.G. Thakare and R.G. Somani
Department of Plant Pathology
Dr. Panjabrao Deshmukh Krishi Vidyapeeth
Akola–444 104, Maharashtra, India

9

Interaction Effect of *Azospirillum* and Phosphate Solubilising Culture on Yield and Quality of Sugarcane

Introduction

The use of organic and inorganic fertilizers have got prime role in maintaining the soil fertility and also to increase the productivity. Organic manures being slow in releasing nutrients and available in insufficient quantities, cannot meet the total nutrient requirements in crop production. Hence the use of chemical biofertilizer became imperative to exploit the further crop potential. However, use of inorganic biofertilizers are becoming scare and costly in recent years. In addition to this, excessive use of inorganic biofertilizers results in environmental pollution and increases salinity and alkalinity of soil. With this view the use of biofertilizers in the form of carrier based incoculants became more relevant and of economic importance.

Azospirillum is a associative symbiotic nitrogen bacterium. Anderson (1985) found that *Azospirillum lipoferum* was able to fix in or on the roots of sugarcane. According to him apart from atmospheric nitrogen and making it available to plants as well as promoting uptake of minerals ions such as nitrate, potassium, phosphate in addition to growth hormones.

Next to nitrogen, phosphorus is vital and non-renewal nutrient for plants and micro-organisms. About 98% Indian soils are poor in available phosphorus (Ghosh and Hasan, 1977) and efficiency of phosphate fertilizers utilization fixation of applied soluble phosphate (Gaur, 1982). Phosphate solubilizing micro-organisms possess the ability to dissolution of insoluble of phosphate to soluble form of phosphate.

Material and Methods

The experiment was conducted during 1992-93 to 1994-95 at three different co-operative sugar factories of Maharashtra *viz.* Vasantdada Shetkari S.S.K. Ltd., Sangli, Terna Shetkari S.S.K. Ltd., Osmanabad and Satara S.S.K. Ltd., Bhuinj. The objective of the present investigation

was to study the interaction effect of Nitrogen fixing bacterium *Azospirillum* and phosphate solubilizing culture on yield and quality of sugarcane var. Co 7219. The trial was conducted on medium black soil in randomised block design with eleven treatments and three replications. For present study the *Azospirillum* and P-solubilizing culture developed by Vasantdada Sugar Institute, Pune is used at the rate of 7.5 kg/ha each at the time of planting by soil application method.

Treatment Details

T_1 = 50% N and 100% P and K
T_2 = 75% N and 100% P and K
T_3 = 50% P and 100% N and K
T_4 = 75% P and 100% N and K
T_5 = 50% N and *Azospirillum* + 100% P and K
T_6 = 75% N and *Azospirillum* + 100% P and K
T_7 = 50% P and P-solubilizing culture + 100% N and K
T_8 = 75% P + P-solubilizing culture + 100% N and K
T_9 = 50% N and P + *Azospirillum* + P-solubilizing culture + 100% K
T_{10} = 75% N and P + *Azospirillum* + P-solubilizing culture + 100% K
T_{11} = Control (Only 100% NPK).

Results and Discussion

(A) *Growth Parameters % (Table 1)*

In case of germination all the treatments remained statistically not significant over control. The significantly highest tillering ratio at 120 days was recorded in the treatment having 75% N and P *Azzospirillum* + P-solubilizing culture, *i.e.*, 2.28 over control (1.97). The same treatment was found to be significantly superior in case of plant population and it was 88.25 × 1000/ha over control (81.45 × 1000/ha) while all other treatments remained statistically non-significant.

As far as girth of cane is concerned the treatments having 75% P + P-solubilizing culture, 50% N and P + *Azospirillum* + P-solubilizing culture and 75% N and P + *Azospirillum* + P-solubilizing culture showed significantly higher cane girth, *i.e.*, 9.55, 9.54 and 9.57 cm respectively over control (8.90 cm). The same treatment was found significantly superior in case of millable cane height.

(B) *Cane and Sugar Yield (Table 2)*

Results of cane and sugar yield of three different locations are pooled and presented in Table 2. Data revealed that the treatment having 50% N and P *Azospirillum* + P-solubilizing culture and 75% N and P + *Azospirillum* + P-solubilizing culture recorded significantly higher cane yield, *i.e.*, 105.31 and 109.74 MT/ha over control (90.29 MT/ha).

Table 1

Mean Growth Parameters as Affected by *Asospirillum* and P-solubilizing culture.

Trial No.	*Germinaion % (60 days)*	*Tillering ratio (120 days)*	*Milalble cane/ ha ('000)*	*Girth of cane (cm)*	*Millable cane height (m)*
T_1	62.54	01.77	75.59	08.81	02.18
T_2	61.83	01.88	78.94	08.76	02.33
T_3	62.15	01.73	80.66	08.72	02.24
T_4	62.96	02.05	83.21	09.04	02.35
T_5	60.52	02.03	80.55	08.92	02.42
T_6	65.23	02.10	85.13	08.99	02.51
T_7	66.60	02.05	81.78	09.28	02.39
T_8	64.85	02.15	83.81	09.55*	02.57*
T_9	65.27	02.19	83.36	09.54*	02.63*
T_{10}	66.08	02.28*	88.25*	09.57*	02.68*
T_{11}	63.07	01.97	81.45	08.90	02.35
C.D.	N.S.	00.28	05.47	00.44	00.17

Table 2

Mean cane yield and CCS (MT/ha) as affected by *Azospirillum* and P-solubilizing culture.

Treat-ment No.	*Cane yield (MT/ha)*			*Pooled Mean*	*CCS (MT/ha)*			*Pooled Mean*
	Terna SSK	*Vasant-dada SSK*	*Satara SSK*		*Terna SSK*	*Vasant-dada SSK*	*Satara SSK*	
1.	76.63	91.00	84.60	81.68	09.69	10.97	11.53	10.73
2.	78.40	87.40	83.88	81.72	10.22	11.20	11.76	11.06
3.	74.80	95.33	86.45	82.10	08.75	10.76	12.08	10.53
4.	80.34	99.33	89.60	85.58	10.57	11.50	12.97	11.68
5.	90.36	77.00	81.24	85.09	12.33	11.45	11.14	11.64
6.	92.70	101.40	91.60	93.78	13.17	13.95	12.54	13.22
7.	98.42*	96.67	89 45	95.14	13.66*	13.73	12.90	13.43
8.	103.33*	100.13	91.90	99.18	14.64*	14.18	12.88	13.90
9.	109.05*	95 20	104.70	105.39*	15.75*	14.54	15.10	15.13*
10.	113.38*	101.67	108.33	109.74*	16.45*	15.16	15.64	15.13*
11.	89.20	92.73	90.70	90.21*	12.41	14.27	12.56	13.08
CD	6.60			9.06	1.07			1.66

This indicates that by use of *Azospirillum* and P-solubilizing culture increased the cane yield by 19.45 MT/ha with saving of 25% chemical nitrogen and phosphorus.

In case of CCS, the significantly highest CCS was recorded in the treatment having 75% N and P + *Azospirillum* + P-solubilizing culture at all 3 locations viz. Terna Shetkari S.S.K., Vasantdada Shetkari S.S.K., Sangli and Satara S.S.K. and it was 16.45, 15.94 and 15.64 MT/ha respectively over control. The pooled data of 3 locations revealed that the treatment having 75% N and P + *Azospirillum* + P-solubilizing culture showed significantly highest CCS, *i.e.*, 15.75 MT/ha over control (13.08 MT/ha).

Summary and Conslusion

An experiment was conducted at three different locations of Maharashtra State viz. Vasantdada Shetkari S.S.K. Ltd., sangli, Terna Shetkari SSK Ltd., Osmanabad and Satara SSK Ltd., Bhuinj during 1992-93 to 1994-95 with an object to find out the interaction effect of *Azospirillum* and phosphate solubilizing culture on yield and quality of sugarcane. It was observed that the application of *Azospirillum* and P-solubilizing culture at the rate of 7.5 kg/ha each at the time of planting by soil application method gave significantly maximum cane and sugar yield (109.74 and 15.75 MT/ha) with saving of 25% inorganic nitrogen and phosphorus while in control the cane yield was 90.29 MT/ha and sugar yield was 13.08 MT/ha.

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Authors

A.M. Thopatel, R.R. More[1] and S.B. Jadhav[2]

1. *Department of Agriculture Microbiology*
2. *Department of Agriculture Science and Technology*
   Vasantdada Sugar Institute
   Manjri (BK.), Dist. Pune, Maharashtra, India

10

Use of Bio-Inoculants for Recycling of Banana Wastes

Introduction

Banana is grown over an area of 1,64,000 hectares in India. This crop is mainly grown in Tamil Nadu, West Bengal, Kerala, Maharashra etc., on a commercial basis, through it is grown in other areas too.

Tiruvaigundam taluk of Tuticorin District is a place wherein Banana is extensively grown on a commercial basis in the Tamiravaruni basin, where there is heavy crop of Banana. The Banana wastes after harvest are found to lie across the water courses causing overflow of channels, leakage due to seepage and loss in water leading to secondary problems like mosquito menace. Moreover the mosquitoes in the presence of high moisture ravage through the deteriorating Banana wastes causing disease as well environment pollution.

Further the hard peduncles after removal of hands mostly of lignin and hemicellulose content are left thrown in the market place causing bruises to the customers (Lavanya, 1997).

Material and Methods

Thus, realising the vulnerability of the situation and to facilitate recycling and to produce a good manure with the available wastes, experiments were conducted in the Department of Soil Science and Agricultural Chemistry, Agricultural College and Research Institute, Killikulam of Tamil Nadu Agricultural University in the year 1995-96 with the locally existing biowastes left in the market place *viz.* Banana wastes.

Fungal bio-inocualnts *viz. Agaricus. Pleurotus* and *Penicilium notatum* were inoculated under different levels of moisture *viz.* 40% and 60% with two different processes of decomposition *viz.*, Aerobic and Anaerobic in a miniature form using pot-culture experiments with Randomised Block design comprising of twelve treatment replicated thrice, *viz.*, Trash, Peduncles, Pseudostem.

The waste were spread in layers and they were detected of the exact moisture content gravimetrically so as to maintain the moisture level, periodically.

The samples were aerated once in 3 days by frequent stirring in aerobic method and in anaerobic method a sandy paste was made over the surface of the pot and water was sprinkled over the sandy layer once in 3 days. The decomposition process was allowed to continue for 2 months until the manure attained a black colour with friable nature.

In anaerobic method, the sandy paste spread over the pot was disturbed once in month to aerate the pot.

After the decomposition period of 2 months, the pots were overturned and the manure was dug from the pot and shade dried and analysed for their nutrient contents.

The bio-inoculants *viz.*, *Agaricus, Pleurotus* and *Penicilium* were inoculated at the rate of 3 g/pot and 3 ml/pot (1 ml = 10^4 cfu, colony forming units) respectively with a capacity of 6 kg wastes per pot.

Initial analysis of the waste materials as well as the nutrient content of the manure at different intervals *viz.*, 1 month after decomposition and 2 months after decomposition were analysed using Piper. 1966. The waste were incorporated initially on a moisture free basis. The treatment included were as detailed below:

1. Control (Aerobic)
2. *Pleurotus* + 40% moisture (Aerobic,
3. *Pleurotus* + 60% moisture (Aerobic)
4. *Penicilium* + 40% moisture (Aerobic)
5. *Penicilium* + 60% moisture (Aerobic)
6. Control (Anaerobic)
7. *Pleurotus* + 40% moisture (Anaerobic)
8. *Pleurotus* + 60% moisture (Anaerobic)
9. *Penicilium* + 40% moisture (Anaerobic)
10. *Penicilium* + 60% moisture (Anaerobic)

Results and Discussion

The nutrient contents of the Banana wastes are depicted in Table 1.

Table 1

Banana wastes and their nutrient (%) on oven dry basis.

Name of the part	*Nutrient content (%)*						
	N	*P*	*K*	*Ca*	*Mg*	*Total carbon*	*Crube fibre*
Pseudo stem	0.18	0.50	2.20	1.48	1.32	54	20
Leaf	0.68	0.86	1.72	0.80	0.65	24	5
Peduncle	0.15	0.30	1.60	0.58	0.47	70	30

Table 2

Effect of different levels of moisture and bio-inoculants on dry matter reduction (kg), moisture content (%), total carbon (%).

Treatment	*Dry matter reduction (kg)*		*Total C (%)*		*Moisture (%)*		*Total Nitrogen (%)*		*Total P (%)*		*Total K (%)*	
	1 month	*2 months*	*1 month*	*2 months*	*1 month*	*2 months*	*1 month*	*2 months*	*1 month*	*2 months*	*1 month*	*2 months*
Control Aerobic (A)	1.98	1.80	62.54	52.30	15.78	8.89	0.27	0.80	0.45	0.48	0.52	0.48
P + 40% M-A	1.00	0.70	48.60	35.70	36.40	30.40	0 68	2.50	1.07	1.45	1.12	0.52
P + 60% M-A	1.00	0.64	52.30	43. 90	45.20	35.90	0.59	1.95	1.03	1.27	1.06	1.75
Pe + 40% M-A	1.37	0.98	52.30	38.80	45.40	28.70	0.58	1.95	0.82	1.34	0.80	1.19
Pe + 60% M-A	1.00	0.67	57.80	45.30	48.30	33.30	0.54	1.85	0.81	1.26	0.76	1.00
Control Anaerobic (AA)	2.03	1.50	80.68	56.30	40.28	26.30	0.25	0.90	0.57	0.45	0.54	0.52
P + 40% M-AA	1.54	0.79	54.60	47.60	57.30	48.40	0.56	1.85	0.95	1.27	1.00	1.61
P + 60% M-AA	0.74	0.32	58.80	49.30	62.40	57.10	0.52	1.75	0.91	1.08	0.90	1.19
Pe + 40% M-AA	1.89	1.01	58.40	49.70	59.70	46.50	0.56	1.75	0.74	0.95	0.74	1.28
Pe + 60% M-AA	1.93	0.86	59.60	50.80	65.30	55.30	0.50	1.55	0.72	0.91	0.68	1.15
Mean	1.45	0.93	58.56	46.92	47.59	37.07	0.51	1.69	0.81	1.05	1.22	0.81
S.D.	0.47	0.39	8.01	5.89	14.05	14.11	0.13	0.50	0.19	0.33	0.20	0.42

A-Aerobic: AA-Anaerobic: P-Pleurotus; Pe-Penicilium: M-Moisture

The results also revealed that (Table 2) there was a drastic reduction in dry matter after 2 months of decompostion viz., 0.93 kg.

Total carbon content in percentage worked out to be on an average 46.9%. Further the moisture content of the manure also got reduced with advent of time, *viz.*, 2 months after decomposition, *i.e.* 37%.

Regarding the total nutrient contents *viz.*, Total N, Total P, Total K, it averaged out to 1.69% N, 1.05% 1.22% K after 2 months of decomposition as compared to one month decomposition period which recorded lower values.

Among the inoculants, the dry matter reduction was higher in Pleurotus, *viz.*, 0.61 kg as compared to that of Penicilium which recorded lower values.

Among the inoculants, the dry matter reduction was higher in *Pleurotus*, *viz.*, 0.61 kg as compared to that of *Penicilium* which recorded 0.88 kg after 2 months decomposition process.

Similarly the total carbon content in (percentage) was found to be lower in *Pleurotus* 44.1% after 2 months of decomposition.

The total nutrient content *viz.*, N, P, K were found be higher in Pleurotus than Penicilium, *viz.*, 2.1%, 1.27% and 1.64% respectively.

Thus among the inoculants *Pleurotus* was found to enrich the manure in a better wary and proved to be an efficient convector of waste with higher order of degradation. The findings are also in agreement with that of Jesylyn Vijayakumari (1986).

Among the moisture regimes, 40% level of moisture was found to be congenial as compared to that of 60% moisture level which could aid faster and better decomposition.

Among the decomposition process the anaerobic method showed higher nutrient contents especially K after 2 months of decomposition as well the total carbon content remained low, *i.e.*, 50.6% after 2 months of decomposition process. Whereas, the nitrogen and phosphorus remained high in aerobic process, though there was not much variation between both the processes (Table 3).

Table 3

Effect of method of decomposition and time interval on the manurial content.

Parameters	*Methods*			
	Aerobic		*Anaerobic*	
	1 month	*2 months*	*1 month*	*2 months*
Dry matter				
Reduction (kg)	1.27	0.96	1.63	0.90
Total carbon (%)	54.71	54.50	62.40	50.60
Moisture (%)	38.20	27.40	56.99	46.70
Total N (%)	0.53	1.81	0.48	1.56
Total P (%)	0.84	1.16	0.78	0.93
Total K (%)	0.85	0.99	0.77	1.15

Thus the use of Bio-softwares will definitely serve as "Bio-Golds" to harness the manurial turnover in India and help a great deal in tackling the fertilizer demand realised nowadays.

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Authors

P.G. Lavanya* and G. Arunachalam**
**Krishi Vigyan Kendra, Kattupakkam–603 203*
***Professor and Head, Dept. of Agricultural Chemistry*
Kllikulam–625 272, Tamil Nadu

11

Application of Pressmud as Plant Growth Promoter and Pollution Arrester

Introduction

Maharashtra and Uttar Pradesh (U.P.) are the major sucrose producing states of India. It, therefore, obviously follows that along with sugar, they generated many byproducts of sugar and distillery wastes which need closer attention for their utilisation on merit. The chief byproducts are bagasse (a potential source of fibre for pulp and paper industry), Pith (an amorphous parenchymatous core tissue, rich in sugar, constitutes 90% calorific value of bagasse, a source of fuel for sugar industry), molasses (a vital raw material for distilleries), stillage from distilleries (as a source of biogas) and pressmud, a multi-ingredient and vital source of macro-micro-nutrients, matrix for microbes, source of phytosterols, PGRs and plant protecting steroids.

On a global scenario, India being the largest producer of sucrose. It is estimated that it generated about 3.2 million tonnes of pressmud (filter mud/scum) (Tandon, 1994). Although, sugar industry looked at it as a waste, with the emphasis on recycling this type of waste through imaginative biotech applications, we feel that pressmud could be analysed for its macro- and micro-nutrients and assigned at least a few value-added applications so that application of waste generated wealth. The present review article examines its chemical characteristics, physical nature and explores the feasibility of assigning it futuristic applications, hopefully for sustained agricultural production.

Material and Methods

Pressmud was obtained from Madhukar Sahakari Sakhar Karkhana. Faizpur and Purushottamnagar Sakhar Karkhana, Shahada during the crushing season December-May.

AR grade chemicals and demineralised water used for analysis. Total and volatile solids were estimated by gravimetry.

Ash contents were determined by burning at 600°C for 30 minutes until constant weight. Cellulose was determined as per Updegraff (1969). Hemicellulose was determined as per Deschatelets and Yu (1986). Lignin was estimated as decribed in Methods of Wood Chemistry (1967). Organic carbon was analysed by Walkley-Black method (1973). Wax was estimated by extraction in benzene and noting down the difference in the weight of pressmud before and after extraction. Protein was estimated as per Loury *et al.* (1951). Total carbohydrates were estimated by phenol sulphuric acid method as per Dubois *et al.* (1956). Reducing sugar was estimated by DNSA method as per Miller (1969). The above methods were routinely used for the analysis of pressmud. Its representative analysis on dry weight basis is summarised in Table 1.

Table 1
Constituents-wise profile of representative pressmud.

Constituents	*%*
Total solids	100
Volatile solids	70
Ash (Non-volatile solids)	30
C/N ratio	30:1
Cellulose	25
Hemicellulose	27
Lignin	4
Organic carbon	24-27
Solubles	38
Wax (Benzene Extract)	9
Proteins	7
Total Carbohydrates	2
Reducing sugars	0.4

About 1.2% nitrogen, 2.5% phosphorus, 2% potassium, 260 mg/kg zinc and 120 mg/kg copper are present.

Results and Discussion

Physically, pressmud is soft, spongy, bulky (light weight) and amorphous blackish brown material.

Literature has reported used of pressmud (Unni *et al.*, 1987, Talegaonkar *et al.*, 1996), in (i) distemper and paint industry, (ii) cement and filter aid industry, (iii) metal polish and animal feed supplement, and (iv) its sterols/steroids in pharmaceutical industry. However, these are only laboratory scale ideas, without estimates of their viability and pollution potential. A lot of work on techno-economic viability of such applications is desired before they mature into a concrete application. Therefore, farm application of pressmud in the near future appears worth exploring, crop-wise, in experimental plots, so that its suitability of scientific basis is established. In this context, its application for the ecofriendly restoration of manganese and coal mine overheads or mine spill soil by Juwarkar *et al.*, (1991) is worth mentioning.

From Table 1, the following facts have clearly emerged upon analysis of pressmud ingredients:

1. The C: N ratio of 30:1 is optimal for the growth of a number of crops (Sharma *et al.*, in press). These includes flowers, vegetables, cereals, pulses, oil seeds, sugarcane, bamboo and eucalyptus.
2. Its cellulose and hemicellulose content in relation to lignin has shown that allelopathic effect, if any, on plant growth is already neutralised (Ramamurthy *et al.*, 1996).
3. Its meagre amount of reducing sugars are hoped to catalyse rapid microbial growth and the same would be promoted over a long period by total carbohydrates and subsequently sustained by composting of both, cellulose and hemicellulose. Thus, a sustained enrichment of organic carbon in the soil appears assured for comparable productivity.
4. The presence of 7% proteins in the pressmud would be a source of sustained release of amino acids by continuous proteolysis through rhizobial microflora. It appears now well established that amino acids facilitate overcoming the physiological constraints and thereby promote increased chlorophyll synthesis, availability of more photo-assimilates and increased growth rate (Sharma *et al.*, 1992, 1993, 1995 and Yadav *et al.*, 1995).
5. Tandon *et al.*, (1994) have found pressmud superior to sewage sludge, biogas slurry, compost, farm yard manure and soil conditioners from municipal wastes. This would not have been possible without pressmud affording physical, chemical, biological and possibly pharmaceutical benefits through porous texture, more aerobicity for the roots, more water holding capacity, possibly sustained release of moisture, sustained release of tricontanol (PGR), harbour of microflora and phytosterols/steroids in wax affording plant protection against plant pathogens.

The above extrapolation of different benefits is merely a reflection of its micronutrients and as yet unidentified ingredients.

Basically, it would afford a porous texture to the soil and thereby make air available for the respiration of the root system, in turn rendering more energy available for absorption of nutrients from the rhizosphere (Rajor *et al.*, 1996). Incidentally, due to its pH 5.0, it has the potential to amend alkaline soils which otherwise are barren. Such amended soils have shown excellent productivity in forestry. Being insoluble in nature, it could serve as a matrix for the growth and sustenance of beneficial microbes. Department of Biotechnology, New Delhi has been examining various matrices for serving as vehicle for the application of biofertilizers. We propose to examine if pressmud could enhance shelf life of nitrogen fixing and/or phosphate solubilizing bacteria/fungi.

Its various inorganic constituents such as nitrogen, phosphorus and potassium are anticipated to supplement fertility of soil and thereby reduce dependence on capital-, energy-, pollution-, and cost-intensive chemical fertilizers. Furthermore it micronutrients, notably zinc and copper,

would supplement micronutrient fertility without additional cost. In fact, Tandon *et al.* (1994) have converted these nutrient values into economic values to Rs. 75 crore/annum.

About 10% wax content of pressmud has the dual potential to serve as a source for plant growth regulators such as tricontanol and phytosterols which have the potential for neutralising the virulence of plant pathogens.

Finally, the unidentified trace nutrients of pressmud would render invaluable fertility value by complementing several physiological processes in plant and rhizobial microflora, providing sustained growth rate. In the light of these facts, potential multiplier effect of pressmud, renders it a most attractive candidate for biotech application. Its use in agriculture/forestry appears to be least polluting and most cost-effective, rewarding and sustainable.

Thus, cumulatively, pressmud appears to be a fountainhead of a number of vital attributes, directly or indirectly, immediately or on sustained basis. Potentially or otherwise and therefore, its value-addition appears within the realm of human efforts, without additional processing/ efforts/cost.

In fact, ignorance of pressmud composition attracted least attention of the business community as yet and hence the putrefied piles on the premises of sugar mills have become a source of aerial pollution. Its utilisation *per se* would not only serve as an avenue for additional income to the sugar industry but its application would control aerial pollution also and simultaneously supplement fertilizer value in turn affording higher agricultural productivity.

In the absence of (a) alternative plans by the sugar industry, (b) competing technology in the offing, and (c) a competing product emerging from or for pressmud, it remains a wealth for farmers. This simple, efficient and economic way of pressmud management could place it rightfully as the biofertilizer of 21st century.

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Authors

S.K. Talegaonkar, S.B. Chincholkar and R.M. Kothari
School of Life Sciences
North Maharashtra University
Jalgaon–425 001, Maharashtra, India

12

Biofertilizer for Multipurpose Tree Species

Introduction

Importance of ectomycorrhizal (EM) fungi in reforestation of eroded lands have been recognised for a long time now. The inoculation of forestry plant seedlings with these fungi has become a standard protocol. Early stage EM fungi are the choice as they have a broad host range. *Laccaria laccata* (Scop. Fr.) Berk. and Br. Is one of the widely distributed early stage fungus with a broad host range forming ectomycorrhiza with *Eucalyptus, Larix, Pinus* and others (Burgess and Malajczuk, 1989; Hung and Molina, 1986; Reddy and Natarajan, 1997; Ramaiah and Perrin, 1990; Sharma and Mishra, 1988; Thomas and Jackson, 1979). Early stage fungi like *Laccaria laccata* are very suitable for inoculation of seedlings which have to be placed in skeletal or mineral soils. With the help of this fungus, time required for the generation of plantable seedling can be reduced to half (Tacon and Bouchard, 1986). Tolerance to the extreme of soil conditions evoked the interest in applying this fungus to develop wastelands into green forest covers (Dixon, *et al.*, 1993; Martins, *et al.*, 1996; Molina, 1982; Molina and Chamard, 1983; Thomas and Jackson, 1983). This fungus exhibited antagonistic behaviour against some potential root pathogens (Krywolap, 1971; Marx, 1969; Perrin, 1985; Sampangi, *et al.*, 1985; Sinclair, *et al.*, 1982; Sylvia, 1983).

Various forms of fungal inoculum can be used for inoculating tree seedlings, but the most recommended form is vegetative mycelium (Trappe, 1977). Vegetative mycelium can be produced by using either solid substrate cultivation or submerged cultivation. Sorghum grain and sphagnum-vermiculite carrier have been used to produce inoculum through solid substrate cultivation (Maul, 1985; Raman, 1988). Inocula produced through these processes are not physiologically consistent and are difficult to maintain (Marx, 1980). Furthermore, substrate that remains at the end of the process is difficult to remove and invites contamination during

application and finally leads to the loss of inoculum (Marx, 1980). Most of these problems can be overcome by producing physiologically consistent mycelium through submerged cultivation (Boyle, *et al.*, 1987; Garg, *et al.*, 1995; Kropacek, *et al.*, 1989; Kuek, 1996; Tacon, *et al.*, 1985; Sasek, 1989). Sturdy inoculum then can be produced by physical entrapment of this mycelium in alginate gel (Kuek, *et al.*, 1992; Mortier, *et al.*, 1989). The objective of this investigation was to examine the possibility of using granulated inoculum of *Laccaria laccata* for inoculation of seedlings of *Eucalyptus* hybrid and *Acacia nilotica* in nursery.

Material and Methods

Species

Laccaria laccata strain ITCC 3334 was obtained from the Indian Type Culture Collection Centre, Indian Agriculture Research Institute (IARI), Delhi. Seeds of *Acacia nilotica* and *Eucalyptus* hybrid were obtained from Tata Energy Research Institute (TERI), New Delhi.

Inoculum Preparation

Laccaria laccata was maintained on agar slants of Modified Melin-Norkan's (MMN) medium (Marx, 1969). This strain was cultivated under submerged conditions in a fermenter Biostat C-22L in chemically defined medium. Mycelium obtained was washed thoroughly with sterile distilled water to remove the traces of remaining nutrients. Rinsed mycelium was homogenised in presterilised Warring blender type homogeniser. Slurry of myceliar fragments was mixed with equal volume of sterile 4% sodium alginate solution and added drop by drop to sterile 0.1 M calcium chloride solution with continuous stirring. After hardening, the beads obtained were rinsed with sterile distilled water. Each bead contained approximately 100 colony forming units. The alginate beads were stored at 4°C in sterile distilled water before using it for inoculation of plant seedlings.

Treatment :

Preparation of soil-vermiculite mixture

One volume of vermiculite was mixed with one volume of soil. Either sterile or non-sterile soil-vermiculite mixture was used in experiment. Sterilisation of soil-vermiculite mixture was carried out by keeping the mixture for 2 hrs at 121°C under 15 psi and allowing it to cool down for 24 hrs. Polyethylene bags were then filled with the sterile or non-sterile mixture.

Inoculation of Acacia nilotica

Seeds of *Acacia nilotica* were graded on the basis of their weight. Seeds of weight ranging from 0.145 to 0.165 g were soaked in 9N sulphuric acid for 15 minutes and washed thoroughly with sterile distilled water. Acid treated seeds were then soaked for 24 hrs in distilled water Inoculum beads at the rate of 10 beads/bag were placed at a depth of six to eight centimetre. Acid treated seeds at the rate of one seed/bag were placed at a depth of one to two centimetres.

Control seedlings did not receive inoculum beads. Three repeats of twenty seedlings treatment were placed in nursery.

Inoculation of Eucalyptus hybrid

Eucalyptus hybrid seeds were sowed in a presterilised soil-vermiculite mixture. Seedlings at the four leaved stage were thinned down to one seedling per bag, either containing presterilised or non-sterile soil-vermiculite mixture. Inoculum beads at the rate of 10 beads/seedling were placed near the root zone while the control seedlings did not receive any inoculum bead. Three repeats of twenty seedlings per treatment were placed in nursery.

Results

Inoculation with the *Laccaria laccata* significantly affected the growth of seedlings. *Acacia nilotica* showed increment of 185% (Figs. 1 & 2) with respect to uninoculated seedlings in sterilised soil-vermiculite mixture. Growth of *Eucalyptus hybrid* was significantly higher when inoculated with Laccaria laccata than the uninoculated plant seedlings (Figs. 3 & 4). In sterile soil-vermiculite mixture, *Eucalyptus hybrid* showed increase up to 147% (Fig. 4, treatment A, B) to inoculation. Inoculation of *Eucalyptus hybrid* with *Laccaria laccata* showed improvement up to 112% in comparison to its non-inoculated seedling in non-sterilized soil-vermiculite mixture (Fig. 4, treatment C, D). contribution of the micro-organism in improvement of plant growth is also apparent (Fig. 4, treatment A. C). Contribution in growth due to inoculation in non-sterilized condition is quite significant but not as high as in sterile soil-vermiculite mixture. Analysis of variance showed that there is no significant difference between the inoculated *Eucalyptus hybrid* seedlings in strilized and non-sterilized soil-vermiculite mixture (Fig. 4, treatment B, D).

Discussion

The alginate entrapped inoculum of *Laccaria laccata* for inoculation of forest tree seedling is very handy and robust in comparison to its counterpart peat-vermiculite based inoculum. We have produced the inoculum based on alginate matrix. In this matrix the inoculum is physiologically consistent and remaining stable for six months. *Laccaria laccata* is an early stage ectomycorrhizal fungus which has been used for inoculation of various tree seedlings. Inoculation of *laccaria laccata* increased the growth of *Acacia nilotica* and *Eucalyptus hybrid* seedlings in comparison to uninocualted seedlings in sterilized and non-sterilized soil. *Endomycorrhiza* is very common in *Acacia* species, but very few reports show the possibility of ectomycorrhizal synthesis in this genus (Natarajan, *et al.*, 1995; Osonubi *et al.*, 1991; Reddell and Waren, 1986). Effect of inoculation on growth of *Eucalyptus* agrees with the previous studies (Ashton, 1976; Barrow, 1977; Bougher, *et al.*, 1990; Heinrich and Patrick, 1986; Lapeyrie and Chilvers, 1985; Malajczuk, *et al.*, 1975; Mulligan and Patrick, 1985; Pryor, 1956). With this work we conclude that the granulated alginate entrapped *Laccaria laccata* can be used for the inoculation of *Acacia nilotica* and *eucalyptus hybrid.*

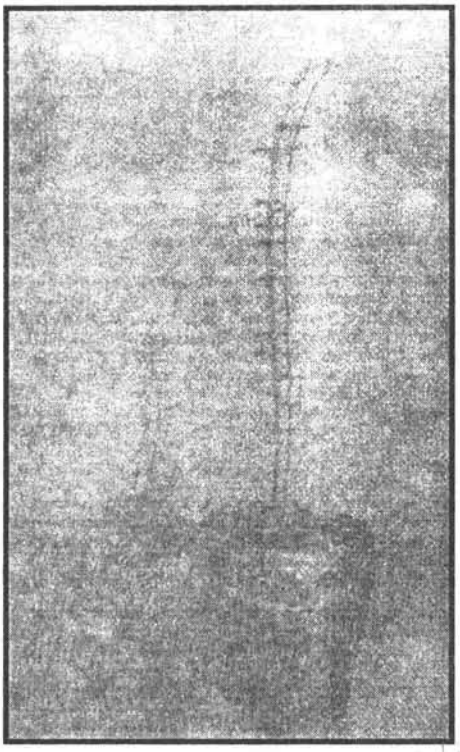

Fig. 1 Effect of inoculation of *Laccaria laccata* on *Acacia nilotica* in sterile soil-vermiculite mixture control (C) seedling did not contain inoculum while inoculated seedling (I) contained 10 beads of *Laccaria laccata.*

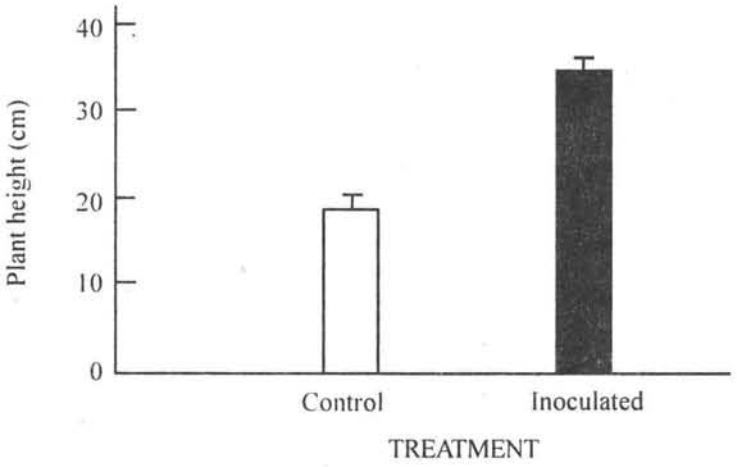

Fig. 2 Effect of inoculation of *Laccaria laccata* on *Acacia nilotica* in sterile soil-vermiculite mixture where control seedling did not contain inoculum while inoculated seedling contained inoculum of *Laccaria laccata* at the rate of ten beads/seedlings.

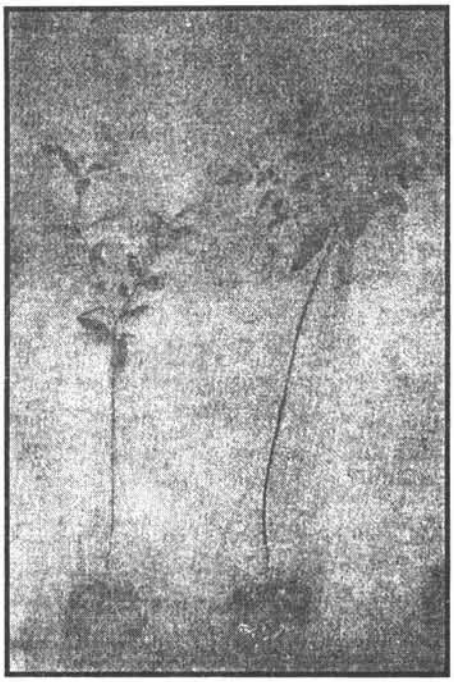

Fig. 3 Effect of inoculation of *Laccaria laccata* on *Eucalyptus* in sterile soil-vermiculite mixture control (C) seedling did not contain inoculum whereas inoculated (I) seedling contained 10 beads of *Laccaria laccata.*

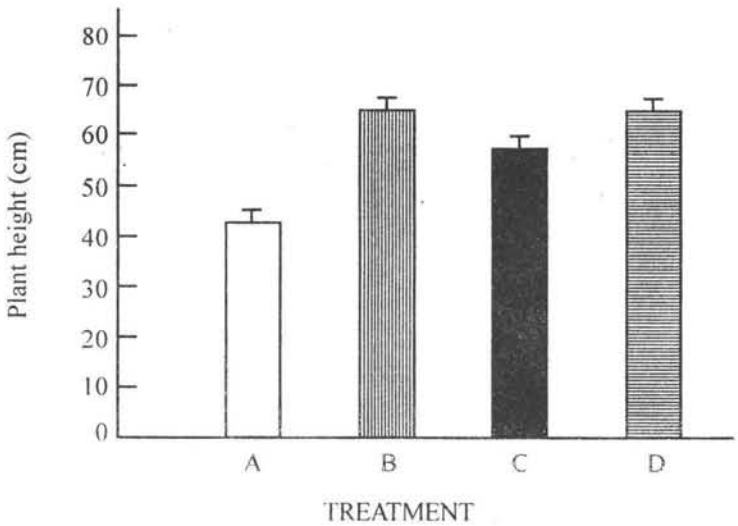

Fig. 4 Effect of inoculation of *Laccaria laccata* on *Eucalyptus* hybrid where various treatments are sterile soil-vermiculite mixture without inoculum (A) and with inoculam (B) and non-sterile soil-vermiculite mixture without inoculum (C) and with inoculum (D).

Summary

Multipurpose tree species have been known for their multifaceted applications, one of the application being reclamation of wastelands. Reclamation of wastelands and introduction of exotic varieties into a new place, operate on the same plane as both provide hostile environment to the introduced plant seedlings. The chances of seedlings survival is very meagre in such regions, and even if they survive, the growth of plant is very poor. In the last couple of years, ecomycorrhizal interface between the environment and plant roots has come up as a potential solution for introducing green cover over these regions. *Laccaria laccata*, one of the early stage ectomycorrhizal fungus, was grown under submerged conditions to produce large amount of biomass. This biomass was homogenised and immobilised in calcium alginate matrix to produce inoculum for tree seedling. The bead form inoculum thus produced, is robust and can be stored at 4°C in sterile distilled water for six months. *Acacia nilotica* and *Eucalyptus hybrid* were selected as tree seedlings for testing the ectomycorrhizal fungus in the nursery. Ectomycorrhizal inoculum, at the rate of 10 beads/seedling, was placed at proximal end of the root of seedlings, sterile and non-sterile soil mix of vermiculite and soil (1:1 V/V) were used in plastic bags to grow the plant seedlings in nursery. After five months, the height of ectomycorrhizal inoculated seedlings differed significantly among the various treatments with respect to the non-inoculated seedlings. In comparison to the non-inoculated seedlings, the inoculated seedlings of *A. nilotica* showed 185% increase in height in sterile soil mix. In case of *Eucalyptus* hybrid, ectomycorrhizal inoculated seedlings in sterile soil mix exhibited 147% increase in height, while in nonsterile soil mix the increase was 112%, with respect to their non-inoculated seedlings.

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Authors

Sandeep Garg, Vandana Gupta and T. Satyanarayana
Department of Microbiology
University of Delhi South Campus
Benito Juarez Road
New Delhi - 110 021, India

13

Response of Tree Legumes Seedlings to Bioinoculation of Endomycorrhizae and *Rhizobium* in Alfisol

Introduction

In general the tree crops establishment in problem soil will be severely interfered by the non-availability of basic nutrient and possible inhibition by the soil borne pathogen and other parasitic attack. For the tree crop establishment none of the inoculants were given and hence the tree crops grown in virgin soil or the wasteland are very much affected by the environmental stresses and certain biotic factors. By the involvement of certain microbial interaction might be possible to alleviate the circumstances to enable them grow and establish in further proliferation. The present investigation in aimed to ascertain the role of certain nitrogen fixing root nodulating bacterial inoculation with P mobilising VAM fungi as co-inoculant in alfisol, since no attempt was made earlier in acid soil. The possibility of these microbes not only created better environment but also slowly makes the soil to be suited for other crops to be introduced at an easiest way.

Material and Methods

A nursery study was conducted to assess the significance of nitrogen fixing *Rhizobium* specific for *Acacia* spp (fast grower) along with and without soil inocorporation of three VAM (*Glomus fasciculatum*, *Glomus mosseae* and *Gigaspora calospora*). *Rhizobium* was given as seed bacteriazation at 600 g/20 kg of *Acacia* seeds (with a cell population of 10^7/g of peat based carrier). VAM fungi was inoculated at the rate of 2 g/plant seed in the polythene pouch (with 75% infective propagule). These microbes were treated on five *Acacias*: (*Acacia mangium. A. crassicarpa, A. concianna, A. holosericea* and *A. hispida-exatic* from Australia). To compare the efficacy of these bioinoculants, N and P controls were also maintained. Each treatment was replicated twenty-five times to obtain the effective information and conducted in a Randomized Block Design.

At 20 days after sowing the seedlings germinated on the treated and untreated pouches were assessed for germinability and vigour index. During 40 days after sowing the *Acaçia* seedlings were ascertained for various growth attributes *viz.*, seedling biomass, nodulation and its biomass, shoot and root growth and percentage of VAM infection by employing the method of Haymen (1970). The data were analysed and tabulated (Tables 1 to 5).

Results and Discussion

The results on the influence of *Rhizobium* along with and without bioinoculation of VAM on the five *Acacias* have revealed that the biogrowth, nodulation shoot and root growth were significantly augmented in the inoculated treatment over their controls. This information found correlated with the report of Murugesan *et al.* (1994) in which in they have reported the response of ten species of *Acacias* to Rhizobial interference. Similar to present investigation the several reports had also indicated the role of VAM and nitrogen fixer on crops (Frezy and Schching. 1993 and Tilak, 1993; Subbarao *et al.* 1985). According to the report of Sekar *et al.* (1994) the inoculation of biofertilizer tends to enhance the seedlings growth, root colonisation and uptake on P nutrient in three trees in Shola forest ecosystem. In the present investigation the dual inoculation of *Rhizobium* with *Glomus fasciculatum* registered better symbiosis and field establishment and nodule formation followed by the combination of *Rhizobium* with either of *Glomus mosseae* by *Gigaspora calospora* in all the five tree legumes in alfisol.

Table 1.
Influence of endomycorrhizae and *Rhizobium* on growth and establishment of *Acacia* mangium.

Treatment	*20 DAS (Initial)*			*40 DAS (day after sowing)*			
	Vigour Index	*Plant Biomass (g/Pl)*	*Nodule No./Pl*	*Nodule Biomass (mg/Pl)*	*Root Growth (cm/Pl)*	*Shoot Growth (cm/Pl)*	*Percent of VAM infection*
Control	640	1.96	1.0	4	7.8	13.2	10
N-control	820	3.21	6.3	19	13.9	21.9	40
P-control	710	2.62	5.0	17	11.6	18.6	60
Rhizobium	790	2.91	14.0	53	12.5	19.6	45
VAM-1	770	2.73	10.0	28	11.9	22.0	90
VAM-2	740	2.61	6.0	19	11.7	16.9	80
VAM-3	700	2.53	5.6	17	11.3	18.7	70
R+VAM-1	870	3.42	16.6	64	14.5	24.9	90
R+VAM-2	830	3.22	12.0	49	12.7	22.3	85
R+VAM-3	810	3.01	10.0	41	12.2	19.7	80
CD (P=0.05)		NS	0.4	2.9	0.7	1.2	

VAM-1 = *Glomus fasciculatum*; VAM-2 *Glomus mosseae*; VAM-3 *Gigaspora calospora*

Table 2
Influence of endomycorrhizae and *Rhizobium* on growth and establishment of *Acacia crassicarpa*.

Treatment	*20 DAS (Initial)*			*40 DAS (day after sowing)*			
	Vigour Index	*Plant Biomass (g/Pl)*	*Nodule No./Pl*	*Nodule Biomass (mg/Pl)*	*Root Growth (cm/Pl)*	*Shoot Growth (cm/Pl)*	*Percent of VAM infection*
Control	600	2.10	4	9	9.8	15.2	15
N-control	790	5.97	10	30	16.7	26.1	30
P-control	700	3.85	8	26	12.5	21.2	45
Rhizobium	780	4.31	17	42	14.3	22.3	45
VAM-1	760	4.11	11	24	15.3	19.8	80
VAM-2	740	3.41	9	18	14.3	19.0	80
VAM-3	755	3.61	8	15	16.3	21.9	60
R+VAM-1	950	6.32	23	78	18.3	29.1	90
R+VAM-2	890	5.11	18	47	14.2	23.8	80
R+VAM-3	870	4.95	16	39	13.7	21.7	76
VAM-1 = *Glomus fasciculatum*; VAM-2 *Glomus mosseae*; VAM-3 *Gigaspora calospora*							
CD (P=0.05)		NS	0.2	0.5	1.2	2.3	NS

Table 3
Influence of endomycorrhizae and *Rhizobium* on growth and establishment of *Acacia holoserisea*.

Treatment	*20 DAS (Initial)*			*40 DAS (day after sowing)*			
	Vigour Index	*Plant Biomass (g/Pl)*	*Nodule No./Pl*	*Nodule Biomass (mg/Pl)*	*Root Growth (cm/Pl)*	*Shoot Growth (cm/Pl)*	*Percent of VAM infection*
Control	710	2.06	2	4	6.6	13.9	15
N-control	960	4.22	5	13	9.8	24.6	40
P-control	840	3.27	4	11	7.8	22.1	45
Rhizobium	920	3.78	13	45	8.9	23.1	35
VAM-1	860	3.54	9	29	8.4	21.7	80
VAM-2	830	3.22	8	21	7.9	19.7	70
VAM-3	845	3.11	7	24	7.9	16.5	60
R+VAM-1	995	4.55	18	76	10.6	27.4	90
R+VAM-2	940	4.66	13	54	9.4	25.5	80
R+VAM-3	900	4.21	10	46	9.8	23.7	78
VAM-1 = *Glomus fasciculatum*; VAM-2 *Glomus mosseae*; VAM-3 *Gigaspora calospora*							
CD (P=0.05)	45	0.12	0.6	1.4	1.8	0.99	NS

Table 4
Influence of endomycorrhizae and *Rhizobium* on growth and establishment of *Acacia conciaana.*

Treatment	*20 DAS (Initial)*			*40 DAS (day after sowing)*			
	Vigour Index	*Plant Biomass (g/Pl)*	*Nodule No./Pl*	*Nodule Biomass (mg/Pl)*	*Root Growth (cm/Pl)*	*Shoot Growth (cm/Pl)*	*Percent of VAM infection*
Control	580	1.80	1.3	3	7.3	16.7	15
N-control	765	3.23	4	11	16.4	29.3	35
P-control	660	2.33	3	7	13.2	22.7	50
Rhizobium	730	3.70	12	32	14.6	26.9	40
VAM-1	655	2.99	10	24	16.4	24.5	80
VAM-2	610	2.66	9	21	15.2	21.1	75
VAM-3	650	2.61	8	19	14.7	23.4	75
R+VAM-1	810	3.99	15	69	18.1	32.0	90
R+VAM-2	796	3.76	11	32	16.3	29.1	80
R+VAM-3	770	3.22	10	31	15.2	26.7	75
VAM-1 = *Glomus fasciculatum*; VAM-2 *Glomus mosseae*; VAM-3 *Gigaspora calospora*							
CD (P=0.05)		0.6	0.2	0.8	1.7	2.1	15

Table 5
Influence of endomycorrhizae and *Rhizobium* on growth and establishment of *Acacia hispida.*

Treatment	*20 DAS (Initial)*			*40 DAS (day after sowing)*			
	Vigour Index	*Plant Biomass (g/Pl)*	*Nodule No./Pl*	*Nodule Biomass (mg/Pl)*	*Root Growth (cm/Pl)*	*Shoot Growth (cm/Pl)*	*Percent of VAM infection*
Control	560	0.99	1	3	5.2	14.2	10
N-control	740	2.24	3	11	10.5	19.3	40
P-control	660	1.75	2	9	9.9	16.9	50
Rhizobium	730	1.94	11	37	10.1	18.5	45
VAM-1	690	1.99	9	29	11.2	15.2	80
VAM-2	670	1.54	8	25	10.3	16.2	80
VAM-3	650	1.77	7	22	9.8	16.1	75
R+VAM-1	795	2.38	14	44	13.9	23.5	90
R+VAM-2	740	2.10	12	34	12.1	21.3	80
R+VAM-3	720	2.16	10	31	12.4	19.7	75
VAM-1 = *Glomus fasciculatum*; VAM-2 *Glomus mosseae*; VAM-3 *Gigaspora calospora*							
CD (P=0.05)	45	0.4	0.1	1.2	1.6	2.4	20

Natarajan *et al.* (1995) indicated effective growth of *Acacia nilotica* by the *in vitro* inoculation of *mycorrhizae*. Thomas *et al.* (1985) indicated the possible increase in root growth by phosphate mobilizing fungi. The role of VAM on uptake of micronutrient with special reference to Zn was reported by Burgres and Robson (1994) in clover and McGarti and Robson (1984) in *Pinus*.

Dual inoculation of favouring the biomass production and yield aspect on various crop along with nutritive mobility were also documented earlier (Rai, 1981; Subbarao *et al.*, 1985; Adhyloya and Joshi, 1986; Azim *et al.*, 1984; Manjunath and Bagyraj, 1987, Gunasekaran and Natarajan, 1991; Leopard and Hopman 1991 and Saravana and Sundaram, 1991). Among the *Acacias* tested the effective symbiosis was observed with *A. holoserecia* and in *A. crassicarpa* under alfisol and able to put forth better wood lot as compared to other *Acacias*.

The possible influence on the enhancement of symbiosis and plant establishment in alfisol might be due to the following reasons:

1. Inherent capacity of nitrogen fixation of *Rhizobium* and mobilisation of fixed P by VAM might have registered better effects over their individual inoculation.
2. The possible approach on arresting the invading soil borne pathogen by VAM proliferation can able to increase the germinability and vigour of the crop, and
3. Under triangular symbiosis plant obtains N and P from these m endosymbionts, whereas *Rhizobium* provides N for VAM and plant it obtain energy from plant and P from VAM. In turn VAM mobilise fixed P (iron and Alumina P) to polyphosphate form and able to supply for host species and able to get energy from it.

Summary

An intensive study was conducted I in and N and P depleted alfisol towards the response of certain tree legumes (*Acacia mangium, A. crassicarpa, A. concianna, A. holoserecia* and *A. hispida*) to the seed bacterisation of *Rhizobium* (600 g for 20 kg of seed) and in combination with three elite VAM (*Glomus fasciculatum, Glomus mosseae* and *Gigaspora calospora*) at 4 g/kg of seed. The results indicated that all the five tree legumes responded to bioinoculants by enhancing biogrowth, root nodulation and root and shoot growth over uninoculated control. However the effect was found to be maximum with co-inoculation of *rhizobia* in combination with *Glomus fasciculatum* followed by the combination of nitrogen fixer with either of *Glomus mosseae* and *Gigaspora* in alfisol.

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Authors

J.Prabakaran S. Mariappan and K. Srinivasan
Department of Environmental Sciences
Tamil Nadu Agricultural University
Coimbatore–641 003, Tamil Nadu, India

14

Infectivity and Efficacy of *Glomus aggregatum* and Growth Response of *Cajanus cajan* (L.) Millsp. CVPT-221 in Cement Dust Polluted Soils

Introduction

Cement dust is a common air and soil pollutant around cement factories and construction sites. It is reported that the particular matter (dust) from kiln exhaust falling on the leaves affect the plant growth by clogging stomata due to formation of crust on leaves, cause foliar injuries, bring changes in photosynthesis and transpiration, turn stigma surfaces to alkalinity and there by reducing the pollen germination and primary, fertilization, all leading to reduce agricultural production (Darley, 1966; Parthasarathi, *et al.*, 1975; Gunamani, *et al.*, 1989 and Swamianthan *et al.*, 1989). As a soil pollutant it is known to decrease water holding capacity of soils and affect the uptake of minerals from soils (Darely, 1966; Parthasarathi *et al.*, 1975). Emanuelssan (1994) has observed, that cement kiln dust, in mild doses inducing lateral root formation at an early stage and reduction of shoot length, number of branches, leaves, inflorescence and fruits. Arul *et al.* (1990) have observed poor rhizobial nodulation in crop legumes in kiln exhaust dust polluted soil.

Gulbarga District alone has 4 major and 5 mini cement industries. The impact of cement dust pollution, and of effluents from these cement factories, mining and quarrying activities for the last two decades in the district has lead to land dereliction (due to pollution and erosion) and considerable damage to vegetation (Naik, *et al.*, 1994).

Vesicular arbuscular mycorrhizae (VAM) formation are now considered to be very important in establishment and growth of plants, particularly in an inhospitable sites (Hall, 1980). Literature survey indicated that the effect of cement pollution on VA mycorrhizal association is very meagre (Arul and Vivekanandan, 1994) and the preliminary survey on the occurrence of VA muycotrophy in plants growing around cement factories has revealed that, the plants from cement polluted soils are more intensely arbusculous than plants from non-polluted areas

(Eshwarlal Sedamkar and Reddy, 1995). Hence an experiment with pigeon pea (*Cajanus cajan*), a major crop of this district, to study the impact of cement on the infectivity and efficacy of a VAM fungus.

Material and Methods

Growth Response of *Cajanus Cajan* to *Glomus Aggregatum* with Cement dust Amendments

A pot experiment was set-up by using a local soil (loamy sand with low fertility). Twelve earthenware pots each with 5kg of autoclaved soil were taken. Three of the pots were treated as control without adding cement dust and in the other pots cement dust was added at the rate of 1g/kg soil (treatment-1), 2g/kg soil (treatment-2) and 4g/kg soil (treatment-3) each in three replicates. All the twelve pots were added with 50g of pure *Glomus aggregatum* inoculum (having the infective propagules level approximately 0.2×10^4 propagules/g soil) as an uniform layer (Jackson, *et al.*, 1972). Ten surface sterilised seeds of *Cajanus cajan* (cv PT 221) were sown in each pot by pushing down to 1 cm depth. All the pots were regularly watered and maintained in green house beds.

After 75 days of growth, the plants were harvested and assessed for percent mycorrizhal association and for the determination of dry weight.

Assessment of per cent mycorrhizal Association:

The percent mycorrhizal association was done by using the simplified slide technique of Giovannetti and Mosse (1980). 40-50 root segments (1 cm) were selected for each treatment and stained by following the root cleaning and staining technique (Phillips and Hayman, 1970) at random and the root segments were mounted on slides at the rate of 10 segments per slide and observed under the microscope to record the absence or presence and to count VAM structures (vesicles and arbuscules) in each root segment and expressed the results as percent VAM association/colonisation (Read *et al.*, 1976).

$$\% \text{ VAM colonisation} = \frac{\text{Number of root bits positive for colonization}}{\text{Total number of root bits observed}} \times 100$$

Estimation of Dry Weight:

The plants after 75 days of the growth from all the sets (control and cement dust treated) were uprooted taking care not to damage the roots. The roots were washed in running water and dipped in water several times till the adhering soil particles were completely removed, then the roots and shoots were separated and oven dried at 70°C for 72 hours. The dry weights of root and shoot were separately recorded.

Results

Infectivity of *G. aggregatum* and its efficacy as judged by the growth response of pigeon pea in 3 different concentrations of cement dust amendment in soils is given in Tables 1 and 2 and Fig. 1.

Table 1
Effect of cement dust amendment in soils infested with *Glomus aggregatum* on the % VAM association and associated fungal structures in *Cajanas cajan* (L.) Millsp. (cv. PT-221) plants at the age level of 75 days.

*Treatment**	*pH*	*% VAM association*	*% Vesicles*	*% Arbuscules*
Glomus aggragatum (control)	8.5	65	65	65
Glomus aggragatum + 1g cement/kg soil	8.7	80	45	80
Glomus aggragatum + 2g cement/kg soil	9.2	90	45	90
Glomus aggragatum + 4g cement/kg soil	9.5	90	35	85

*F = 10.6 (P<0.01).

Infectivity

It is clearly evident from the present investigation that the % VAM association in .pigeon pea roots increased with the increase of cement content in the soil (Table 1). The VAMF structures, both arbuscules and vesicles were also found to vary quantitatively. The formation of vesicles inside the host root in cement treated soils was found gradually declined depending upon the concentration as compared to control. On the contrary the intense arbuscule formation was observed inside the roots of plants growing in cement dust polluted soils and this arbusculous nature was found to be very dense as the concentration of cement dust increased. The pH of the soil was also found increased due to the addition of cement in soil.

Efficacy

The effectiveness of *G. aggregatum* under the influence of cement dust in a sterilised 'p' deficient soil when assessed by studying the growth response of pigeon pea after 75 days growth showed that the cement dust amendment in soils could affect the growth of the plants and *vis-à-vis* the effectiveness of mycobiont (Table 2 and Fig. 1). There is a significant (at 5% level) decrease in shoot length and dry weight of both root and shoot systems due to cement addition in soil, and it was found to be in relation with the cement content in soil.

Similarly, the proliferation of roots system in cement dust treated plants was also high as compared to control (Fig. 1). However, the dry weight of the root system of cement treated plants decreased when compared to control plants.

Discussion

The results obtained on the effect of cement dust amendment in soils in the infectivity and effectiveness of VAM fungus (*G. aggregatum*) clearly indicated that the cement dust pollution

enhanced the degree of VAM fungal colonisation in pigeon pea as compared to control (Table 1 and Fig. 1). It has been reported by Arul, *et al.,* (1994) that the kiln exhaust induced alkaline soil did not affect the existence of mycorrhizal spores and their colonisation of a few popularly grown legumes in Tamil Nadu. Phosphorus deficiency in an alkaline pH and high levels of Ca associated (calcarious) soils may practically explain the higher levels of VAM colonisation as reported by Lesica and Antibus (1986). However, the associated structures, both arbuscules and vesicles inside the root cortex differed quantitatively. The intensity of formation of arbuscules increased significantly (significant at 1% level) with the addition of cement dust and equally there is a precipitous decline in the formation of vesicles (Table 1). This indicates that there is a tilt in the degree of dependency and it is an indicative of change in the degree of benefit that plant species receives when grown at various soil conditions.

The growth stimulation in plant due to VAM fungal association is well documented (Gilmore, 1971 and Bagyaraj and Manjunath, 1980) and the growth promoting ability of *G. aggregatum* in pigeon pea is known (Rajendra Singh, 1993). Cement dust pollution in soils was found to affect the growth of pigeon pea and there is a significant (significant at 5% level) decrease in shoot length and dry weight of both shoot and root systems (Table 1 and Fig. 1). Similarly effect on rhizobial nodulation was observed dry Arul *et al.* (1990) in crop legumes in cement kiln exhaust dust polluted soil.

Table 2
Effect of cement dust amendment in soils infested with *Glomus aggregatum* on the growth of *Cajanas cajan* (L.) Millsp. (cv. PT-221) plants at the age level of 75 days.

*Treatment***	*Shoot length (cm*)*	*Dry weight**	
		Root	*Shoot*
Glomus aggragatum (Control)	31.31 ± 6.63	4.66 ± 0.16	2.30 ± 0.57
Glomus aggragatum + 1g cement/kg soil	26.44 ± 2.83	4.62 ± 0.19	2.51 ± 0.71
Glomus aggragatum + 2g cement/kg soil	24.42 ± 1.87	3.20 ± 0.96	1.70 ± 0.57
Glomus aggragatum + 4g cement/kg soil	21.00 ± 2.03	2.96 ± 0.98	1.32 ± 0.43

**Average value of 3 replicate with 10 plants in each replicate
* $P<0.05$.

Host plant growing normally in soils with relatively low nutrient status are predisposed to effective endophyte fungal invasion (Russel, 1973). In the present study similar situation was observed in the loamy sand soil with low fertility where extensive root proliferation and endophyte invasion is evident (Fig. 1). Similar observation of intensive VAM development in

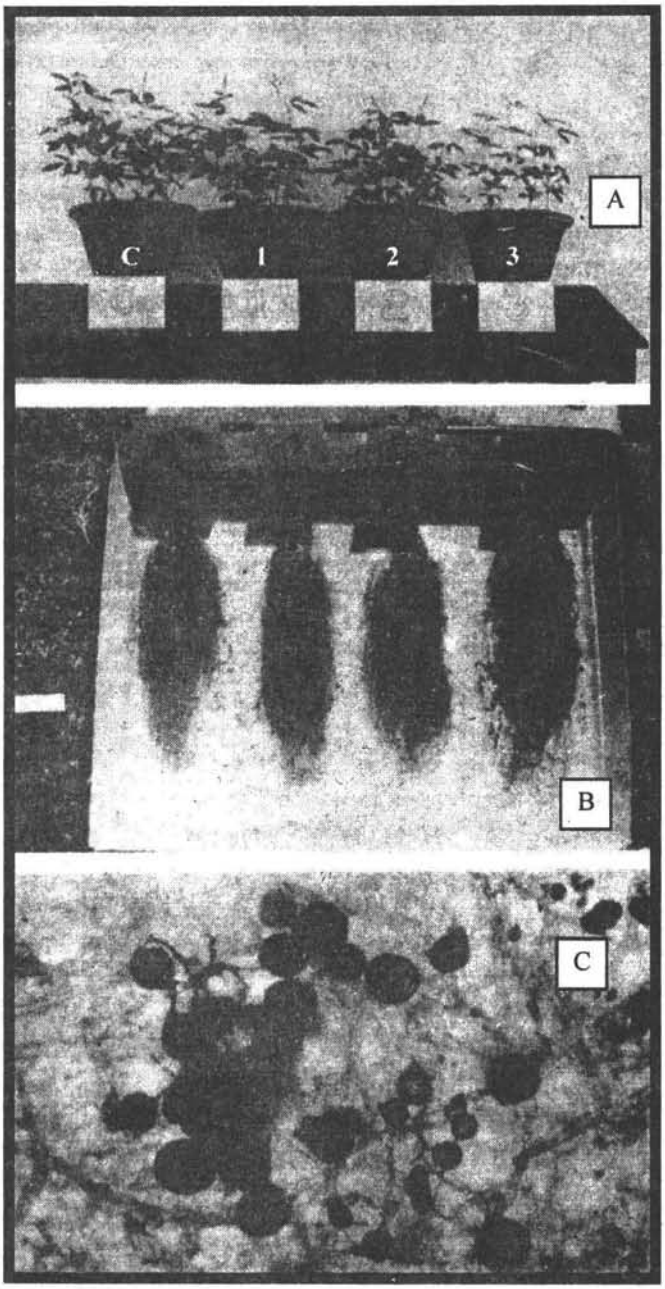

Fig. 1. (A) Effect of cement dust amended soils infested with *Glomus aggregatum* on the growth of pigeon pea *(Cajanus cajan)* (L.) Millsp. cv. PT-221. (B) High proliferation of root system of *C. cajan* due to cement dust amendment in soil; *C. G. aggregatum* (control); 1. *G. aggregatum* +1g cement/kg soil 2. *G. aggregatum* +2g/kg soil 3. *G. aggregatum* +4g cement/kg soil (C). Chlamydospore of *Glomus aggregatum* (×20).

Cassia occidentalis, C. sericea plants was observed in loamy sand soil by Narayana Reddy and Ramchander Goud (1989). The importance of soil type and the pollutants like heavy metals or sewage sludge in affecting VAM formation and function was discussed earlier by Sreeramulu and Bagyaraj (1986) and Heckman (1986) and Dakessian *et al.* (1986).

Summary

Infectivity and efficacy of *G. aggregatum* as judged by the growth response of pigeon pea (*Cajanas cajan*) cv. PT-221 in three different concentrations of cement dust amended soils was studied by conducting a pot culture experiment. It is clearly evident from the present investigation that the % VAM association in pigeonpea roots increased with the increase of cement content in the soil. The VAMF structures, both arbuscules and vesicles were also found to vary quantitatively. The formation of vesicles inside the host root in cement treated soils was found gradually declined depending upon the concentration as compared to control. The effectiveness of *G. aggregatum* under the influence of cement dust in sterlised, low fertile soils when assessed by studying the growth response of pigeon pea after 75 days growth showed that the cement dust amendment in soils could affect the growth of the plants and *vis-à-vis* the effectiveness of mycobiont. There is a significant decrease in shoot length and dry weight of both root and shoot systems due to cement addition in soil and it was found to be in relation with the cement content in soil.

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Authors

Eshwarlal Sedamkar and C. Narayana Reddy
Department of Botany
Plant Pathology and Mycorrhiza Laboratory
Gulbarga University
Gulbarga–585 106, Karnataka, India

15

Saline Soil Tolerance of *Sapindus emarginatus* (Vahl) Seedlings with Established *Glomus fasciculatum* Infection

Introduction

Saline, saline-alkali soils commonly referred as salt affected soils are quite extensive in India and occupy over 12 million hectares (Archana, 1987). Salt affected soils contains various cations and anions which interact with Na^+ and Cl^- and influence the effect on plant response (Carter, 1981; Singh, 1994). The plant species mostly crop plants differ in their response to salinity level. Salt affected soils are widely distributed in India and occupy an area of 7 million hectares. In Andhra Pradesh it comprises an area of 2.40 lakh hectares.

Endomycorrhizae (*Vesicular Arbuscular Mycorrhizae*) from an intimate association with plant roots. Majority of plant species depend upon *mycorrhizae* associates for adequate nutrient uptake, those lacking *mycorrhizae* can be severely stunted with low growth (Chen, 1985). In addition to greatly enhanced uptake of nutrients they confer other benefits to their host. They are known to increase drought resistance of young seedlings (Bowen, 1980) detoxify certain soil toxin, enable seedlings to with stand high soil temperature (Marx, 1980) extreme acidity and alkalinity (Mark, 1975). Thus these fungi play and important role in difficult soils and has a potential application in afforestation and reforestation programmes.

Sapindus emarginatus is an economically important tropical deciduous species, usually confined to dry deciduous forests. A moderate hand some tree, it is frequently cultivated for ornamental purpose or for the sake of its fruits, the pulp of which is used as a substitute for soap and also have medicinal value. The fruit is emetic, tonic, astrigent and anthelmintic. It is used in the treatment of asthma, colic due to indigestion, diarrhoea, cholera and paralysis of the limbs and lumbago. Fruit powder is taken with honey in the treatment of tonsils. Roots and bark are employed as mild expectorant and demulcent.

The knowledge on response of *Sapindus emarginatus* to salinity is scanty. In the present study efforts had been made to study the response of *Sapindus emarginatus* to various levels of saline alkaline soils in a pot experiment at nursery level after establishing infection with *Glomus fasciculatum*.

Material and Methods

Fruits of *Sapindus emarginatus* were obtained from the Forest Department of Tirupati Division, India. The seeds were separated carefully without affecting the seed coat. The seeds were surface sterilised by 0.1% H_2O_2 for 15 minutes, rinsed with tap water followed by sterile distilled water. Half strength Murashige and Skoog medium was prepared as described by Narayanaswamy (1994). Single surface sterilised seed was placed in each slant containing MS medium under aseptic condition. The tubes were placed in seed incubator for germination.

Culture of *Glomus fasciculatum* (Thaxt) Gerd and Trappe (obtained from ICFRI, Dehra Dun, India) was established in 4-L pots on Sorghum (Co_{26}) in sand, soil, red earth (1:1) rooting medium for several 21 days cycle to increase the *mycorrhizal* inoculum potential of the medium. The spores of *Glomus fasciculatum* were isolated from the rooting medium by sieving and decanting technique described by Gerdman and Nicolson (1963). Each individual spore is carefully picked up by observing under dissecting microscope placed in an inoculation chamber under aseptic conditions to the emerged radicle of *Sapindus emerginatus* on MS medium. The tubes were incubated for establishing infection with *Glomus fasciculatum* to the young roots for one month.

Saline alkaline lands were selected from three areas *viz.*, Akasaganga, Jeevakona and Thumburavanam in Rayalseema region of Andhra Pradesh. Soil samples from different horizons *i.e.*, 0 cm., 30 cm., 60 cm., and 90 cm., were collected from each area separately. The soil from different horizons was mixed individually, dried, processed and passed through 2 mm sieve for further analysis. Analysis of soil samples was done for particle size distribution (Black, 1965), $CaCo_3$. (Piper, 1952), cation exchange capacity, organic matter, exchangeable cations and composition of saturation extract (Richard, 1954). The soil from each area was autoclaved at 121°C for 1h for three consecutive days and filled earthen pots of 25 cm and 13 cm depth. Thirty earthen pots were used for experiment and 10 were used as control with fertile autoclaved soil. The seedlings grown on MS medium were uprooted and washed with sterile distilled water to remove the roots. Two seedlings were transplanted to each pot filled separately with the saline-alkaline soil collected from the different areas and also to the fertile soil collected from agricultural field. All the forty pots were placed in green house for four months and watered with 700 ml sterilised tap water on every alternate day. The percentage of survival in control and experimental pots was recorded.

The seedlings were carefully uprooted to avoid the possible loss of root system and were thoroughly washed first with tap water than in 0.1 N HCl followed by distilled water. The root system of four plants per each type of soil was separated and stored in preserving and fixing solution of 37% formaldehyde solution, glacial acetic acid and 95% ethanol with a 0.05/0.05/

0.9/v/v/v ratio until *mycorrhizal* colorization could be assessed. Spore density in the pots with three different saline alkaline soils were determined by the procedure described by Gerdman and Nicolson (1963). For VA *mycorrhizal* fungal colorisation assessment. The designated root samples were cleared and stained for fungal structures as described by Brundrett *et al.* (1984) and stored in glycerine. The VA *mycorrhizal* fungal assessment method is described by McGonigle *et al.* (1990). Approximately 100 intersects of each species were assessed for the population of the root colorized. The shoot height and root height of root height of the seedlings were measured. The leaves were separated and leaf area was measured using Skye leaf area meter. The fresh weight of the seedlings were determined. The plant material was dried at 80°C and dry matter yield was recorded. The data obtained was subjected to ANOVA.

Results and Discussion

The saline alkaline soils collected from three different areas were characterised by high pH 8.9 to 9.6, ESP 32-46, ECE 6.1-11.6 dSm-1, water soluble salts are CO_3^{-2}, 43.8-62.5 and HCO_3^- 43.8-62.5 Cl^- 9.11—14.93 and SO_4^{-2} 8.14-10.63 (Table 1). The amount of nitrogen and organic carbon was found significantly more ($P<0.05$) in the soil collected from Jeevakona. The amount of sodium was significantly high ($P<0.05$) in the soil collected form Akashaganga and Thumburavanam than the soil from Jeevakona. The other cations Ca^{+2}, Mg^{+2}, K^+ were also found more in the soils collected from other than Jeevakona area.

Maximum percentage of survival (94%) of *Sapindus emerginatus* seedlings were found in fertile soil (pH 6.8) followed by soil collected from Jeevakona area (pH 8.9). However, the percentage of germination between these two soils is not significant at 5% level. The minimum percentage of germination was found in the soil from Akashaganga (pH 9.6) Seventy-six per cent of germination was found in the soil of Thumubravanam area (pH 9.2). there was no significant difference of shoot height and root height between the fertile soil and soil collected from Jeevakona. Whereas the shoot and root height was found to be inhibited significantly ($P<0.05$) in the two soils other than Jeevakona. Maximum leaf area (193 cm) was found in the seedlings grown on fertile soil, followed by soil from Jeevakona, Thumburavanam and Akashaganga. The fresh and dry matter yield of the seedlings were found significantly lower ($P<0.05$) in the soils from Akashaganga and Thumburavanum. Similarly there was no significant difference in percentage of VAM infection and number of spores between soil from Jeevakona and fertile soil. The percentage of VAM infection and occurrence of number of spores inhibited in the soils of Thumburavanam and Akashaganga. The difference was significant at 5% level. A positive relationship was observed between the percentage infection, fresh and dry matter yield of the seedlings.

In the present experimental conditions VAM treatment to the *Sapindus emarginatus* could able to increase the tolerance of the seedlings to pH 8.9 and high slat concentration. Establishment of VAM infection to the seedlings is therefore essential to undertake national development programmes of afforestation in saline alkaline soils to maintain ecological balance and environmental stability.

Table 1
Composition of saturation extract, pH, $CaCO_3$ of saline alkaline soils collected from different areas

Location	*Ece dSm* ^{1}	*Cations (me/L)*				*Anions (Me/L)*				*pH*	*ESP*	*CaCo₃ %*
		Ca^{2+}	Mg^{2-}	Na^{+}	K^{-}	CO_3^{2-}	HCO_3^{-}	C^{l-}	SO_4^{2-}			
Akasaganaga	11.6	0.19	0.09	88.0	0.06	81.36	62.5	14.93	10.63	9.6	46.0	4.5
Jeevakona	6.1	0.11	0.05	69.0	0.02	59.40	43.8	9.11	8.14	8.9	32.0	3.6
Thumbura vanam	8.4	0.14	0.07	73.0	0.04	67.89	59.0	11.63	9.76	9.2	40.0	8.0

Table 2
Physical and chemical properties of saline alkaline soils collected from three different areas

Location	*Particle size distribution*			*Organic matter*	*Total nitrogen*	*Exchangeable cations*			
	Sand %	*Silt %*	*Clay %*	*%*	*%*	Ca^{2+}	Mg^{2+} *(mol (P+)).*	Na^{+} kg^{-1}	K^{+}
Akasaganaga	63.81	22.24	13.95	0.31	0.028	6.92	4.6	14.37	0.92
Jeevakona	58.90	24.98	16.12	0.46	0.048	4.68	2.4	10.63	0.58
Thumbura vanam	60.13	21.63	18.24	0.26	0.032	5.79	3.2	13.60	0.89

Table 3
Percentage of survival, shoot height, leaf area, fresh and dry matter yield of *Sapindus emarginatus* seedlings.

Area of soil Collection	*% of survival*	*Shoot height (cm)*	*Root height (cm)*	*Leaf area (cm)*	*Fresh matter yield(g)*	*Dry matter VAM(g)*	*% of VAM infection*	*No. of spores g^{-1} soil*
Akasaganaga	58	11.6	5.0	116	9.2	4.3	52	24
Jeevakona	91	19.4	7.6	184	14.3	8.0	76	38
Thumbura vanam	76	14.8	5.9	138	10.6	5.2	59	29
Fertile soil	94	19.8	7.9	193	15.1	7.9	78	41

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Authors

T.Vijaya* and B.V. Prasada Reddy
Biotechnology Cenre for Tree Improvement
Tirupati-517 507, Andhra Pradesh
**Presently at Department of Biotechnology*
S.V. University, Tirupati-517 502, Andhra Pradesh

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Importance of Vesicular Arbuscular Mycorrhizae in Transplanted Crops

Introduction

Vesicular arbuscular mycorrhizal (VAM) fungi are known to improve growth and yield of many crop plants mainly through phosphorus nutrition. However application of this technology in commercial crop production is minimal as VAM fungi are obligate symbionts and are not cultured under laboratory conditions (Jeffries, 1987). The obligate symbiotic nature of VAM fungi presently dictates that all VAM inoculum be grown on roots of an appropriate host plant (Sreenivasa and Bagyaraj, 1988). The best way to utilise VAM fungi for crop production may be to concentrate on transplanted crops like chilli and tomato which are normally raised in nursery beds. They can be easily inoculated to nursery beds at the time of sowing and precolonised seedlings can be transplanted to main field to harness the benefits of mycorrhization. Some researchers have observed wide variations among and within different species of VAM fungi in their ability to promote plant growth (Rao *et al.*, 1983). Recently some scientists have noticed host preference for VAM endophytes (Hetrick, 1984). Looking into these findings it seems advantageous to select VAM fungus suitable for a particular host-soil-climate combination (Powell, 1982). Hence the present investigations were carried out to select an efficient VAM fungus each for chilli and tomato, to determine their optimum dares of inoculation and to assess their performance on plant growth, and yield at different P levels in versitol.

Material and Methods

Glomus fasciculatum G. macrocarpum, Gigaspora margarita, Acaulospora laevis, and *Sclerocystic dussii* were tried for the selection of efficient VAM fungi for chilli var. Byadagi and tomato var L-15 based on their inoculum potential. Earthen pots of 30 cm diameter were filled with unsterile vertisol which was P-deficient (28 kg P_2O_5 per hectare). VAM inocula were inoculated to pots with uniform number of infective propagules. Comparable uninoculated control pots were maintained. Fertilizer PNK were given at the recommended dose (Chilli 150:

75: 75 and Tomato 115: 100: 60 kg NPK/ha). Bold healthy seeds of chilli var. Byadagi or tomato var L-15 were sown to pots. All agronomic practices were followed. After crops harvest, shoot dry weight and weight of fruits were recorded. Percentage mycorrhizal root colonisation (Phillips and Hayman, 1970) and mycoirrhizal spore counts (Gerdemann and Nicolson, 1963) were estimated. Shoot P concentration was determined (Jackson, 1967) by vanadomolybdate phosphoric yellow method.

After analysing the results of this pot trial, nursery beds of chilli and tomato were raised. The efficient VAM inoculum for each crop was tried at different levels (0, 0.5, 1.0, 1.5, 2.0 and 2.5 kg per bed) to know the optimum does of inoculum. The size of each nursery bed was one square meter. Comparable uninoculated control beds were maintained. Precolonised seedlings were transplanted to microplots of size 5.4 × 5.4 square meters for chilli and 4.5 × 3.75 square meters for tomato. Fertilizer NPK were given at recommended level (as mentioned above) for each crop. The plant growth, yield and mycorrhizal parameters were estimated by following standard procedures (as mentioned above).

After determining the optimum level of efficient inoculum for each crop, another field trial with varied levels of P (0, 20, 40, 60, 80 and 100% of recommended dose for each crop) was conducted to workout the P-savings with inoculation of efficient VAM fungi. All agronomic practices and parameters determined were similar as in the previous trial.

Results and Discussion

Chilli and tomato responded well to the inoculation of VAM fungi. Among different VAM fungi tried, *Glomus macrocarpum* and *G. fasciculatum* caused significantly maximum root colonisation, spore number, shoot dry weight, shoot P concentration and fruit yield in chilli and tomato respectively (Tables 1 and 2) as compared to other VAM fungi. Inoculation of respective efficient VAM fungi to the nursery beds resulted in precolonised seedlings. Percentage root colonisation and spore numbers increased with increase in inoculum levels in both the crops. Both these parameters did not differ significantly between 2.0 and 2.5 kg (Tables 3 and 4). A matching trend was observed in plant dry weight fruit yield and plant pconcentration also (Tables 3 and 4) in both the crops. Thus inoculation of 2.0 kg of respective efficient VAM inoculum was found be optimum.

In the last trial, percentage root colonisation and spore counts were found to increase with increase in P-level up to 80 per cent of recommended dose beyond which these parameters decreased in both the crops (Tables 5 and 6). The plant dry weight, plant P concentration and fruit yield increased with increase in P level. However these parameters did not differ significantly between 80 and 100 per cent of recommended P in both the crops (Tables 5 and 6).

Glomus macrocarpum and *G. fasciculatum* exhibited host preference in chilli and tomato respectively perhaps because of optimum inflow rates of P. Two kilogrammes of respective efficient VAM inoculum seems to contain optimum number of infective propagules to cause maximum plant growth and yield in both chilli and tomato. Thus it was found to be and economical and optimum dose. Inhibition of percentage root colonisation and spore counts at higher P level is well documented (Sreenivasa *et al.*, 1993 and Sreenivasa, 1994). Thus may be

Table 1
Effect of different VAM fungi on *mycorrhizal* and Plant parameters in Chilli var. Byadagi.

VAM Fungi	*Per cent root colonization*	*Sporecount per 50g soil*	*Shoot P conc. (%)*	*Shoot dry weight (g/plant)*	*Weight of green fruits (g/pot)*
Glomus fasciculatum	88	446	0.19	19	106
Gigasora margarita	77	396	0.16	15	89
Acaulospora lacvis	70	361	0.14	11	73
Sclerocystic dussii	71	369	0.14	11	76
Glomus macrocarpum	90	478	0.24	22	135
Uninoculated Control	41	98	0.06	6	48
CD at P: 0.05	4.5	101.8	0.02	1.06	4.89

Table 2
Effect of different VAM fungi on *mycorrhizal* and Plant parameters in Tomato var. L-15.

VAM Fungi	*Per cent root colonization*	*Sporecount per 50g soil*	*Shoot P conc. (%)*	*Shoot dry weight (g/plant)*	*Weight of green fruits (g/pot)*
Glomus fasciculatum	93	476	0.35	23	1.10
Gigasora margarita	29	455	0.27	20	0.98
Acaulospora lacvis	86	439	0.24	19	0.98
Sclerocystic dussii	82	438	0.22	19	0.85
Glomus macrocarpum	90	470	0.33	22	1.00
Uninoculated Control	38	86	0.17	15	0.60
CD at P: 0.05	4.1	9.8	0.02	3.0	0.26

Table 3
Effect of different levels of efficient VAM fungus, *Glomus macrocarpum on mycorrhizal* and plant parameters under field conditions in chilli var Byadagi.

Inocolum level (kg/bed)	*Per cent Colonization*	*Spore count 50g Soil*	*Plant P conc. (%)*	*Plant dry biomass (g/plant)*	*Fruit (g/plant)*	*Yield (Q/ha)*
0	26	70	0.18	49	132	20.2
0.5	47	103	0.23	63	137	20.5
1.0	61	141	0.30	72	151	21.5
1.5	71	183	0.40	80	166	24.0
2.0	79	202	0.46	87	182	26.2
2.5	79	202	0.47	89	185	26.6
SE m(±)	1.13	2.19	0.01	0.97	2.03	0.43
CD at P: 0.05	3.42	6.61	0.02	2.94	6.10	1.31

Note: Size of Nursery bed 1m × 1m

Table 4
Effect of different levels of efficient VAM fungus, *G. fascilulatum on mycorrhizal* and plant parameters in Tomato var L-15 under field conditions.

Inocolum level (kg/bed)	*Per cent Colonization*	*Spore count 50g Soil*	*Plant P conc. (%)*	*Plant dry biomass (g/plant)*	*Fruit (g/plant)*	*Yield (t/ha)*
0	27	72	0.18	46	1950	40.1
0.5	49	125	0.26	54	1980	41.0
1.0	65	164	0.32	67	2096	43.4
1.5	73	195	0.38	75	2232	46.2
2.0	79	224	0.48	83	2318	48.06
2.5	79	225	0.48	83	2328	48.27
SE m(±)	1.36	2.09	0.01	0.96	13.6	0.74
CD at P: 0.05	4.10	6.30	0.012	2.90	48.9	2.22

Table 5
Performance of efficient VAM fungus, *G. macrocarpum* at different P levels in Chilli var. Byadagi under field conditions.

P-level (% Rec. dose)		*Per cent root colonization*	*Spore count 50g Soil*	*Plant P conc. (%)*	*Plant dry biomass (g/plant)*	*Fruit (g/plant)*	*Yield (Q/ha)*
Inoculated	0	49	70	0.09	32	95	15.5
	20	51	71	0.14	37	103	18.3
	40	65	92	0.22	62	151	26.5
	60	73	139	0.31	81	196	34.5
	80	82	189	0.40	93	254	44.2
	100	69	144	0.41	96	258	45.0
Unino-culated	100	44	54	0.36	91	230	39.0
SE m(±)		1.71	5.9	0.01	1.5	6.2	1.3
CD at P: 0.05		5.30	18.3	0.04	4.6	19.0	3.9

Table 6
Performance of efficient VAM fungus, *G. fasciculatum* at different P levels in tomato var L-15 under conditions

P-level (% Rec. dose)		*Per cent root colonization*	*Spore count 50g Soil*	*Plant P conc. (%)*	*Plant dry biomass (g/plant)*	*Fruit (g/plant)*	*Yield (t/ha)*
Inoculated	0	53	79	0.08	26.4	403	15.1
	20	59	112	0.15	38.5	619	24.6
	40	70	152	0.27	54.7	1091	34.3
	60	84	196	0.34	65.7	1790	49.9
	80	69	135	0.42	71.4	2395	56.7
	100	68	114	0.45	78.0	2513	58.2
Unino-culated	100	44	35	0.39	69.8	2334	53.7
SE m(±)		1.8	6.4	0.01	2.4	55.2	0.7
CD at P: 0.05		5.6	19.8	0.04	7.5	170.3	2.3

ascribed to decrease in membrane mediated root exudated (Ratnayake *et al.*, 1978). The benefits of mycorrhization in terms of plant growth and yield can be obtained at 80 per cent of recommended P in both the crops suggesting the possibility in P to an extent of 20 per cent of recommended dose.

Thus, these trials clearly revealed *Glomus macrocarpum* and *G. fasciculatum* to be efficient VAM fungi or chilli and tomato, two kg of respective efficient VAM inoculam to be optimum dose to harness the benefits of inoculation in terms of P savings to an extent of 20 per cent in both the crops.

Summary

The screening and selection of efficient vesicular arbuscular mycorrhizal (VAM) fungi was carried out for chilli and tomato in vertisol. Among various VAM fungi tried, *Glomus macrocarpum* and *G. fasciculatum* were found to be efficient for chilli and tomato respectively which promoted uptake of P, plant growth and yield. The optimum dose of VAM inoculum was found to be two kg per nursery bed of one square meter size. Upon inoculation of optimum dose of respective VAM inoculum to nursery beds and transplantation of precolonised seedlings to main field, a net P saving to an extent of 20 per cent of recommended doses was achieved.

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Authors

M.N. Sreenivasa
Deptt. of Agricultural Microbiology
University of Agricultural Sciences
Dharwad-580 005, Karnataka

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Biochemical and Genetic Characterisation of Mineral Phosphate Solubilizing *Enterobacter asburiae*

Introduction

Phosphate solubilizing microorganisms (PSMs) are considered to play a significant role in making available soil phosphates for the growth of plants (Subba Rao, 1982; Goldstein, 1986; Kucey *et al.*, 1989; Nareshkumar, 1996). PSMs solubilise mineral phosphates by bringing about a drop in the pH of the surrounding either by proton extrusion (Roos and Luckner, 1984) or by the secretion of mono, di and tricarboxylic acids (Kucey, *et al.*, 1989; Cunningham and Kuiack, 1992; Nahas, 1996).

We have earlier reported that two PSMs, *Citrobacter koseri* and *Bacillus coagulans*, could not solubilize mineral phosphates present in alkaline vertisol soil when carbon and nitrogen sources were provided (Gyaneshwar, *et al.*, 1998). They also did not solubilize rock phosphate when the medium was buffered. These PSMs secreted low levels of organic acids whereas 20-50 fold higher concentration of organic acids are necessary for solubilizing soil phosphates. This factor may therefore be responsible for the variations in the efficacy of PSMs in plant-PSMs *inoculation* experiments (Kucey, *et al.*, 1989).

Since alkaline soils have a high buffering capacity, we screened rhizobacteria which could grow and solubilize rock phosphate under buffered media conditions. We could isolate three isolates from pigeon pea (Cajanus cajan) rhizosphere (Gyaneshwar, *et al.*, 1996) which secrete approximately 50mM gluconic acid. These bacteria have been identified as *Enterobacter asburiae* and could solubilize soil phosphates when they were grown in the presence of glucose as C source and ammonia or nitrate as the N source.

It is known that *pyrroloquinoline quinone* (PQQ)-dependent glucose dehydrogenase (GDH) (EC 1.1.99.17) is responsible for gluconic acid secretion in *Erwinia herbicola, Pseudomonas cepacia* and *Acinetobacter calcoaceticus*. *A. calcoaceticus* has two GDH isozymes. GDH-A is

a monomer or 82 Kd and is localized in the plasma membrane whereas GDH-B is a homodimer of 50 Kd and is present on the outer side of periplasmic membrane. While both the enzymes can use glucose as the substrate, GDH-B can also act on disaccharides like maltose and lactose whereas deoxyglucose is a specific substrate of GDH-A (Cleton-Jansen, *et al.*, 1989). The GDH from *E. coli* (Cleton-Jeasen, *et al.*, 1990) and *Pseudomonas* is like GDH-A. GDH from *P. aeruginosa* is 20% active with galactose as substrate as compared to GDH from *P. cepacia* which is as active with galactose as with glucose (Lessie and Phibbs, 1984).

In this paper, we show that the activity of GDH of P-solubilizing *E. asburiae* is increased by about 5-fold under P starvation conditions and protein synthesis is required for this increase. The GDH is similar to the B type enzyme found in *A. calcoaceticus*.

Material and Methods

Culturing Procedures

The *E. asburiae* strains were cultured on media containing 100mM glucose, 25mM $MgSO_4$, 10mM NH_4Cl and the following micronutrients (mg/L) $FeSO_4$. $7H_2O$ (3.5), Zn SO_4 $7H_2O$ (0.16), $CuSO_4 5H_2O$ (0.08), H_3BO_3 (0.5), $CaCl_2 2H_2O$ (0.03) and $MnSO_4 4H_2O$ (0.4). Phosphate source was 0.1mM KH_2PO_4. The medium was buffered with 100mM Tris-Cl pH 8.0. These cells were used to inoculate to fresh medium as described below.

Induction of GDH and Alkaline Phosphatese (AP) of Soil Isolates

The isolates were inoculated in minimal medium containing 100mM glucose, 10mM NH_4Cl, 10mM K_2HPO_4 and the micronurients. The medium was buffered with 100mM Tris-HCl pH 8.0. The cells were harvested after growth to 0.4 O.D. and washed twice with normal saline. The cells were then resuspended in some medium with 100μM P. To monitor the effect of protein synthesis inhibitor on the induction of GDH, chloramphenicol was added at 170μg/ml. The cells were harvested at different time intervals, washed thrice with saline and resuspended in 50mM Tris-HCl pH 8.0 and were used for GDH and alkaline phosphatase assay. To study the levels of P concentration that was sufficient for GDH repression, different concentrations of K_2HPO_4 were added. The cells were harvested after 6 hours washed and used for GDH assay. Glucose was replaced with other carbon sources at 100mM concentration for dermining their effect on GDH. The cells were harvested by centrifugation in a table top centrifuge, washed twice with 0.9% saline and resuspended in the above described media either with 10mM P as P sufficient or with 10mM P as P deficient conditions.

Enzyme Assays

The assay mixture for GDH consisted of 1 ml of 50mM Tris-HCl buffer pH 8.75, 0.1 ml of 6.7 mM 2,6 Di-chlorophenolindophenol (DCIP), 0.1 ml 20mM phenazine methosulphate (PMS) and 0.1 ml of cells in a final volume of 3 ml. GDH activity was determined by measuring the decrease in absorbence at 600 nm of DCIP mediated with PMS using glucose to begin the reaction (Matsushita and Ameyama, 1982). For alkaline phosphatase assay 0.1 ml of the cells were added in the assay system consisting of 1 mg/ml P-Nitrophenylphosphate (pNPP) in 30mM Tris-HCl pH 9.0. The activity was determined by estimating P-Nitrophenol liberated from

pNPP (Smart *et al.*, 1984) and comparing it to absorbence of standard P-nitrophenol at 420 nm. Protein estimations were done by the dye binding method (Bradford, 1976).

Results

Induction of GDH and AP

The specific activity of GDH was found to increase upon P starvation. There was basal level of GDH activity which was increased to 5 fold in 6 hours. By P starvation in all the isolates (Fig. 1). The increase in GDH activity correlated with the decrease of media pH. GDH of these isolates was induced only upon P starvation and was independent of the carbon source used for the growth of the cells (Table 1). The increase in activity upon P starvation required *de novo* protein synthesis as addition of chloramphenicol abolished the induction of GDH (Table 2).

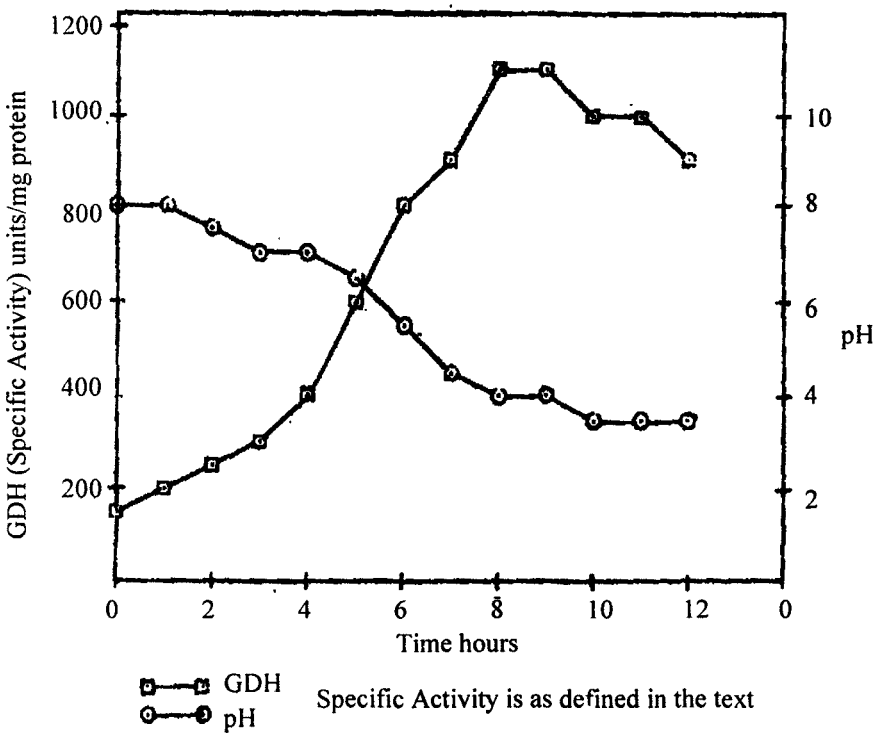

Fig. 1. Induction of GDH of soil isolate 3.

Substrate Specificity of GDH of Soil Isolates

In order to determine whether the GDH of these isolates was of GDH-A or GDH-B type the GDH activity was assayed with different substrates. The whole cells of all the three soil isolates could show activity with maltose and galactose as substrates but could not use deoxyglucose as a substrate. The activity with these substrates also increased upon P starvation similar to the increase in GDH (Table 3).

Table 1
GDH activity of soil isolates with various C sources.

C Source	*GDH activity (units/mg total protein)*	
	+P	*–P*
Glucose		
Isolate 1	262±4.1	1390±149
Isolate 2	265±7.5	1737±125
Isolate 3	285±8.5	1500±55
Glycerol		
Isolate 1	242±7.5	1303±100
Isolate 2	274±10	1600±60
Isolate 3	236±5.1	1418±125
Mannitol		
Isolate 1	225±6.0	1193±130
Isolate 2	250±6.5	1395±75
Isolate 3	240±5.0	1158±100
Gluconate		
Isolate 1	200±10.0	1000±100
Isolate 2	220±5.5	900±65
Isolate 3	250±5.0	1200±120

Values are expressed as mean ±S.D. of three independent experiments.

Table 2
Effect of Chloramphenicol on induction of GDH.

	GDH activity (Units/mg total protein)	
	Chl+	*Chl–*
Isolate 1	300±55	1000±100
Isolate 2	250±40	1200±125
Isolate 3	200±50	900±75

Values are expressed as mean ±S.D. of three independent experiments.

Table 3
Substrate specificity of GDH of High-Potency PS bacteria.

Substrate	*GDH activity (Units/mg total protein)*					
	Isolate 1		*Isolate 2*		*Isolate 3*	
	+P	*P*	*+P*	*–P*	*+P*	*–P*
Maltose	100	600	150	700	150	750
Galactose	50	200	50	150	60	250
Lactose	UD	UD	UD	UD	UD	UD

Values are expressed as mean of three independent experiments.

Discussion

The P-solubilizing *E. asburiae* isolates under study, secrete high concentration of gluconic acid and showed the induction of glucose dehydrgenase activity upon P starvation. The induction required *de novo* protein synthesis and different concentration of free P was required to repress the GDH activity with 5mM P repressing the activity to about 50% for isolates 1 and 2 and more than 75% for isolate 3. This indicates that the GDH of these isolates is regulated by the P concentration in the media and could be part of phosphate starvation inducible mechanism of these bacteria. That the soil isolates expressed genes induced for P starvation under conditions allowing maximum GDH activity is seen from the induction of alkaline phosphatase under the same conditions. It is known that various strains of *Rhizobium* are P limited at 100-150μm concentration of P as determined by the depression of AP activity (Smart *et al.*, 1984).

In *E. herbicola* the P solubilizing property was shown to be inducible by P starvation (Goldstein and Liu, 1987). 20mM P was required to completely abolish HAP solubilization by *E. herbicola.* The mineral phosphate solubilizing gene involved in PQQ biosynthesis cloned from *E. herbicola* in *E. coli* also showed P induction/repression phenotype indicating it to be part of phosphate starvation system. Similarly the DCP solubilization by *E. coli* is repressed by P concentration. However, the Ed pathway enzymes are not induced by P starvation in *E. coli.* In Pseudomonas GDH is induced by gluconate or 2-ketogluconate (Lessie and Phibbs, 1984). The GDH of the soil isolates reported here was induced only by P starvation as the activity was similar with all the C sources used for the growth of the bacterial strains.

The GDH of these isolates was active with maltose as substrate and could not use deoxyglucose indicating it to be like GDH-B of *A. calcoaceticus* which is a periplasmic protein. The whole cells also showed activity with galactose although it was substantially less as compared to glucose.

It would be of interest to characterise further the gluconic acid secretion by these *E. asburiae* strains and also clone the gene(s) involved in the overproduction of gluconic acid from these soil isolates. The cloned gene(s) could then be transferred to PGPRs like *Rhizobium* and *Pseudomonas* allowing them to provide P to the plants they colonize.

Summary

We have isolated *Enterobacter asburiae* strains capable of releasing Pi from alkaline vertisol and which can secrete 50mM gluconic acid. We show here that the phosphate solubilization is P-starvation inducible and correlates with an increase in the glucose dehydrogenase (GDH) activity of these strains. The GDH enzyme is similar to the B-type GDH of *Acinetobacter claocoaceticus*. GDH activity reaches a maximum value in 6 hours at 0.1mM P concentration. This is five-fold higher than that in the presence of 10 mM Pi and requires *de nova* protein synthesis.

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Authors

P. Gyaneshwar, G. Naresh Kumar and L.J. Parekh
Department of Biochemistry
Faculty of Science, M.S. University
Vadodara-390 002, Gujarat, India

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Effect of Phosphobacterium on Growth and Seed Yield of Swordbean (*Canavalis ensiformis* L.) Under Graded Levels of Phosphorus

Introduction

Swordbean, otherwise called Jackbean is a dual crop and contains a protein content of around 24.5 per cent. It can play a vital role in filling up the protein shortage. It is nontraditional pulse and relatively an unexplored legume with higher productivity and wider adaptability. The tender pods are delicious and become fibrous on maturity (Selvraj *et al.*, 1994; Sivasubramaniam, 1992). Seeds are large and contain an alkaloid canavalin. This crop can also be grown as an ornamental hedge or border plant or as a forage legume.

Material and Methods

The present study on swordbean, variety SBS-1 (Swordbean Selection-1), was designed to study the effect of phosphobacterium (*Bacillus megaterium*) under graded levels of phosphorus at the farm of Gandhigram Rural Institute (Deemed University), Tamil Nadu. during the year 1995-96. The experiment was carried out in randomised block design with three replications. There were three methods of phosphobacterium application *viz.*, seed treatment (500 g of inoculum for 120 kg of seed per hectare), soil application (2000g of inoculum per hectare mixed with dried cowdung manure) both basal along with an untreated control. Three levels of phosphorus were tried *viz.*, 25, 50, 75kg P_2O_5 per hectare with a common dose of 25 kg N as basal. Sowing was done in plots of size 4.5 × 3.0m at a spacing of 45 × 30 cm under ridges and furrow system. Observations on 25 randomly selected plants per plot were recorded for plant height (cm), number of flowers and fruits per plant, fruit setting percentage, number of seeds per pod and seed yield (kg per hectare). One hundred seed weight (g) and germination percentage were assessed from each replication. Seeds were sown in germination trays in incubator at 25°C and 95% RH. Seedling count was taken after seven days and germination was expressed in percentage.

Results and Discussion

Results obtained indicated that there was a significant effect of seed inoculation of phosphobacterium on swordbean in increasing the fruit setting percentage (49.27%), number of seeds per pod (10.52) and seed yield (1410 kg per ha) as compared to soil application and untreated control (Table 1). This may be attributed to the presence of seed borne inoculum within rhizosphere where root hairs absorb the nutrients (Singaram & Kothandaraman, 1993). Different levels of phosphorus application showed no significant difference except in influencing the number of fruits and fruit setting percentage. They were found to be more with either 50 or 75kg P_2O_5 application as compared to 25kg P_2O_5 per hectare. One hundred seed weight and germination percentage of seeds were not influenced by different treatments.

Application of phosphobacterium seemed to be environment friendly (Goswami & Kamath, 1984) and the study revealed the efficiency of seed inoculation of phosphobacterium in reducing the phosphorus loss due to insolubility thereby increasing the seed yield of swordbean.

Table 1

Effect of Phosphobacterium on growth and seed yield of swordbean

Treatment	*Plant height at harvest (cm)*	*No. of flowers per plant (75^{th} day)*	*No. of fruits per plant (75^{th} day)*	*Fruit setting percentage*	*No. of seeds per furit*	*One hundred seeds weight (g)*	*Seed yield (kg/ha)*	*Germination (%)*
Phosphobacterium								
Po – Untreated control	111.2	36.18	14.73	40.28	8.67	132.60	1050	98.7
P_1 – Seed inoculation	113.1	39.44	18.33	49.27	10.52	136.70	1410	99.1
P_2 – Soil application	112.1	36.98	15.20	40.64	9.83	136.20	1250	99.6
CD (P = 0.05)	NS	0.58	0.89	1.64	0.04	0.51	140	NS
Phosphorus levels								
F_1 – 25 kg/ha	111.7	37.69	15.47	41.91	9.63	132.60	1200	99.1
F_2 – 50 kg/ha	111.2	37.20	16.33	43.51	9.70	133.80	1250	99.6
F_3 – 75 kg/ha	111.6	37.71	16.47	44.74	9.66	133.80	1260	99.7
C.D. (P = 0.05)	NS	NS	0.89	1.64	NS	0.51	NS	NS

NS = Not Significant.

Summary

The study on Swordbean variety SBS-1 was designed to study the effect of phosphobacterium (*Bacillus megaterium*) under graded levels of phosphorus. There were three methods of phosphobacterium application *viz.*, seed treatment (500g of inoculum per hectare), soil application (2000g per hectare) and an untreated control. The levels of phosphorus tried were 25, 50 and 75 kg P_2O_5 per hectare. The results revealed that the seed inoculation of phosphobacterium increase fruit setting percentage (49.27%), number of seeds per pod (10.52) and seed yield of swordbean (1410 kg) as compared to soil application and untreated control.

Different levels of phosphorus application showed no significant difference except in influencing the number of fruits and fruit setting percentage. They were found to be more with either 50 or 75 kg P_2O_5 application as compared to 25 kg P_2O_5 per hectare. One hundred seed weight and germination percentage of seeds were not influenced by different treatments.

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Authors

K. Govindan and P. Arjunan
Faculty of Agriculture and Animal Husbandry
Gandhigram Rural Institute
Gandhigram-624 302. Dist. Dindigal
Tamil Nadu

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Effect of Inoculation of Phosphomicrobes on Yield and Nutrient Uptake in Groundnut

Introduction

Groundnut requires large amount of phosphorus for growth and high phosphorus availability to the crops in the form of different phosphatic fertilizer is of great economic importance, especially in country like ours, where the soils are poor in available phosphorus content and super phosphate in expensive. Insoluble inorganic compounds of phosphorus are largely unavailable to the plants. Phosphomicrobes have a capacity in bringing the insoluble phosphate into solution (Shinde and Patil, 1985). Apart from increasing the availability in soil, they are known to produce growth promoting substances which in turn enhance growth and yield (Sattar and Gaur, 1987). The fertility status of the soils is likely to go down unless adequate quantities of plant nutrients are added to the soil. The information on the use of phosphomicrobes on the growth and yield of crops in vertisols of Karnataka is scanty and inconsistent. Therefore long-term field trial investigation was undertaken to know the performance of phosphomicrobes on yield and nutrient uptake in groundnut.

Material and Methods

A field experiment conducted at Main Research Station, University of Agricultural Sciences. Dharwad for three consecutive years of Kharif 1992, 1993 and 1994 on medium black soils. The soil having a pH of 8 organic carbon 0.54%, available N, P_2O_5 and K_2O were 254, 35 and 335 kg/ha respectively. The treatments consisted of phosphorus application (50kg P_2O_5/ha) through two sources *viz.*, Mussoorie rock phosphate (MRP) and single super phosphate (SSP) with 0, 25, 50, 75 and 100 per cent of the recommended dose. The phosphomicrobes includes *Aspergillus awamori* and *Pseudomonas striata*. Total of 18 treatments were replicatd thrice in a Randomised Block Design. The fertilizers N and K_2O were given as per the recommended

dose (each 25 kg/ha). After harvest of the cop yield and yield components were recorded. The nodule number and weight was taken at 45 DAS. The nutrient uptake studies were made at harvest. The nitrogen in the plant sample was estimated by modified Kjeldahl's method (Jackson, 1967) and the phosphorus by vanadomolybdo phosphoric yellow colour method using spectrophotometer at 470 nm as described by Jackson (1967).

Results and Discussion

Yield and Yield Components

The pod yield recorded with inoculation of *A. awamori* (3399 kg/ha) of *P. striata* (3416 kg/ha) alone were 12 and 13 per cent higher respectively when compared to control (3026 kg/ha). The beneficial effect of P-solubilizers on grain yield of rice have been reported by Gaur *et al.* (1980). Inoculation of *P. striata* along with the application of 75 per cent MRP + 26 per cent SSP recorded (4648 kg/ha) and differed significantly over uninoculated treatments (Table 1). Similar studies were corroborated by Manjaiah (1989). The number of pods per plant and pod weight per plant followed the similar trends as that of pod yield per hectare.

The haulm yield of groundnut was unaffected due to various treatments. But inoculation of phosphomicrobes significantly influenced the nodule number and nodule dry weight at 45 DAS. Inoculation of *P. striata* with the application of 75 per cent MRP + 25 per cent SSP recorded significantly higher nodule number per plant (110.20) when compared to uninoculated treatments. Nodule dry weight was highest in *Pseudomonas striata* with 100 per cent SSP (199 mg/plant). Similarly Manjaiah (1989) reported that phosphorus application increased the nodule number and nodule dry weight and SSP was more effective than MRP.

Uptake on Nitrogen and Phosphorus

Inoculation of *P. striata* with 75 per cent MRP + 26 per cent SSP recorded significantly highest nitrogen uptake (83.29 kg/ha) over control (46.80 kg/ha) indicating 1.7 fold increase in N uptake by groundnut (Table 2). The increased in N-uptake by groundnut crop was ascribed due to the application of phosphorus and their synergistic interaction (Pantamkumar and Bhathkal, 1967).

Inoculation of *A. awamori* or *P. striata* alone recorded 20 (7kg/ha) and 30 (8.3 kg/ha) per cent higher total P-uptake by groundnut plant than control (5.85 kg/ha). The higher P-uptake may be due to the increased availability of phosphorus in the soil due to solubilization of native phosphorus by phosphomicrobes. Ahmed and Jha (1977) and Mohd *et al.* (1989) reported increased P-uptake by wheat and gram with the inoculation of phosphobacterin. Among all the treatments, inoculation of *P. striata* with 75 per cent MRP + 25 per cent SSP recorded highest P-uptake (13.00 kg/ha).

The results clearly indicate the use of phosphomicrobes to augment the yield and nutrient uptake in groundnut crop. The Indian farmers succumbs to a greater loss due to the high cost incurred by application of phosphorus through SSP. Hence application of 75 per cent of the recommended dose of P through MRP and 25 per cent through SSP along with the inoculation of *P. striata* is more lucrative than applying 100 per cent of phosphorus through SSP alone.

Table 1

Number of pods per plants, pod weight (g) per plant, pod yield (kg/ha) and haulm yield of groundnut as influenced by phosphomicrobes (Average of three years).

Treatments	*Pod numbers Per plant*	*Pod weight (g) per plant*	*Pod yield (kg/ha)*	*Hauim yield (kg/ha)*
1. Control	12.86	14.23	3026	3137
2. MRP O: SSP 100	17.93	19.16	3543	3743
3. MRP 25: SSP 75	7.20	20.10	3492	3627
4. MRP 50: SSP 50	18.10	20.10	3413	3637
5. MRP 75: SSP 25	16.60	20.63	3368	3605
6. MRP 100: SSP 0	16.63	17.90	3336	3445
7. *Aspergillus awamori* (A)	17.70	19.80	3399	3360
8. MRP O: SSP 100 + A	18.63	22.03	3613	3871
9. MRP 25: SSP 75 + A	17.96	21.76	4100	3504
10. MRP 50: SSP 50 + A	17.73	20.70	3600	3761
11. MRP 75: SSP 25 + A	20.70	20.36	3869	3173
12. MRP 100: SSP 0 + A	17.36	20.43	3518	3187
13. *Pseudomonas striata* (P)	18.43	21.23	3416	3623
14. MRPO: SSP 100 + P	20.40	22.53	4445	3853
15. MRP 25: SSP 75 + P	18.66	21.70	3842	3731
16. MRP 50: SSP 50 + P	19.30	21.43	3865	3531
17. MRP 75: SsP 25 + P	22.40	23.90	4648	3900
18. MRP 100: SSP 0 + P	10.06	20.50	3571	3464
S.Em. ±	1.40	0.47	139	300
C.D. at 5%	4.10	1.35	401	NS

MRP = Mussoorie rock phosphate; SSP = Single super phosphate; NS = Not significant.

Summary

A field experiment was conducted during Kharif seasons of 1992, 1993 and 1994 in vertisols of the Main Research Station, University of Agricultural Sciences, Dharwad to know the effect of phosphomicrobes on yield and nutrient uptake in groundnut. The results vividly indicates that, inoculation of *Pseudomonas striata* along with the application of the recommended phosphorus (50 kg P_2O_5/ha) through 75 per cent Mussoorie rock phosphate (MRP) and 25 per cent single super phosphate (SSP) recorded significant higher pod number, pod weight and yield compared to control and all other uninoculated treatments. The former recorded more

number of nodules and nudule dry weight. The uptake of nutrients were significantly superior in the inoculated treatments than the uninoculated treatments.

Table 2

Nodule number, nodule dry weight (mg/plant), nitrogen and phosphorus uptake as influenced by phosphomiicrobes (Average of three years.)

Treatments	*Nodule number /plant at 45 DAS*	*Nodule dry weight (mg/pl at 45 DAS)*	*N-uptake (kg/ha)*	*P-uptake (kg/ha)*
1. Control	49.73	46	46.80	5.85
2. MRP O: SSP 100	78.73	81	66.20	9.00
3. MRP 25: SSP 75	64.66	75	63.00	8.50
4. MRP 50: SSP 50	80.83	65	60.25	8.22
5. MRP 75: SSP 25	90.73	50	55.40	6.90
6. MRP 100: SSP 0	75.50	83	59.50	7.26
7. *Aspergillus awamori* (A)	66.00	75	61.25	7.00
8. MRP O: SSP 100 + A	98.80	104	71.00	10.50
9. MRP 25: SSP 75 + A	77.20	68	68.25	9.25
10. MRP 50: SSP 50 + A	90.86	70	60.00	9.90
11. MRP 75: SSP 25 + A	93.93	99	63.28	10.25
12. MRP 100: SSP 0 + A	81.83	76	57.94	10.00
13. *Pseudomonas striata* (P)	73.06	84	64.00	8.30
14. MRP O: SSP 100 + P	115.46	119	80.25	11.31
15. MRP 25: SSP 75 + P	90.60	69	70.20	10.20
16. MRP 50: SSP 50 + P	102.60	80	69.60	10.70
17. MRP 75: SsP 25 + P	110.20	89	83.29	13.00
18. MRP 100: SSP 0 + P	88.06	93	69.40	9.65
S.Em. ±	4.07	2.50	0.84	0.40
C.D. at 5%	11.72	7.45	2.50	1.20

MRP = Mussoorie rock phosphate; SSP = Single super phosphate; NS = Not significant.

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Authors

C.A. Agasimani, Mudalagiriyappa and G.D. Radder
University of Agricultural Sciences
Dharwal-580 005, Karnataka, India

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Production and Evaluation of Phosphocompost from Neem with Rock Phosphate

Introduction

Decomposition of organic matter is a natural process responsible for the breakdown of complex organic compounds. During this process, inorganic minerals locked up in organic residue released are made available to plants. The activity of microorganisms and various physico-chemical agencies bring about change in the structure and chemical composition of organic matter which in turn determines species composition of late colonizing microorganisms. Microbial succession continues until all the organic substances are mineralized.

The composting of neem along with low grade rock phosphate and 'P' solubilizers is known to increase the solubility of insoluble phosphates. Although the extent of solubilization can vary with the kind of wastes (Gaur, 1982), Neem compost enriched with rock phosphate and *Aspergillus awamori* produced a good quality of phosphocompost.

Material and Methods

Neem leaves were collected from the university campus and placed in specialized pots for composting.

Phosphate solubilizing microorganism: *Aspergillus awamori* was selected as it is an efficient solubilizer of insoluble phosphates.

Phosphatic fertilizer: Mussorie rock phosphate served as the phosphate source and was added at 100 μg/g concentration to the compost on the 17th day.

Mycorrhizal fungi: *Glomus fasciculatum* was used to treat the plants along with the compost. *Abelmoschus esculentus* (Okra, bhendi) was used as a test plant.

The treatments given were as follows:

1. C (Control)

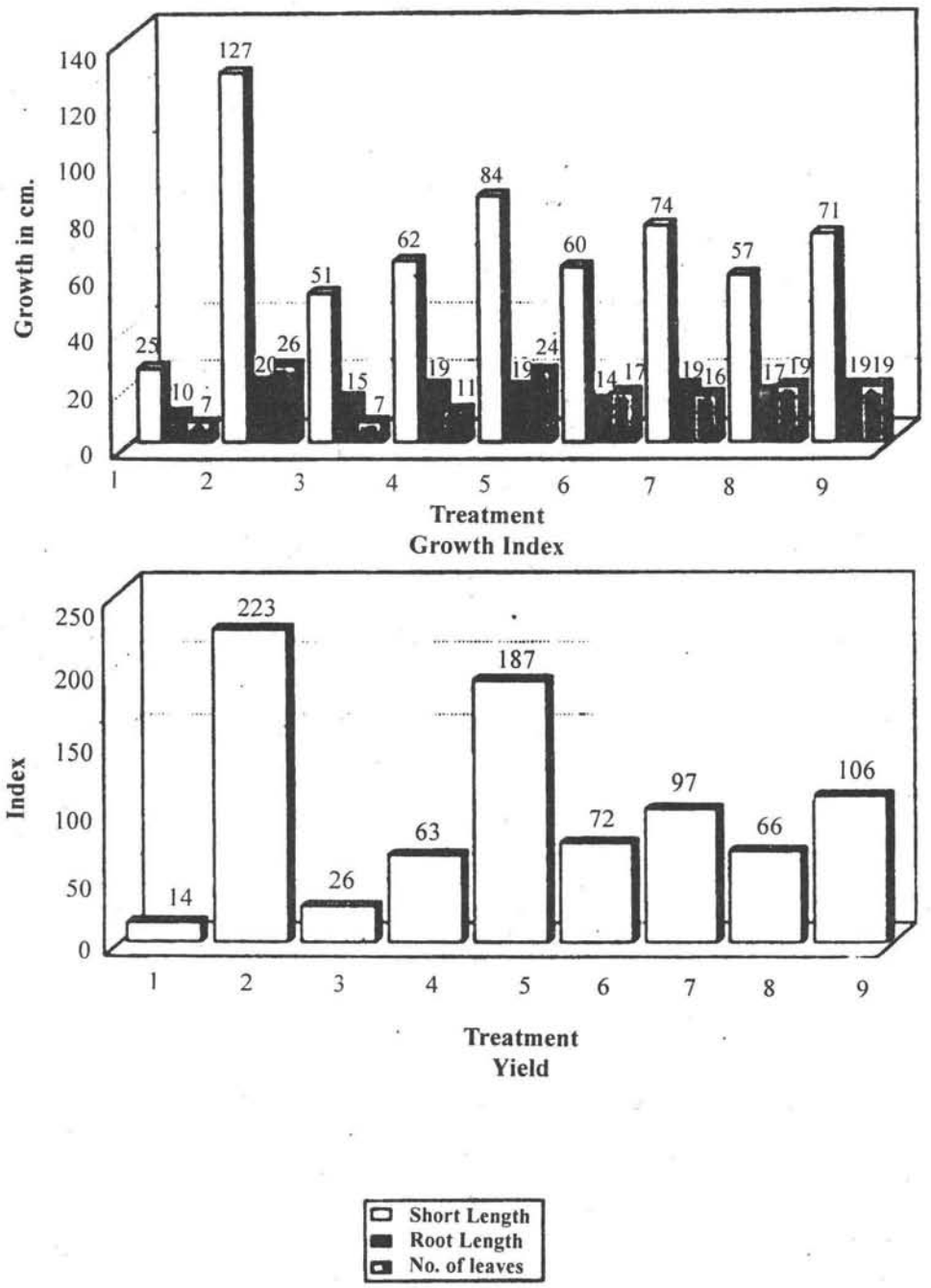
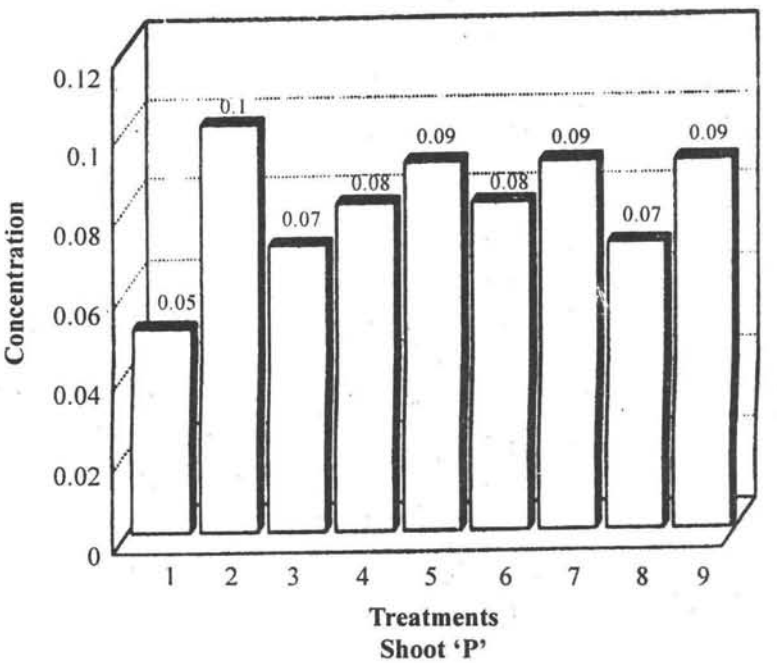


Fig. 1. Growth index and yield of *Abelmoschus esculentus* L (1. C, 2. NC + Aa + M, 3. NC + R ?, 4. NC + RP + M, 5. M, 6. PSM + M, 7. PSM, 8. NC, 9. NC + Aa)



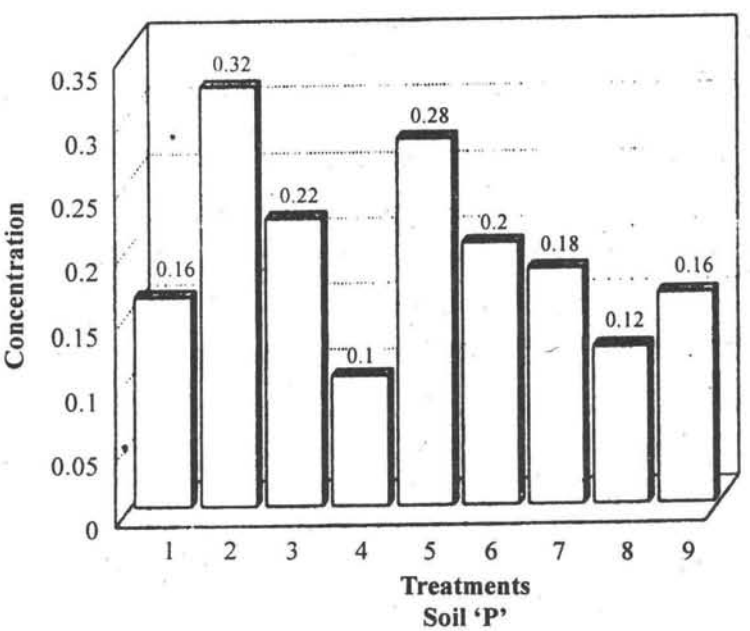


Fig. 2. Concentration of phosphorus in shoot and soil (1. C, 2. NC + Aa + M, 3. NC + RP, 4. NC + RP + M, 5. M, 6. PSM + M, 7. PSM, 8. NC, 9. NC + Aa)

2. NC + A.a + M (Neem compost inoculated with *Aspergillus awamori* and *Glomus fasciculatumi*).
3. NC + RP (Neem compost with rock phosphate)
4. NC + RP + M (Neem compost with rock phosphate and *Glomus fasciculatum*)
5. M (Mycorrhiza alone-*Glomus fasciculatum*)
6. PSM + M (*Aspergillus awamori* + *Glomus fasciculatum*)
7. PSM (*Aspergillus awamori* alone)
8. NC (Neem compost alone)
9. NC + A.a (Neem compost with *Aspergillus awamori*)

Experiments were carried out in replicated randomized block design with 3 replications for each treatment. Plant growth, 'P' content and yield were recorded at the maturity of the crop (70 days). The yield obtained were compared with respective treatment control.

Results and Discussion

The data obtained showed that when bhendi plants inoculated with neem compost along with *Aspergillus awamori* and VA Mycorrhiza, as given in the histogram showed highest fruit yield (Fig. 1).

Neem compost along with VAM showed significant increase in 'P' content of soil and shoot 'P' than did alone (Fig. 2).

The data indirectly show that the insoluble rock phosphate incorporated to neem waste is solubilized and is available to bhendi plants due to biochemical and microbial activity during decomposition of organic wastes. Since the neem compost also contain efficient 'P' solubilizers (Meera Madan, 1974) it improves the phosphorus used efficiently and VAM activity for maintenance of soil productivity which is very much essential for ecofriendly and sustainable agriculture.

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Authors

N.M. Vanitha and D. Anusuya
Department of Botany Bangalore University
Banglore-560 056, Karnatka, India

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Effect of Cyanobacteria on Soil Characteristics and Productivity of Gram Grown in Salt Affected Soil

Introduction

Soil salinity has become an alarming problem in Indian agriculture. Sufficient technology is developed for reclamation of such soils in Indo-Gangetic plains. The salinity problem is however of different nature in peninsular region (Varade, *et al.* 1985) needing some different integrated approach. Apparently salinity research has acquired many dimensions (Mehta, 1993). The response of cyanobacteria (BGA) as biological ameliorant is being increasingly understood (Sing, 1961; Kaushik, 1989 and Venkataraman, 1993). In Maharashtra the salt affected area is 30,000 ha. In Vidarbha Region, it is located in Purna River basin mostly under rainfed cropping, having Black Cotton soil with an average annual rainfall 827mm. The conventional recuperative measures are long known, and are found to suffered from limited adoptability due to economic reasons.

The object of present studies is endeavoured to assess the feasibility of using cyanobacerial incoculant (Blue Green Algae) as a bioameliorant in problem soil under protective irrigation conditions as a possibility of harmless, low cost, renewable, easily adoptable measure to augment conventional methods.

Material and Methods

A field experiment was conducted in problem saline soil at Washim road Block of P.K.V. Akola in Rabi 1994, using randomized block design, with five replications and eight treatments combinations. Gram crop was sown in October 1994 and harvested in February 1995. Treatments consisted four levels of Blue Green Algae (0;25; 50 and 75 kg/ha) incorporation with three levels of gypsum (0,1 and 2t/ha) addition to soil at sowing.

Soil based inoculant culture of BGA was obtained from Agricultural Microbiologist,

Mahatma Phule Krishi Vidyapeeth, Pune-5 which consisted mixture of *Tolypothrix* sp. (G4), *Calothrix* sp. (C3) and *Nostoc calcicola* (B5).

Soil samples were collected from surface layer up to 9 cm depth from each of the plot. Samples from five replications were mixed thoroughly to get a composite sample for each of the treatment. Soil parameters *viz.* pH, EC, Organic Carbon, ESP, available N and available P were quantified using standard procedures. Pretreatment soil sample was used as a check. Grain yield of Gram Cv. CHAFFA from each of the next plot was recorded on sun drying for 4 days after harvest (20.2.95). Weight of 1000 grains from each of the plot was measured to assess effect on quality/boldness.

Results and Discussion

Yield Responses

Increase in grain and biomass yield of Gram (*Cicer arietinum*) crop due to single application of BGA and/or gypsum as soil ameliorant are recorded with a synergistic response (Table 1). The rise in grain yield being 3.94 to 5.85 q/ha due to BGA and 3.72 q/ha due to gypsum with an additive effect of 0.232 q/ha under protective irrigation cultivation on a problem patch of soil. Increase in yield of rice and sugarcane after first year of reclamation of Usar soils with native algal flora are reported by Singh (1961); and in paddy in saline soils by Kaushik and Subhashini (1985). Kaushik (1989) have reported a significant yield increase in paddy in second and third year of algalization to sodic soils of Delhi. Similar promising yield response to paddy crop in salt affected soils at Govt. Experimental farm, Gohad in M.P. in 1985 and 1986 due to algalization is reported by Sharma, Bharadwaj and Chauhan (1989).

Grain quality is noticed to have improved in present studies as evidenced by statistically, significant increase in weight of grains. It was 154.02g and 153.97g when B 25 G1 and B 75 G2 is applied respectively as compared to 152.26g in control (Table 1).

Soil Properties

Promising improvement in soil properties due to application of BGA and/or gypsum are observed in gram crop in these studies. pH is reduced to 9.30 due to BGA 75, and 9.24 due to gypsum application at the rate of 2 tonnes as against 9.70 in control. EC is also reduced from 0.66 dSm^{-1} to 0.50 dSm^{-1} when BGA was applied and 0.44 dSm^{-1} when gypsum was applied. The ESP is also markedly reduced in present trial from 25.24% in control to 19.93% when B75 G2 is applied and 19.83% with BO G2 application. Reduction in soil pH due to algalization in salt affected soils is reported by Hemantkumar and Kaushik (1990) and Prasad and Verma (1987).

Summary

Feasibility of using cyanobacterial inoculant (BGA) as bio-ameliorant to a problem saline soil was tried under rainfed (arid) field conditions of PKV Akola (Maharashtra) during 1994-95. Four levels of BGA inoculant with and without gypsum were assessed using Randomised Block Design.

Table 1

Effect of BGA and Gypsum incorporation to saline soil on yield of gram crop (Rabi 1994) and soil parameters.

Treatments	*Grain yeld kg/ha*	*Weight of grains g/1000*	*pH*	*EC dSm^{-1}*	*Org. carbon*	*ESP %*	*Available N kg/ha*	*Available P kg/ha*
BO GO	1312	152.2	9.70	0.66	0.42	25.24	191.2	8.9
B25 GO	1706	152.8	9.48	0.59	0.46	23.33	206.9	10.6
B50 GO	1833	153.2	9.36	0.55	0.52	22.50	213.2	10.6
B75 GO	1897	153.2	9.30	0.50	0.58	20.98	225.7	12.4
BO G1	1684	153.7	9.35	0.49	0.46	21.80	197.5	11.5
BO G2	1731	154.0	9.25	0.44	0.48	19.83	202.1	16.9
B25 G1	1729	154.0	9.34	0.47	0.48	21.80	206.9	12.1
B75 G2	1845	154.0	9.24	0.40	0.50	19.93	222.6	13.5
Pre treatment count:			9.72	0.68	0.40	25.48	190.4	8.8
SE (m) ±	11.2	0.2						
CD at 5%	32.3	0.577						

Increase in grain and biomass yield of gram (*Cicer arietinum*) due to single application of BGA was evidenced. The rise in grain yield being 3.94 q/ha due to BGA (soil application at the rate of 25 kg/ha) 3.72 q/ha due to gypsum (at the rate of lt/ha). The yield in control being 13.13 q/ha. The weight of grains was 154.02 g/1000 and 153.97 g/1000 when B 25 GI and B75 G2 was applied respectively as compared to 152.26 g in control. Soil pH was reduced to 9.30 and 9.24 when BGA at the rate of 75 kg/ha and gypsum at the rate of 2t/ha respectively was applied as against the initial pH 9.70. Similarly EC was also reduced from 0.66 dSm^{-1} to 0.50 dSm^{-1} when BGA was applied and 0.44 dSm^{-1} when gypsum was applied. Improvements in available C, N, and P were also observed.

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Authors

K.D. Thakur, K.G. Thakare, A.U. Narayana and S.O. Kolte
Department of Plant Pathology
Dr. Panjabrao Deshmukh Krish Vidyapeeth
Akola-444 104, Maharashtra, India

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Crop Response to Algalization in Rice Variety BPT-5204

Introduction

Incorporation of Blue Green Algae in standing water in paddy crop have been considered as a sure remedy for scarcity of chemical nitrogen (Venkataraman, 1981; Hamdi, 1982). The performances of B.G.A. application in rice fields have aptly been reviewed (Goyal, 1982) and further emphasized the need for more thrust through state extension agencies. The technology has already been introduced in many states since 1975. The impact could not reach to many rice growing areas in Tamil Nadu due to absence of emphatic extension propaganda (Sandaran, 1990).

Material and Methods

Blue Green Algae was applied to paddy crop one week after transplanting by broadcasting in July 1991 in a replicated field trial at Tharsa, Distt. Nagpur. BGA inoculant was obtained from the National Facility Centre for BGA; Indian Agricultural. Research Institute, New Delhi, which consisted *Anabaena* sp.; *Nostoc* sp.; *Tolypothrix* sp.; and *Cylindrospermum* sp. Grain and straw yield from each net plot was recorded on harvesting in November 1991. Weight of 1000 grains; and protein content were also recorded by routine methods.

Results and Discussion

Results (Table 1) indicated that grain yield was increased from 11.84% to 41.58% with different treatments and the straw yield increased from 6.05% to 63.96% over control. Incorporation of BGA evoked a statistically significant response in plant height; number of tillers per hill; grain weight and chlorophyll content; both with and without chemical nitrogen. The substitution effect was however; not noticed perhaps due to slow availability of biologically fixed N in the cropping system. Highest residual nitrogen *i.e.* 82 kg^{-1} and 41 kg^{-1} was recorded in two graded

Table 1
Response of Kharif rice to BGA with and without chemical nitrogen.

Treatment (quantities /ha)	*No. of tillers/ plant*	*Plant height (cm)*	*Chl. (mg. g^{-1})*	*Grain yield ($q.ha^{-1}$)*	*Straw yield ($q.ha^{-1}$)*	*Grain weight (g/1000)*	*Protein (g%)*	*Residual N in soil over initial ($kg.ha^{-1}$)*
Control (B_0N_0)	15.7	81.2	1.35	45.0	51.1	16.10	8.51	–08.00
BGA 10 kg (B_1N_0)	17.4	86.8	1.56	54.1	54.4	16.31	9.21	11.00
BGA 20 kg (B_2N_0)	17.9	89.9	1.83	53.7	56.3	16.35	9.47	23.00
BGA 10 kg+ N-50 (B_1N_1)	19.2	93.6	2.06	54.4	67.9	17.33	10.33	41.00
BGA 20 kg+ N-50 (B_2N_1)	19.4	98.4	2.20	63.8	77.0	17.40	10.75	82.00
N 50 alone (B_0N_1)	18.6	19.4	2.00	50.4	58.6	16.64	9.50	–02.00
N 100 alone (B_0N_2)	20.6	99.5	2.22	61.9	83.7	17.69	9.82	–01.00
SE	0.08	0.10	0.07	1.42	1.17	0.06	0.18	
CD at 5%	0.25	0.32	0.23	4.50	2.50	0.20	0.56	

B_0 = No BGA B_1 = BGA @ 10kg B_2 = BGA @ 20 kg
N_0 = No Chemical N_1 = 50 kg N ha^{-1} N_2 = 100 kg N ha^{-1}
Nitrogen through urea through urea Chl. = chlorophyll

doses of BGA respectively as compared with –01.00 in chemical N and –08.00 in absolute control.

Dar and Ganaie (1990) reported 20% increase in grain yield; 12.4% in straw yield; besides appreciable improvement in grain weight; plant height and tillering. Singh *et al.* (1989) put review on crop response to rice. Ahluwalia *et al.* (1990) indicated that BGA plays a very important role in supplementing part of chemical N. Mandhare and Patil (1990), Bobde *et al.* (1990) and Rokade *et al.* (1992) observed in a field trial for three years that the highest yield of paddy was obtained due to application of 20 kg BGA + 100% chemical N. Application of BGA could save 25% of chemical N required for paddy. However, Roy *et al.* (1990) through similar experiments for three years reported a saving of 50% chemical N. Similar trends are reported in saving of chemical N; plant height; tillering; chlorophyll content over two years at Pusa in Bihar by Jha *et al.* (1990).

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Authors

V.W. Tarsekar, K.D. Thakur and S.O. Kolte
Department of Plant Pathology
Dr. Panjabrao Deshmukh Krish Vidyapeeth
Akola-444 104, Maharashtra, India

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Biofertilizers in Banana Fields: A Case Study from Anekal Taluka, Bangalore District, Karnataka

Introduction

Studies on soil algal have received much attention in these days because of its eco-friendly behaviour with other organisms. Investigations on soil algae have done by Stokes (1940), Lund (1947), Okauda and Yamaguchi (1952), Gonzalves and Yalvigi (1959), Singh (1961), Shields and Durell (1964), Bharati and Pai (1972), and Kaushik (1985). Algae are the important constituent of the soil microflora of temperate and tropical regions. Native soil-algal floristics and their distribution in relation to soil fertility have been studied in detail across the globe (Marathe and Navalkar, 1963; Pandey, 1965; Kamar and Patel, 1973; Khan and Mathur, 1976; Krishnakumari, 1982 and Bongale, 1986). From the point of view of morphology, physiology and nutritional behaviour they are heterogeneous and create variation in the elemental requirements (O, Kelley, 1974). The native algal flora of crop fields, other than paddy fields is not well documented, except few references (Raju, 1967; Gonzalves and Yalvigi, 1959; Marathe, 1964; Dutta and Venkataraman 1958; Bongale, 1986 and Shakuntala, 1991).

The present exploratory survey was undertaken to fill the lacuna of native algal composition in the Banana plantations.

Material and Methods

The Bangalore urban district includes eleven talukas, the present study area is located in Anekal Taluk with 533.9 sq km area and situated south-eastern part of the district. It receives moderate rainfall (80cm/yr) and the mean annual temperate is 23.6°C. The overall soil characteristic is red-"ragi soil", black soils are also found in some pockets. The climate is mostly semi-arid type. The common crops are ragi, paddy, beans, horse gram, tomato, sunflower, groundnut and maize. The commercial crops like Papaya and Banana are also of common occurrence in this region.

The surface soil samples were collected from wet-land as close to the vicinity and also between rows of plants from different points all over the field and a composite sample was made. The composite samples were studied in the laboratory for their physico-chemical properties using standard methods. Whereas, for assessment of the native algal flora, soil-water biphasic medium and moist cultures were maintained in triplicate using De's modified Beneck medium (Singh, 1961). Cultures were maintained in natural day-light at north-east window of the laboratory for a period of three months. The periodic harvesting of algae started on 15th day of incubation and was continued up to ninety days. The voucher specimens are preserved in Lugol solution. Algae were identified with the help of camera lucida drawings and photographs by making use of standard reference books (Desikachary, 1959; Prescott, 1951 and Hustedt, 1962).

Results and Discussion

Physico-Chemical parameters of the soils are presented (Table-1). It is clear that the pH ranges from 6.3 to 7.8, indicating moderately acidic to alkaline. The other nutrients like Nitrogen, phosphate, potassium and sodium are in low quantities, whereas carbonates, chlorides and organic carbon occur moderately.

The distribution pattern of algae in the study area is given (Table-2). Into the number of algal species is 39, out of these, 34 belong to Cyanophyceae, 4 to Cholorophyceae and 1 to Bacillariophyceae. Among these, *Oscillatoria* and *Nostoc* had appeared in all types of soils and represented with four species each, whereas *Chroococcus indicus* present only in ABS_1 soil. On the other *Chlorella vulgaris* and *Nitzschia diserta* from ABS_4 soil only.

It was observed that during the growth of algae, the species cyanophyceae exhibited maximum growth and predominated over other groups in all the soils. An increase in the number of cyanophyceae may be attributed to the presence of elevated levels of pH and carbonates (Table-1). Whereas the sample ABS_2 and ABS_4 showed maximum amount of nitrogen and had harboured 21and 23 species respectively. Cameron and Wallace (1960) opined that the abundance of algal species may have direct bearing on soil moisture and the extent of time it is available. Banana plantations needs no waterlogging unlike paddy soils.

In the present investigation, the predominance of blue green algae was observed and it is in agreement with paddy maize, jowar, wheat and sugarcane soils (Shakuntala, 1991). This may be due to neutral alkaline nature of these soils. During culture, it was observed that Cyanophyceae always dominated over Chlorophyceae and Bacillariophyceae.

It is too early to conclude that, the banana crop may lodge more number of cyanophyceae members in the absence of literature on algae of banana fields.

Summary

Soil samples from Anekal banana plantations indicated the pH range from 6.2 to 7.8. Total 4 samples from Anekal were subjected to Physico-chemical and biological characteristics. Totally 39 species were enumerated out which, 34 species were assigned to Cyanophyceae, four to Chlorophyceae and one to Bacillariophyceae. The abundance of native algal flora were correlated to nutritional requirements.

Table 1
Physico-chemical composition of Banana field soils from Anekal. (Chemical composition in terms of % of dry soil except pH)

											Agal spp. per sample		
Sample	*pH*	*CO_3*	*PO_4*	*Cl*	*Org.C*	*K*	*Na*	*N*	*Ca*	*C/N*	*Cy*	*Ch*	*Ba*
ABS_1	6.2	0.08	0.002	0.40	0.54	0.0062	0.0110	0.080	0.009	6.78	7	1	0
ABS_2	7.3	0.40	0.004	0.34	0.34	0.0050	0.0090	0.128	0.006	2.34	21	2	0
ABS_3	6.7	0.32	0.006	0.036	0.90	0.0572	0.0501	0.064	0.018	9.47	9	1	0
ABS_4	7.8	0.62	0.005	0.044	1.20	0.0357	0.0550	0.095	0.025	18.75	23	3	1

Cy = Cyanophyceae
Ch = Chlorophyceae
Ba = Bacillariophyceae

Table 2

Distribution pattern of Algal forms from Anekal soil

	Species	*Soil collection number*
1.	*Chroococcus turgidus* (Kutz.) Nag	2, 4
2.	*C. indicus* Zeller	1
3.	*Gloeocapsa decorticans* (A. Br.) Richter	1, 2, 4
4.	*G. quaternata* (Breb.) Kutz.	3
5.	*Gloeothece membranancea* (Rabenh.) Bomet	2
6.	*Aphanocapsa biformis* A. Br.	4
7.	*Aphanothece pallida* (Kutz) Rabenh	1, 2
8.	*Spirulia subsalsa* Oerst ex. Gomont	2
9.	*Oscillatoria curviceps* Ag. ex Gomont	2, 4
10.	*O. cholrina Kutz.* Ex Gomont	1, 2, 4
11.	*O. proboscidea* Gomont	1, 3, 4
12.	*O. limosa* Ag. ex Gomont	
13.	*Phormidium foveolareum* (Mont) Gomont	2, 4
14.	*P. retzii* (Ag.) Gomont	2, 4
15.	*P. favosum* (Bory) Gomont	2, 4
16.	*P. tenue* (Menegh) Gomont	1, 3
17.	*Lyngbya kuetzingiana* Kirchner	2, 4
18.	*L. trunicicola* Ghose	3, 4
19.	*L. corticola* Bruhlet Biswas	2, 3, 4
20.	*L. limnetica* Lemmermann	3, 4
21.	*L. rubida* Fremy	2
22.	*Microcoleus paludosus* (Kutz) Naegeli	2, 4
23.	*Cylindrospermum musicola* Kutzing ex Born. et Flah	4
24.	*Nostoc muscorum* Ag. ex Bomet Flah	2, 3, 4
25.	*N. linckia* (Roth) Bomet ex Born et Flah	2, 4
26.	*N. piscinale* Kutzing ex Born et Flah	2
27.	*N. entophytum* Born et Flah	4
28.	*Anabaena anomala* Fritsch	4, 2
29.	*Plectonema puteale* (Kirchner) Hansgirg	
30.	*Scytonema dilatatum* Bhardwaja	3, 4
31.	*S. schmidtii* Gom.	2
32.	*S. hofmanni* Ag. ex born. et Flah	4
33.	*Tolypothrix distorta* Kutz. Ex Born et Flah	2

34.	*Calothrix atrica* Fremy	2, 3
35.	*Cholorococcum humicola* (Naegeli) Rabenhorst	1, 3, 4
36.	*Chlorella vulgaris* Biejerinck	4
37.	*Protococcus viridis* Aqandh	2, 4
38.	*Oedogonium* sps	2
39.	*Nitzschia diserta* Hustedt	4

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Authors

N. Venugopal and K. P. Sreenath
Department of Botany
Bangalore University
Banglore-560 056, Karnataka, India

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Isolation of Pesticides and Heavy Metal Tolerant Strains of *Azotobacter chroococcum* from the Rhizospheric Region of Wheat Crop

Introduction

Use of industrial effluent and sewage sludge in agricultural fields has become a common practice. The effluents contain a variety of organic and inorganic chemical like pesticides and heavy metals which affect microbial population in soil (Ayanaba, 1981) as well as the processes mediated by these organisms. The biological effects vary depending on the types of these pollutants, their concentration and the duration of treatment (Tu, 1981).

The common pollutants of soil *viz.* pesticides and heavy metals adversely affect the growth and survival of the plant as well as its rhizosphere microflora. One of the most important nitrogen fixing microorganisms, the *Azotobacter* has been shown to be sensitive to these toxicants (Kheri *et al.*, 1993).

In the present paper an attempt has been made to overcome the problem of toxicity of pesticides and heavy metals by isolating the tolerant strains of *Azotobacter chroococcum*. These studies can serve as an appropriate biofertilizer in the polluted soil.

Material and Methods

Experimental Background

The soil in which the study was conducted is a sandy clay loam having a pH of 8.0. The physico-chemical properties of soil are given in Table 1. The soil has received no exogenous input of metals. The soil was properly mixed, sieved and heavy metals like Zn^{2+}, Pb^{2+}, Cu^{2+}, Ni^{2+}, Cd^{2+}, Cr^{3+} were added in solution to the soil before sowing. Sufficient water was added to bring the soil to 50% water holding capacity, pesticides like Mancozeb, 2, 4-D and Malathion were also added in solution after ten days of sowing of wheat seeds.

Table 1

Properties of the experimental soil

Property	*Nature/Value*
Type	Alluvial
Soil class	Sandy clay loam
Water holding capacity	29%
pH	8.0
Organic carbon	0.62%
Cation exchange capacity	
CEC/cmol kg^{-1}	11.7

Pot Experiment

Three and a half kilogram of soil was taken in each pot for different treatments and three replicates were taken for each treatment. The soil was fertilized with 120:60:50 kg/ha of nitrogen, phosphorus and potassium (NPK). The carrier based strain inoculant of *Azotobacter chroococcum* obtained from IARI, New Delhi was used to treat the seeds of wheat (*Triticum aestivum* L. var. PDW 154). Wheat seeds were surface sterilized in 0.1% mercuric chloride solution and washed with six changes of sterile distilled water. Four seeds of wheat were sown in each pot. Seedlings were thinned to two plants per pot. Plants were grown for three months. Pots were watered as and when required.

Isolation of Azotobacter Chroococcum from Rhizosphere Region of Wheat

A total of fifteen *Azotobacter* strains were isolated from the rhizospheric region of wheat plant in the flowering stage by soil dilution and plate count method on Jensen's agar from the soil containing different amounts of heavy metals and pesticides (Timonin, 1940). The isolates were characterized up to the species level by different biochemical tests and pigment production.

Determination of Minimum Inhibitory Concentration of *Azotobacter Chroococcum* Isolates

MIC of all the isolates was determined on Jensen's agar. The medium was solidified with 2% agar and the pH was adjusted to 7 with NaOH. Filter sterilized solution of heavy metals and pesticides were added to molten Jensen's medium and the plates were allowed to solidify. The individual isolates (10^4 cells) was spot inoculated onto the metal amended plates and kept for five days at 28 ± 2°C. The minimum concentration of each metal and pesticides that inhibited the growth of *Azotobacter* was defined as MIC.

Results and Discussion

MIC values of various strains of *Azotobacter chroococcum* to heavy metals and pesticides from metal and pesticide contaminated soil is summarized in Tables 2 and 3. It is evident from the tables 2 and 3 that the isolates exhibited high MIC and some were found to be multiple

tolerant to high concentration of heavy metals and pesticides. Thus, the order of decreasing toxicity of the metals was as follows:

$Cd^{2+}>Ni^{2+}>Zn^{2+}>Cu^{2+}>Pb^{2+}>Cr^{2+}$

Table 2
MIC values of *Azotobacter chroococcum* strains for heavy metals.

Heavy metals	*MIC (ppm)*
Ni^{2+}	150.8
Cu^{2+}	182.5
Zn^{2+}	158.6
Cd^{2+}	75.0
Pb^{2+}	1500
Cr^{3+}	1750

Table 3
MIC values of *Azotobacter chroococcum* strains for pesticides

Pesticides	*MIC (ppm)*
Mancozeb	225
2,4-D	125
Malathion	200.7

The pesticides toxicity was in the following order Mancozeb Malathion 2,4-D. Growth of all kinds of organisms including both gram positive and gram negative bacteria, actinomycetes and fungi was affected by Cd(II) (Babich and Stotzkyk, 1977). The Cd(II) toxicity is believed to be the consequence of inactivation and inhibition of certain Vital enzymes of living cells (Jacobson and Turner, 1980). It seems that the multiple metal resistance possessed by these isolates might be due to the increased production of extracellular polysaccharide. The production of polysaccharide is thought to be responsible for binding the Cr(III) thus making it non-toxic (Aislabie and Loutit, 1986). Moreover, it was suggested to be necessary adjunct to the survival of bacterial cells in polluted environment (Costerton *et al.*, 1981). Heavy metals were found to be more toxic to these microorganisms which are involved in the biogeochemical cycles *e.g.* nitrogen fixation, nitrification etc. (Brierley and Thornton, 1983).

The data presented in Tables 2 and 3 show that 2,4-D is the least toxic pesticide. Earlier reports also demonstrated the degradation of endosulphan and 2,4-d by *Azotobacter chroococcum* thus making them least toxic (Balajee and Mahadevan, 1990).

The application of industrial effluents and indiscriminate use of pesticides to agricultural land which are used for cultivation of cereals require caution. However, these isolates can serve as suitable biofertilizers in highly polluted agricultural field for seed inoculation.

Summary

Azotobacter is a free living heterotropic bacterium which helps in nitrogen fixation and thus enhances the crop production. However, very little information is available regarding the effect of agro-chemicals *viz.* pesticides and heavy metals on the free living microbes. Therefore, a study was conducted to examine the toxic effect of these agrochemicals on the population of *Azotobacter* in the rhizosphere region of wheat crop and to isolate the tolerant strains of *Azotobacter chroococcum*. Pesticides and heavy metals in the soil significantly affected the *Azotobacter* population and the inhibition varied with the nature of toxicant. BHC and Cd were found to be the most toxic to the *Azotobacter* population. Minimum inhibitory concentration (MIC) of the tolerant isolates were found to be much higher than the amount of pesticides and heavy metals added to the soil. The results show that these isolates were multiply tolerant to different pesticides and can be used as biofertilizers in the pesticides treated fields and/or where the fields are irrigated with sewage sludge or industrial effluent. Molecular biological studies of the plasmids present in the tolerant isolates are in progress.

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Authors

Rana Athar*, Shams Tabrez Khan* and Dr. Masood Ahmad**

* *Institute of Agriculture, A.M.U., Aligarh, U.P. India*

** *Department of Biochemistry, Faculty of Life Sciences, A.M.U., Alighrh, U.P., India*

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Characterization and Identification of *Azotobacter* strains Isolated from Mulberry (*Morus alba L.*) Rhizosphere Soil

Introduction

Free living nitrogen fixation is closely associated with the growth of the nitrogen fixing micro-organisms (Rovira, 1965). Among free living nitrogen fixers importance of *Azotobacter chroococcum* has been stressed since the time it was discovered by Beijerinck 1901 (Tchan and New, 1984). Members of the *Azotobacteraceae* are primarily characterized as non-symbiotic, aerobic, heterotrophic bacteria whose main property is the ability to fix molecular nitrogen in nitrogen free or poor medium with an organic carban as energy source (Becking, 1992).

In the present investigation efforts have been made to isolate, characterize and identify the *Azotobacter* strain isolated from mulberry rhizosphere soil and to screen efficient nitrogen fixing *Azotobacter* strain.

Material and Methods

Rhizosphere soil samples of mulberry was collected at six locations two each from Kolar, Mysore and Ramanagar as per the procedure outlined by Dobereiner *et al.* (1972). The collected soil samples were brought to the laboratory, air dried, powdered passed through 0.27mm sieve and used for isolation. Seven *Azotobacter* strains from Kolar five each from Mysore and Ramanagar were isolated by employing dilution plate technique and maintained on Walksman No. 77 medium.

Characters like colony growth, intensity and pigmentation were studied by streaking the purified cultures on Walksman No. 77 medium. The isolation were further tested for gram reaction (Hucker's modified method). Formation of microcysts studied in three weeks old cultures stained with acridine orange and observed under oil immersion. Utilisation of various

carbohydrates-Mannitol, Glucose, Fructose, Sucrose, Rhamnose, Malate and Citrate as carbon sources were studied separately by adding to Walksman No. 77 medium at 1 per cent w/v. The total nitrogen fixed per gram of carbon source utilized was estimated by microKjeldahl method (Jackson, 1973).

The authentic *Azotobacter* culture obtained from collection of the Department of Agricultural Microbiology, University of Agricultural Sciences, G.K.V.K. Bangalore was used for comparison and identification of isolated cultures. This standard isolate was gram negative, microcyst former, produced brown black pigment and had fixed 6.6 mg of Nitrogen per gram of Mannitol used.

Results and Discussion

Cultured cells were morphologically found to be ovoid, motile, gram negative and all were microcyst formers these observations are comparable to that of Parker and Socolofsky (1966). All the strains produced brown black pigment which was water insoluble. The details are given in Tables 1, 2 and 3. Among the seven carbohydrates tested as a carbon source, Mannitol and

Table 1

Growth characters for authentication and Nitrogen fixing ability of *Azotobacter* strains isolated from Kolar soil.

Azotobacter Strains	*Gram stain*	*Formation of Microsysts*	*Pigmentation*	*Cultural characters*	*mg N-fixed/g of C source (Mannitol)*
K1	Gram-ve	+	on fourth day light brown	Good growth flat, entire, wrinkled at the edge of the colony	6.1
K2	Gram-ve	+	On third day dark brown	Good growth, flat entire, wrinkled at the edge of the colony	6.9
K3	Gram-ve	+	On fifth day pale brown	Good growth, flat entire, wrinkled at the edge of the colony	6.3
K4	Gram-ve	+	On eighth day dark brown	Moderate growth, flat entire	5.8
K5	Gram-ve	+	On sixth day light brown	Moderate growth, flat entire	5.6
K6	Gram-ve	+	On seventh day dark brown	Good growth, flat, entire, wrinkled at the edge of the colony	5.8
K7	Gram-ve	+	On fourth day pale brown	Moderate growth, flat entire	5.2
SEM±					0.1046
CD at 5%					0.3078

All values are means of four replications. Gram-ve = Gram negative, + = Microcyst formed.

Table 2
Growth characters for authentication and nitrogen fixing ability of *Azotobacter* strains isolated from Mysore soil.

Azotobacter Strains	*Gram stain*	*Formation of Microsysts*	*Pigmentation*	*Cultural characters*	*mg N-fixed/g of C source (Mannitol)*
M1	Gram-ve	+	on fifth day pale brown	Good growth flat, entire, wrinkled at the edge of the colony	6.3
M2	Gram-ve	+	On sixth day dark brown	Good growth, flat entire, wrinkled at the edge of the colony	6.0
M3	Gram-ve	+	On fourth day dark brown	Good growth, flat entire, wrinkled at the edge of the colony	6.8
M4	Gram-ve	+	On third day light brown	Moderate growth, flat entire, wrinkled at the edge of the colony	6.3
M5	Gram-ve	+	On seventh day light brown	Moderate growth, flat entire	5.6
SEM±					0.0658
CD at 5%					0.1984

All values are means of four replications, Gram-ve = Gram negative, + = Microcyst formed.

Glucose were the most preferred carbohydrates, whereas Fructose and Sucrose were moderately preferred carbon sources and none of the strains were able to utilize Rhamnose, Malate and Citrate as carbon source Table 4, which is in accordance with the results described by Tchan and New (1984) and as observed by Rao and Gaur (1988).

Based on these studies the isolates were identified as *Azotobacter chroococcum* and labelled as K1, K2, K3, K4, K5, K7, M1, M2, M3, M4, R1, R2, R3, R4, and R5.

The nitrogen fixed per gram of carbon source varied among the strains. It ranged from 5.3 mg to 6.9 mg in isolates of Kolar, 5.6 to 6.8 mg in Mysore and 5.3 to 6.8 mg in Ramnagar soils.

Table 3
Growth characters for authentication and nitrogen fixing ability of *Azotobacter* strains isolated from Ramnagar soil.

Azotobacter Strains	*Gram stain*	*Formation of Microsysts*	*Pigmentation*	*Cultural characters*	*mg N-fixed/g of C source (Mannitol)*
R1	Gram-ve	+	on fifth day dark brown	Good growth flat, entire, wrinkled at the edge of the colony	6.2
R2	Gram-ve	+	On sixth day dark brown	Good growth, flat entire, wrinkled at the edge of the colony	6.8
R3	Gram-ve	+	On fourth day dark brown	Good growth, flat entire, wrinkled at the edge of the colony	6.1
R4	Gram-ve	+	On third day light brown	Moderate growth, flat entire	5.4
R5	Gram-ve	+	On seventh day light brown	Good growth, flat entire, wrinkled at the edge of the colony	5.3
SEM±					0.0707
CD at 5%					0.2131

All values are means of four replications, Gram-ve = Gram negative, + = Microcyst formed.

The nitrogen fixing ability of seventeen *Azotobacter* strains was low to moderate. *Azotobacter* strain CK_2 fixed maimum amount of nitrogen followed by CM_3 and CR_2 strains. However, this was not up to the maximum level of fixation (Goswami, 1976, and Rai and Gaur, 1986).

Summary

An investigation was conducted to isolate, characterize and identify *Azotobacter* spp. from mulberry rhizosphere soil. The seventeen *Azotobacter* strains isolated were gram negative, motile, avoid rods, produced brown black pigment. Formed microcyst and indicated marked variations in growth between different carbohydrates. They were identified as *Azotobacter*

Table 4

Utilization of different carbon sources and authentication of *Azotobacter* strain isolated from soils of Kolar, Mysore and Ramanagar

Azotobacter	*Carbon Source*						
	Man-nitol	*Glu-cose*	*Fru-ctose*	*Suc-rose*	*Rham-nose*	*Mal-ate*	*Cit-rate*
K1	4+	3+	2+	2+	-	-	-
K2	4+	4+	3+	3+	-	-	-
K3	4+	4+	2+	2+	-	-	-
K4	3+	3+	2+	2+	-	-	-
K5	3+	3+	+	+	-	-	-
K6	3+	2+	2+	2+	-	-	-
K7	3+	3+	+	+	-	-	-
M1	4+	4+	2+	2+	-	-	-
M2	3+	3+	2+	2+	-	-	-
M3	4+	4+	2+	3+	-	-	-
M4	3+	3+	+	+	-	-	-
M5	3+	3+	+	+	-	-	-
R1	4+	4+	2+	2+	-	-	-
R2	4+	4+	2+	3+	-	-	-
R3	3+	3+	+	+	-	-	-
R4	3+	3+	2+	2+	-	-	-
R5	3+	3+	+	+	-	-	-

4+ = Maximum

3+ = Good growth

2+ = Moderate growth

+ = Less growth

- = No growth

chroococcum. The maximum nitrogen fixed per gram of carbon source (Mannitol) was 6.9 mg by *Azotobacter* strain CK_2 followed by CM_3 and CR_2 with 6.8 mg of nitrogen each.

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Authors

T.H. Shankarappa and A.R. Madhav Rao
Department of Agricultural Microbiology
Department of Agricultural Sciences
G.K.V.K. Compus
Bangalore-560 065, Karnataka, India

26

Effects of Sulphatic Biofertilizer on Pigeonpea [*Cajanus cajan* (L.) Millsp.]

Introduction

The sulphur requirements of legume plants are comparable to that of phosphorus and therefore, it is recognized the fourth major nutrient in Indian Agriculture. In India 25 million hectare crop land have been reported as sulphur deficient. Plants utilize the sulphur in the form of sulphate (SO_4^{-2}) and microiorganisms are mainly responsible for oxidizing unavailable, elemental or reduced sulphur to available sulphate. There is a possibility of using heterotrophic sulphur oxidizing of sulphur fertilizer in soil which are slow to oxidize. Sulphur ozidizing fungi appear particularly useful for this purpose since their spores or mycelium can be produced economically in large quantities and have good survival characteristics, both in the inoculant and soil. However, no information is available regarding the presence of heterotrophic oxidizing micro-organisms in soil, their sulphur oxidizing potentiality and effect on plant growth. Therefore, present study was undertaken to assess the effect of artificial inoculation of different sulphur oxidizing micro-organisms on growth parameters, yield and quality of Pigeonpea.

Material and Methods

A pot culture experiment was conducted in the glass house during 1992. The design of experiment was a factorial and completely randomized (FCRD) with five replications. The treatments comprised, seven cultures as main factors and two sulphur levels (Table-1) as sub-factors. Ten seeds of *Cajanus cajan* CV. BDN-1 were sown per pot and after germination only five plants were finally kept to record the growth parameters. The recommended dose of NPK (25:50:0 kg/ha) and 100 kg. elemental sulphur/ha was distributed evenly in the soil before sowing. Five most efficient microorganisms which were isolated from 150 soil samples of

different types and locations were multiplied and inoculated in the soil at the rate of 250 ml/pot having 10^7 cells or spores/ml in liquid medium culture.

The soil had an electrical conductivity of 0.5 dSm^{-1} exchangeable sodium percentage of 8.54, pH 7.7 and sulphate content of 9.0 ppm. The soil and plant samples were analysed for different chemical properties by standard methods (Chopra and Kanwar, 1980). The microbial population in soil *viz.*; bacteria and nitrogen fixers were recorded by Most Probable Number (MPN) method (Alexander 1965) and fungi and actinomycetes were recorded by dilution plating technique (Pramer and Schmidtt, 1964 and Wainwright, 1978).

Results and Discussion

The effects of different sulphur oxidizing culture with and without sulphur on the growth and yield of pigeonpea (Table 1) revealed that all the six cultures with and without sulphur addition increased significantly the total number of nodules, dry matter and grain yield/plant over control. However, inoculation of soil with culture along with sulphur reported more than the culture without sulhur. The maximum response was observed in composite culture with sulphur, followed by *T. thiooxidans, A. terreus, T. thioparus, M. cinctum, S. constrictum,* sulphur alone and minimum with the uninoculated control.

The total number of nodules and dry matter of nodules/plant were increased from 19.0 to 29.5 and 170 to 300 mg/plant, respectively, due to inoculation of soil with the various cultures with sulphur over uninoculated control.

The sulphur had significant role in increasing nodulation and nitrogen fixation in grain legumes (Kaul *et al.* 1978; Rey *et al.* 1982; Jain *et al*, 1984 and Aulakh and Pasricha, 1986).

The dry matter weight and grain yield of pigeonpea were found to be increased, significantly over control due to inoculation of culture plus sulphur. The dry matter weight ranged from 3.80 to 5.70 g/plant whereas grain weight ranged from 7.00 to 8.90 g/plant. These results are in agreement with the results reported by Badiger, *et al.* (1982) and Tandon (1986). They reported that application of sulphur resulted in increasing the dry matter and grain yield of different pulses, including pigeonpea from 20 to 50 per cent.

Aulakh and Pasricha (1986) reviewed that, elemental sulphur and other forms of sulphur which could improve the straw and grain yield as well as quality of grain of pigeonpea.

The results regarding sulphur uptake by pigeonpea and protein per cent in grains (Table 2) indicated that sulphur application and culture inoculation both together could increase the sulphur uptake ranging from 260 to 390 mg/100 mg dry weight. Whereas protein percentage ranged from 22.60 to 23.85. These results are in agreement with the results reported by Saraf (1988); Mehta and Singh (1979) and Aulakh and Pasricha (1986). They reported that sulphur had a vital and important role in grain legumes for their protein synthesis.

The effects of cultures with and without sulphur on microbial population (Table 3) revealed that sulphur oxidizing bacteria, fungi, actinomycetes as well as nitrogen fixers were increased due to inoculation of soil with cultures and application of sulphur. However, culture in conjunction with sulphur was more effective that without sulphur. Further, it was noticed that total microbial population was more at 60 days than at harvesting stage of the crop. The composite culture of bacteria and fungi with sulphur treatment showed maximum microbial population

Table 1

Effects sulphur oxidizing micoroorganisms with and without sulphur on the growth of Pigeoopea.

Treatments	*No. of Nodules plant*			*Dry matter weight of nodsules (mg plant)*			*Dry matter weight (g plant)*			*Grain weight (g plant)*		
	With S	*With-out S*	*Mean*	*With S*	*With-out S*	*Mean*	*With S*	*With-out S*	*Mean*	*With S*	*With-out S*	*Mean*
Scolecobasidium constricutus	24.5	20.0	22.25	250	175	212	5.00	3.90	4.45	8.35	7.20	7.77
Myrothecium cinctum	25.0	20.0	22.50	260	180	220	5.15	4.05	4.60	8.50	7.25	7.87
Aspergillus terreus	26.0	21.0	23.50	280	185	232	5.40	4.25	4.82	8.65	7.45	8.05
Thiobacillus thioparus	25.0	20.5	22.75	265	180	222	5.20	4.10	4.65	8.50	7.30	7.90
Thiobacillus thiooxidans	27.0	21.5	24.25	285	190	237	5.50	4.30	4.90	8.70	7.50	8.10
Composite culture	29.5	23.0	26.25	300	210	255	5.70	4.45	5.07	8.90	7.65	8.27
Control	24.0	19.0	21.50	220	170	195	4.60	3.80	4.20	7.80	7.00	7.40
Mean	25.8	20.7		265.7	184		5.22	4.12		8.48	7.33	
	S.E.±	C.D.5%		S.E.±	C.D.5%		S.E.±	C.D.5%		S.E.±	C.D.5%	
Culture	0.31	0.96		3.53	10.60		0.07	0.21		0.08	0.25	
Sulphur	0.52	1.67		1.92	5.77		0.31	0.90		0.26	0.86	
Culture x Sulphur	0.44	1.35		1.10	3.31		0.09	0.30		0.15	0.48	

Table 2
Effects of sulphur oxidizing microorganisms with and without sulphur on sulphur uptake and protein content of Pigeonpea

Treatments	*Sulpur uptake (mg/100g)*			*Protein (%)*		
	with Sulphur	*without sulphur*	*Mean Sulphur*	*With Sulphur*	*Without*	*Mean*
Scolecobasidium constrictum	330	265	297	23.35	22.70	23.02
Myrothecium cinctum	340	270	305	23.40	22.72	23.06
Aspergillus terreus	360	285	322	23.60	22.85	23.22
Thiobacillus thioparus	345	270	307	23.50	22.90	23.12
Thibacilhus thiooxidans	375	290	332	23.70	22.90	23.30
Composite culture	390	300	345	23.85	23.00	23.42
Control	310	260	285	23.15	22.60	22.87
Mean	350	277		23.50	22.78	
	S.E.±	C.D.5%		S.E.±	C.D.5%	
Culture	3.85	11.48		0.38	N.S.	
Sulphur	2.20	6.56		0.46	N.S.	
Culture x Silphur Interaction	3.21	9.71		0.68	N.S.	

Table 3
Effect of inoculation on soil microbial population in Pigeonpea crop

Treatments	*At 60 days*				*After harvesting*			
	Bacteria ($\times 10^5$)	*Fungi* ($\times 10^3$)	*Actinomycetes* ($\times 10^5$)	*Nitrogen fixers* ($\times 10^4$)	*Bacteria* ($\times 10^5$)	*Fungi* ($\times 10^5$)	*Actinomycetes*	*Nitrogen fixers* ($\times 10^5$)
C_1 *Scolecobasidium constrictum*	13.16	16.50	5.16	7.83	10.33	12.83	4.83	6.50
C_2 *Myrothecium cinctum*	14.50	18.00	5.33	8.16	11.50	15.00	5.16	7.50
C_3 *Aspergillus terreus*	14.83	21.83	6.16	8.66	12.00	18.83	6.00	7.83
C_4 *Thiobacillus thioparus*	18.33	10.50	6.16	8.33	16.00	9.66	5.83	8.00
C_5 *Thibacilhus thiooxidans*	20.16	12.66	6.33	8.83	17.16	10.33	6.00	8.00
C_6 Composite culture	21.66	22.16	7.66	9.16	19.33	20.00	6.83	8.50
C_1 + Sulphur	17.66	21.83	6.83	8.16	15.16	17.50	6.50	8.00
C_2 + Sulphur	18.50	22.16	7.16	9.50	16.50	19.33	6.83	8.33
C_3 + Sulphur	20.66	25.50	8.16	9.66	17.16	22.00	7.50	8.83
C_4 + Sulphur	23.66	13.00	8.33	9.50	21.00	11.50	7.50	8.83
C_5 + Sulphur	26.33	14.66	8.50	9.83	22.66	13.50	8.00	9.16
C_6 + Sulphur	28.16	26.33	8.83	10.16	25.50	23.66	8.16	9.83
C_0 + Sulphur	10.50	8.33	4.16	6.00	9.16	8.00	3.83	5.50
C_0 + S_0 (control)	7.50	6.50	3.50	4.16	7.16	6.00	3.16	4.00

amongst all the cultures under study. Similar, results were also noticed by Moser and Olson (1953); Germide *et al.* (1984) and Wainwright (1984).

Summary

A pot culture study on sulphur oxidizing micro-organisms in pigeonpea crop revealed that all the growth parameters *viz*; total number of nodules on roots, dry matter weight of nodules and plants and grain yield were increased, significantly due to inoculation of sulphur oxidizing micro-organisms with or without sulphur. The composite culture of two bacteria viz; *Thiobacillus, Thioparus, Thiooxidans* and three fungi *viz; Scolecobasidium constrictum, Myrothecium cinctum* and *Aspergillus terreus* in conjunction with elemental sulphur found to be significantly, superior over individual culture and sulphur treatment. The effects of bacterial culture (*Thiobacillus thiooxidans*) were on par with fungal culture (*Aspergillus terrous*). The increase in yield up to 27.14% sulphur uptake to 50% and protein content ranging from 22.60% to 23.85% due to inoculation of composite culture with sulphur fertilizer over uninoculated control.

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Authors

D.B. Shinde[1], P.L. Patil[2] and K.K. Khode[3]

1. *Asstt. Microbiologist*
   Central Sugarcane Research Station
   Padegaon, P.O. Nira, Dist. Pune
   Maharrashtra – 412 102, India.
2. *Ex. Associate Dean and Principal of College of Agriculture*
   Dhule-424 004, Maharashtra.
3. *Sugarcane Specialist*
   Central Sugarcane Research Station
   Padegaon, P.O. Nira, Dist. Pune
   Maharashtra – 412 102.

27

Effect of Gamma Irradiation on the Biomass Production, Nodulation and Nitrogen Fixation by Stem Nodulating *Sesbania rostrata*

Introduction

Stem nodulating *Sesbania rostrata* growing as wild plants on water-logged soils in Senegal has recently received particulars attention due to its profuse stem nodulation, fast growth and more active N_2 fixation than most root nodulating, legumes (Singh, *et al.*, 1991). *S. rostrata* can grow in flooded as sell as in dry conditions and can nodulate and fix N_2 at high levels of combined nitrogen (Rinaudo *et al.*, 1988; Kalidurai and Kannaiyan, 1988 and Balasubramani and Kannaiyan, 1990). *S. rostrata* is the fastest N_2 fixing leguminous green manure plant known, fixing 70-90% of the total plant N during 45-55 days (Pareek, *et al.*, 1990). Fifty days old *S. rostrata* could accumulate as high as 190-267 kg N ha^{-1} (Ladha, *et al.*, 1989). The nitrogenase activity and the nodule dry weight were higher in stem nodules than in root nodules of *S. rostrata* (Balasubramani, *et al.*, 1992 and Saraswati, *et al.*, 1992). However, the potential value of *S. rostrata* as nitrogen fixing leguminous green manure could be realized by genetic improvement for important traits. Hence, an attempt was first made to study the effect of different doses of gamma irradiation on the biomass production, nodulation and nitrogen fixation by stem nodulating *Sesbania rostrata*.

Material and Methods

Well filled seeds of *Sesbania rostrata* were hand picked to obtain seed samples of 100g each for gamma irradiation. The sample seeds were packed in butter paper covers and placed in the gamma cell and exposed to gamma irradiation at 50, 55, 60, 65, and 70 kR at various time intervals depending on the dose and intensity of gamma irradiation and the half life period of ^{60}Co. The treated seeds were then sown separately in well poloughed and finely levelled plots of size 14 sq with 30 × 30 cm spacing in randomized block design with 3 replications.

Non-irradiated seeds served as control. The plots were flooded throughout the crop growth period.

The plant height, number of leaves plant^{-1}, number of branches plant^{-1}, biomass (g plant^{-1}) and root and stem nodule numbers plant^{-1} were recorded at 45, 70 and 95 days aftger sowing (DAS). The nitrogenase activity of root and stem nodules were estimated at 45, 70 and 95 DAS as described by Hardy, *et al.* (1968).

Results and Discussion

In general, gamma irradiation resulted is reduced plant height compared to control. Maximum plant height of *S. rostrata* was recorded in control. The plant height decreased with increased levels of gamma irradiation. However, the number of compound leaves plant^{-1} and number of branches plant^{-1} were significantly increase by gamma irradiation than control. Maximum number of leaves and branches plant^{-1} were recorded at 50 kR followed by 55 kR. However, gamma irrigation at 70 kR showed no significant increase in the number of leaves and branches plant^{-1} than control at initial stages of growth (45 DAS). Interestingly gamma irradiation significantly increased the biomass production of *S. rostrata* than control. Maximum biomass of both fresh and dry weight plant^{-1} was recorded in treatment with 50 kR followed by 55, 60, 65 and 70 kR. However, the biomass plant^{-1} at 70 days after sowing with 70 kR was on par with that of control. In general, the growth and biomass production of *S. rostrata* were decreased with increased doses of gamma irradiation (Tables 1 and 2). Branches per plant were found to be negatively correlated to plant height but are positively correlated to biomass per plant.

Table 1

Effect of γ-irradiation on the growth of *Sesbania rostrata*

Treatment	*Plant height (cm)*			*Compound leaves/plant*		
	45 DAS	*70 DAS*	*95 DAS*	*45 DAS*	*70 DAS*	*96DAS*
Control	95.7	225.4	306.4	29.8	63.7	123.5
50 kR	91.5	214.2	297.1	35.0	114.2	189.4
55 kR	89.1	208.8	293.6	33.4	106.5	179.0
60 kR	85.7	202.0	288.3	32.1	95.0	165.4
65 Kr	83.0	195.3	283.6	30.3	88.8	156.2
70 kR	78.8	187.4	275.5	28.6	79.4	143.7
SEd	2.36	5.20	4.04	1.06	6.38	8.37
CD	4.75	10.47	8.13	2.14	12.85	16.83

DAS = Days after sowing

Table 2

Effect of γ-irradiation on the number of branches and biomass of *Sesbania rostrata.*

Tredatment	*Branches/plant*			*Biomass (g/plant)*			
				Fresh weight		*Dry weight*	
	45DAS	*70 DAS*	*95 DAS*	*70 DAS*	*95DAS*	*70 DAS*	*95 DAS*
Control	1.1	2.2	7.9	163.5	273.5	45.4	97.7
50 kR	3.3	11.6	18.4	342.7	592.4	98.8	219.3
55 kR	2.8	10.2	16.6	306.2	529.2	87.7	194.5
60 kR	2.4	8.0	14.2	251.0	441.7	71.5	160.0
65 kR	1.9	7.1	13.8	224.7	399.4	63.3	145.1
70 kR	1.6	5.4	12.4	177.5	323.6	49.6	115.7
SEd	0.38	1.41	1.97	9.81	14.27	3.23	5.73
CD	0.76	2.83	3.97	19.76	28.73	6.50	11.54

DAS = Days after sowing

Gamma irradiation significantly increased stem nodules plant^{-1} over control at 70 and 95 DAS. Maximum stem nodulation was noticed at 50 kR irradiation followed by 55, 60, 65 and 70 kR. With higher doeses of gamma irradiation, stem nodules plant^{-1} decreased but was significantly higher than the control (Table 3). The nitrogenase activity of stem nodules was significantly higher in treatments with 50, 55 and 60 kR than control. However, gamma

Table 3

Effect pf γ-irradiation on the root and stem nodulation of *Sesbania rostrata*

Treatment	*Nodules per plant*					
	45 DAS		*70 DAS*		*95 DAS*	
	Root Nodules	*Stem Nodules*	*Root Nodules*	*Stem Nodules*	*Root Nodules*	*Stem Nodules*
Control	72.4	48.0	26.6	209.3	7.4	478.6
50 kR	69.5	46.5	29.4	445.5	9.2	1011.2
55 kR	71.0	43.7	27.0	391.9	8.5	915.0
60 kR	68.3	40.1	25.1	318.7	7.6	768.9
65 kR	66.2	37.6	24.5	283.2	6.3	694.9
70 kR	64.0	36.3	23.9	230.0	6.0	566.3
SEd	3.75	4.63	3.23	11.80	2.16	24.52
CD	7.56	9.33	6.50	23.76	4.36	49.39

DAS = Days after sowing

irradiation at 65 and 70 kR had no significant effect on stem nodule nitrogenase activity over control. Maximum stem nodule introgenase activity was shown in treatment with 50 kR followed by 55 and 60 kR (Table 4). Gamma irradiation had no significant influence on root nodulation and root nodule nitrogenase activity of *S. rostrata.*

Table 4

Effect of γ-irradiation on the nitrogenase activity of stem and root nodulation of *Sesbania rostrata.*

Treatment	*Nitrogenase activity (η moles produced h $^{1}g^{-1}$ nodules dry weight*					
	45 DAS		*70 DAS*		*95 DAS*	
	Root Nodules	*Stem Nodules*	*Root Nodules*	*Stem Nodules*	*Root Nodules*	*Stem Nodules*
Control	139.5	256.3	89.1	384.4	60.7	338.5
50 kR	136.7	253.2	94.6	432.9	59.5	396.2
55 kR	138.2	257.5	93.0	418.6	61.4	381.6
60 kR	135.5	248.4	92.3	412.1	57.0	373.5
65 kR	133.1	243.6	89.8	393.7	56.9	357.8
70 kR	135.0	240.9	88.5	380.5	57.8	348.0
SEd	3.66	8.70	3.24	12.40	2.37	11.53
CD	7.79	18.53	6.91	26.43	5.06	24.58

DAS = Days after sowing

Gamma irradiation of cowpea at 60 kR was found to be lethal whereas 10 kR significantly increased the number of flowers, pods, pod length and seed yield per plant while the same decreased at 20 kR and above (Louis and Kadambavanasundaram, 1973). Dose dependent decrease in seedling emergence, plant height, survival pollen fertility, pods per plant and pod length of greengram (Rathinaswamy et al., 1978) and cowpea (Packiaraj, 1988) were recorded. Growth variations in cowpea upon gamma irradiation were attributed to alterations in the nucleotides (Lukas, 1971). Moreover, the mutagenic sensitivity of any biological material can be attributed to the level of differentiation and development of embryo at the time of treatment and also to the extent of damage to the growth processes like rate of cell division, cell elongation, hormone production and various stages of biosynthetic pathways (Scholz and Lehman, 1962).

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Authors

S. Sundaravarathan and S. Kannaiyan
Department of Agricuture Microbiology
Tamil Nadu Agricultural University
Coimbatore-641 003, T.N., India.

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Activity of Ammonia Assimilating Enzymes in Nodules of *Sesbania rostrata* Mutants

Introduction

Within the legume nodules, dinitrogen fixation occurs wholly inside the bacteroids by the nitrogenase enzyme (Raju, 1982). Ammonia formed during fixation has to be transferred from the fixation site for its uninterrupted formation of amino acids and other proteinaceous compounds. Ammonia produced as a result of dinitrogen fixation is incorporated into organic compounds before transportation to roots and shoots. Assimilation of ammonia by various ammonia assimilating enzymes is an integral part of the overall biological nitrogen fixation process and is an essential process like nitrogenase reaction. Hence, an attempt was made to determine the activity of ammonia assimilating enzymes in *Sesbania rostrata* mutants *viz.*, MB-SS-SK, DB-SS-SK, PF-SS-SK and TP-SS-SK in comparison with that of wild parent.

Material and Methods

One gramme of nodules were extracted with 10 ml of ice-cold 100 mM phosphate buffer (pH 7.5) containing 1 mM disodium EDTA, 1 mM dithioerythritol and 1 per cent polyvinyl pyrolidone (PVP). The extract was centrifuged at 10,000 rpm for 30 min at 4°C. The supernatant was collected and used as enzyme source for assay of Glutamine synthetase (Shapiro and Stadman, 1970), Glutamate synthase (Vandecastelle *et al.*, 1975) and Glutamate dehydrogenase (Doherty, 1970) activity. The protein content in the enzyme extract was estimated as per the method of Lowry, *et al.* (1951).

Glutamine synthetase was expressed in η moles of gamma-glutamyl hydroxamate formed min^{-1} mg^{-1} protein. Glutamate synthase and Glutamate dehydrogenase were expressed as η moles of NAD (P) H oxidized min^{-1} mg^{-1} protein.

Results and Discussion

The glutamine synthetase (GS), glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH) activities in stem nodules of MB-SS-SK mutant were significantly higher than in wild type. The activity of ammonia assimilating enzymes in stem nodules of all other mutants was on par with that of wild type. In general, the activity of ammonia assimilating enzymes in root nodules of *S. rostrata* mutants did not differ significantly with that of wild type. Maximum activity of ammonia assimilating enzymes was recorded in nodules of MB-SS-SK mutant (Table 1). Increase in the activitiy of ammonia assimilating enzymes in stem nodules of MB-SS-SK mutant could be attributed to increased nitrogen fixation and the proximity to photosynthate and higher phosphoenolpyruvate carboxylase (PEPCase) activity in the nodular tissue. The nonphotosynthetic CO_2 fixation occurs via PEPCase and acts as a mechanism for recovering some of the respired CO_2, thus increasing nodule efficiency (Layzell, *et al.*, 1979) and maintaining pools of tricarboxylic cycle intermediates required for ammonia assimilation and amino acid biosynthesis (Coker and Schubert, 1981).

Table 1

Activity of ammonia assimilating enzymes in roots and stem nodules of *Sesbania rostrata.*

Mutants	*GS**		*GOGAT***		*GDH***	
	Root Nodule	*Stem Nodule*	*Root Nodule*	*Stem Nodule*	*Root Nodule*	*Stem Nodule*
DB-SS-SK	366.8	448.7	204.1	246.5	62.7	79.0
MB-SS-SK	375.7	458.5	210.0	254.3	67.4	87.6
TP-SS-SK	363.9	440.4	199.7	235.8	56.5	74.4
PF-SS-SK	369.2	451.0	206.8	251.0	65.1	82.1
Wild Plant	365.1	443.8	201.5	239.2	59.6	76.2
SEd	5.68	5.82	5.31	6.35	4.71	5.06
CD	12.38	12.69	11.57	13.83	10.27	11.03

GS = Glutamine synthetase; GOGAT = Glutamate synthase;

GDH = Glutamate dehydrogenase; *=η moles of γ-glutamy hydroxamate formed $min^{-1}.mg^{-1}$ protein;

**η moles of NAD (P) H oxidized $min^{-1}.mg^{-1}$ protein.

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Authors

S. Sundarvarathan and S. Kannaiyan
Department of Agricultural Microbiology
Tamil Nadu Agricultural University
Coimbatore-641 003, T.N., India.

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Development of *Sesbania rostrata* Mutants by Gamma Irradiation

Introduction

Nitrogen fixation and biomass production by stem nodulating *S. rostrata* is variable and depends on physical, environmental, nutritional and biological factors. The potential value of *S. rostrata* as nitrogen fixing leguminous green manure cannot be realized without the genetic improvement for important traits. The genetic improvement of *S. rostrata* could be accomplished by different processes such as traditional selection and breeding, sexual hybridization through protoplast fusion and induced mutagenesis.

Induced mutations are considered as alternative to spontaneous mutations in plant improvement programmes. Mutagens are considered as powerful tool in the hands of plant breeders for improving and evolving new crop varieties. Since 1970, the use of mutation breeding as an adjunct to the conventional crop improvement programme is on the increase. Besides providing new material for evolution, mutagens also provide materials for recombination and selection of genetically altered strains. Mutation breeding is necessary for qualitative and quantitative characters for rectifying the specific defects in an otherwise will adapted commercial variety (Stubbe, 1959). Hence, an attempt was made to develop mutants of *Sesbania rostrata* by gamma irradiation for higher biomass production, nodulation and nitrogen fixation.

Material and Methods

Well filled seeds of *Sesbania rostrata* were hand picked to obtain seed samples of 100g each for gamma irradiation. The sample seeds were packed in butter paper covers and placed in the gamma cell and exposed to gamma irradiation at 50, 55, 60, 65, and 70 kR at various time intervals depending on the dose and intensity of gamma irradiation and the half-life period of ^{60}Co. The treated seeds were then sown separately in well ploughed and finely

levelled plots of size 14 sq. in with 30 × 30 cm spacing in randomized block design with 3 replications. Non-irradiated seeds served as control. The plots were flooded throughout the crop growth period.

The plants were observed for any morphological variation throughout the crop growth period. Plant height, number of leaves per plant, number of branches per plant, biomass (g/plant) and root and stem nodule numbers per plant were recorded at 90 days after sowing (DAS). The nitrogenase activity of root and stem nodules was estimated at 90 DAS as described by Hardy *et al.*, (1968). The total nitrogen (Humphries, 1956), phosphorus and potassium (Jackson, 1973) were estimated at 90 DAS. Available calcium and magnesium in leaves of *S. rostrata* mutants were estimated as per the method of Jackson (1973). The micronutrients such as iron, manganese, zinc and copper were estimated by feeding triple acid extract in the atomic absorption spectrophotometer (AA 120 using 2483.3 A°, 2794.8 A°, 2138.6 A° and 3247.5 A° wavelength respectively (Jackson, 1973).

Results and Discussion

Four morphologically different *S. rostrata* mutants *viz.*, (i) dual branching plant, (ii) multiple branching plant, (iii) twisted plant, and (iv) purple flowered plant were selected based on the mutagenic effect of gamma irradiation and designated as DB-SS-SK, MB-SS-SK, TP-SS-SK, and PF-SS-SK respectively. The plant height of mutants was significantly less than that of wild type which recorded the maximum plant height. Among the mutants DB-SS-SK and PF-SS-SK showed higher plant height followed by twisted plant and multiple branching type. However, the number of leaves per plant and branches per plant were maximum in MB-SS-SK followed by PF-SS-SK mutants respectively (Table 1). The number of leaves and branches per plant in

Table 1

Selection of mutants of *S. rostrata* based on phenotypic characters at 90 days after sowing

Mutants	*Plant height (cm)*	*No. of Compounds Leaves/plant*	*No. of branches plant*	*Biomass (g/plant)*	
				Fresh wt.	*Dry Wt.*
DB-SS-SK	310.5	157.9	13.1	418.3	149.9
MB-SS-SK	293.3	218.5	21.0	627.1	222.5
TP-SS-SK	295.0	132.3	9.7	329.9	118.7
PF-SS-SK	304.1	174.1	15.9	486.5	173.7
Wild Plant	321.7	129.0	8.3	317.0	112.1
SEd	4.35	9.81	1.50	8.51	3.73
CD	9.47	21.38	3.26	18.54	8.13

DS-SS-SK–Dual branching plant; MB-SS-SK – Multiple branching plant;
TP-SS-SK–Twisted plant; PF-SS-SK – Pumple flowered plant.

TP-SS-SK were on par with that of wild type which recorded least number of leaves and branches per plant. Maximum biomass per plant was produced by MB-SS-SK followed by PF-SS-SK and DB-SS-SK mutants respectively. The biomass produced by the mutant TP-SS-SK was on par with wild type. In general, the mutants were short but produced more number of leaves, branches and biomass per plant compared to wild type. The higher biomass production was attributed to more number of branches per plant, compound leaves per plant, increased stem nodulation and nitrogen fixation. Joshua and Ramani (1993) have irradiated *S. rostrata* seeds with gamma rays and selected an induced mutant with extended vegetative phase. The mutant was nonphotosensitive and by virtue of its longer vegetative phase produced higher biomass irrespective of the time of sowing. Kumar (1994) recorded higher biomass, lesser doubling time and higher relative growth rate of *Azolla* mutants and attributed them to the desirable changes in the frond characters.

The MB-SS-SK mutant showed maximum stem nodulation followed by PF-SS-SK, DB-SS-SK and TP-SS-SK respectively. The wild type produced less stem nodules than the mutants. The stem nodule nitrogenase activity of MB-SS-SK, PF-SS-SK and DB-SS-SK mutants was on par with each other but significantly higher than the wild type and TP-SS-SK mutant (Table 2). However, the root nodulation and root nodule nitrogenase activity in mutants were not significantly higher than that of wild type. Carroll, *et al.* (1985a) produced 15 EMS induced soybean mutants with increased nodulation, nitrogen fixation and nitrate tolerance and designated them as nitrate tolerant symbiotic (nts) mutants or supernodulators which were defective in the autoregulatory control of nodulation (Delves, *et al.*, 1986 and 1987). Genetic analysis indicated that the increased nodulation in soybean was controlled by a single Mendelian recessive gene

Table 2
Nodulation and nitrogen fixing activity of *S. rostrata* mutants at 90 days after sowing.

Mutants	*Nodule numbers/plant*		*Nitrogenase activity**	
	Root nodule	*Stem nodule*	*Root Nodule*	*Stem nodule*
DB-SS-SK	12.1	776.5	66.1	388.7
MB-SS-SK	15.5	1224.3	69.7	407.5
TP-SS-SK	10.0	556.7	64.3	353.0
PF-SS-SK	13.9	852.0	67.5	395.9
Wild Plant	10.7	531.1	65.0	361.3
SEd	3.15	17.04	2.83	10.86
CD	6.87	37.14	6.17	23.66

*= η moles ethylene produced h^{-1} g^{-1} nodule.

operating through the shoot (Delves, *et al.*, 1986 and Lee, *et al.*, 1991). There was no evidence of host and rhizobial strain specificity affecting expression of the supernodulation trait (Carroll, *et al.*, 1985b and Gremaud and Harper, 1989).

The nitrogen content of MB-SS-SK mutant was on par with PF-SS-SK but was significantly higher than the mutants DB-SS-SK, TP-SS and wild type. The phosphorus and potassium content of the mutant MB-SS-SK was significantly higher than TP-SS-SK mutant and wild type but was on par with the mutants PF-SS-SK and DB-SS-SK. Significantly increased calcium and magnesium content was recorded in MB-SS-SK mutant than wild type (Table 3). However, there was no significant increase in the micronutrients *viz.*, iron, manganese, zinc and copper content of *S. rostrata* mutants than wild type (Table 4). In general, maximum nitrogen,

Table 3
Nutrient content of *S. rostrata* mutants at 90 days after sowing.

Mutants	*Nitrogen (%)*	*Phosphorus (%)*	*Potassium (%)*	*Calcium (%)*	*Magnesium (%)*
DB-SS-SK	3.73	0.57	1.59	1.12	0.89
MB-SS-SK	3.84	0.59	1.63	1.14	0.91
TP-SS-SK	3.59	0.54	1.53	1.11	0.89
PF-SS-SK	3.79	0.58	1.61	1.12	0.90
Wild plant	3.61	0.55	1.56	1.10	0.86
SEd	0.045	0.012	0.019	0.017	0.015
CD	0.098	0.027	0.043	0.038	0.033

Table 4
Micronutrient content of *S. rostrata* mutants at 90 days after sowing

Mutants	*Iron (ppm)*	*Manganese (ppm)*	*Zinc (ppm)*	*Copper (ppm)*
DB-SS-SK	336.0	193.9	59.1	48.5
MB-SS-SK	346.7	198.5	61.3	47.3
TP-SS-SK	332.5	188.0	58.5	45.1
PF-SS-SK	340.1	194.7	63.1	50.9
Wild plant	330.3	190.1	60.5	46.0
SEd	9.11	5.64	3.46	3.17
CD	19.84	12.29	7.55	6.91

phosphorus, potassium, calcium and magnesium content was noticed in mutants MB-SS-SK followed by PF-SS-SK, DB-SS-SK, TP-SS-SK and wild type. Similar increase in the accumulation of nutrients was observed in soybean (Day, *et al.*, 1986; Hansen, *et al.*, 1992 and Ohyama, *et al.*, 1993) and Azolla mutants (Kumar, 1994) than their wild parent. The present study has helped to identify *S. rostrata* mutants with increased nodulation, nitrogen fixation and nutrient content compared to the wild parent which may be exploited as a potential biofertilizer for augmenting the rice production.

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Authors

S. Sundaravarathan and S. Kannaiyan
Department of Agricultural Microbiology
Tamil Nadu Agricultural University
Coimbatore-641 003, T.N., India.

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Biofertilizer from Sludge of Distillery Waste Treatment Plant: A Laboratory Study

Introduction

In India distilleries mainly use sugarcane molasses as raw material which contains high amounts of sugars, non-sugar organic matter proteins, salts and vitamins. The process of alcohol manufacture is peculiar and it becomes necessary to use large volumes of water to dilute this raw material (sugarcane molasses) and about 90% of the fermenting material is discharged as a waste. The waste carries a big pollutional load its BOD and COD values being 30,000 – 70,000mg/L and 65,000 – 1,30,000mg/L, respectively, in the case of batch process of alcohol production and 90,000-95,000mg/L and 2,00,000-2,20,000mg/L, respectively in the case of continuous process of alcohol manufacture (Table-1). The waste in addition has a deep dark brown colour which persists after degradation and thus makes difficult, the discharge of this treated effluent in the running waters.

Initially anaerobic treatment studies on the waste were carried out in this laboratory (Pathade G.R.) using waste under concern to recover biogas, results of which indicate that although good quality and quantity of biogas is recovered, the final effluent contains significant amount of organics (Table 2).

Attempts were made in our laboratory to treat anaerobically digested distillery effluent by aerobic treatment method using developed mixed microbial seed culture of yeasts (*Schizosaccharomyces pombe* and *Leucosporidium scottii*) and *bacteria* (*Bacillus macerans, Lactobacillus agilis* and *Citrobacter freundii*).

Material and Methods

Apparatus: The aerobic treatment apparatus consisted of aeration tanks of dimensions 7 × 15 × 24 cms with working volume of 1 litre (Bench scale) and 14-L capacity rectangular reactor

Table 1
Characteristics of distillery wastes

Sr. No.	*Characteristics*	*Process*	
		Batch	*continuous*
1.	Colour	Dark brown	Dark brown
2.	Odour	Jaggery smell	Jaggery smell
3.	Temperature (at exit) degrees C1	80-105	80-105
4.	pH	3-5.4	3-5.4
5.	BODs (mg/L)	30,000-70,000	90,000-95,000
6.	COD (mg/L)	65,000-1,30,000	2,00,000-2,20,000
7.	Total solids (mg/L) (TS)	30,000-1,00,000	2,70,000-2,80,000
8.	Total volatile solids (TVS) mg/L)	50,000-60,000	-
9.	Total suspended solids (TSS) mg/L	350	-
10.	Total Dissolved solids (TDS) mg/L	80,000-90,000	-
11.	Total Inorganic solids (ash) mg/L	20,000-30,000	-
12.	Total volatile acids mg/L	5000-6000	-
13.	Total sugar (TS) (as acitic acid) mg/L	8000-10,000	-
14.	Total nitrogen (Kjeldahl) mg/L	1000-2000	2000-2500
15.	Free ammonia (as N) mg/L	20-40	-
16.	Total acidity (as Ca Co_3) mg/L	15,000-19,000	-
17.	Mineral acidity (as Ca Co_3) mg/L	1500-2000	-
18.	Potassium (K) mg/L	8000-12,000	18,000-20,000
19.	Phosphorous (as PO_4) mg/L	8000-12,000	1000-1500
20.	Sulphates (as SO_4) mg/L	2000-6000	15000-18000
21.	Chlorides (as Cl)mg/L	3000-5000	13,000-15,000
22.	Acid insolubles (mg/L)	100-150	-
23.	Sodium (as Na) mg/L	150-200	300-500
24.	Calcium (as Ca) mg/L	500-600	2600-2700
25.	Magnesium (as Mg) mg/L	2000-2500	-
26.	Iron (as Fe) mg/L	100-300	-
27.	BOD: N: P ratio	100:3.5: 26.7-100:2.86: 17.2	100:2.2: 1:1-100:2.7: 1.58

Table 2
Characteristics of effluent from anaerobic digester run on distillery waste.

Characteristics	*Value*
pH	7.3
COD (mg/L)	31000
BOD_5 (mg/L)	7000
Total nitrogen (mg/L)	1100
Total phosphorous (mg/L)	10-28
BOD: N:P ratio	100: 15.7: 1.71
Average volume of biogas	
Produced litres/litre/waste/day	30

(scale-up studies) with working volume of 6.9-L. Aeration was done through aerators with perforated diffuser stones and electrically operated stirrer to maintain DO levels of 3.0mg/L.

Chemical analysis: COD, BOD, MLSS, SS, DO, total nitrogen, total phosphorus and sulfates were analysed by methods described in APHA (1985) and Trivedy and Goel (1984). The pH was measured and monitored on a Systronics-335 model digital pH meter.

Biodegradation studies: MLSS was developed using developed mixed microbial seed culture and HRT was optimized using optimized MLSS levels (Table 3).

Table 3
Results of extent of biodegradation at bench and scale up level studies.

Studies	*MLSS (mg/L)*	*HRT (days)*	*Residual COD (mg/L)*	*Residual BOD (mg/L)*	*%COD reduction*	*%BOD reduction*
Bench level Studies (1-L)	5700-5860	6 days	3690	131	87.6	98.0
Scale-up studies (6.9-L)	5000	6 days	3400	160	88.47	97.68

Results and Discussion

Table 1 and Table 2 indicate high organic content of the distillery wastes while Table-3 indicates significant % BOD reduction at bench scale (98%) and scale up level (97.68%) studies using 6 days HRT and 5000-5860mg/L MLSS levels.

The sludge accumulated in the aerobic treatment of anaerobically digested distillery waste has high drainability (12-14hrs) and contains 4.0-5.0% total nitrogen and 0.3-0.4% potassium.

Table 4
Characteristics of sludge accumulated during aerobic biodergadation of anaerabically digested distillery waste.

Sludge parameters	*Values*	
	Range	*average*
1. SV –30 (ml/L)	90-95	93
2. SVI (ml)	15.4-16.5	15.9
3. Drainability (hrs)	12-14	12.6
4. %dry solids	6-6.5	6.3
5. % total nitrogen	4.0-5.0	4.7
6. % Potassium	0.3-0.4	0.37

Conclusion

The anaerobically digested effluent contains significant amount of organics as well as nitrogen and phosphorus. At 6-days retention time, 5000-5860mg/L MLSS and 3.0mg/L DO levels, residual BOD values of effluent coming out of aerobic treatment of anaerobically digested distillery waste were in the range of 97.68-98%.

The sludge of this aerobic treatment showed high drainability and whose total nitrogen was on an average 4.7% and % K was 0.37.

Studies indicate that the sludge accumulated can be used as a good biofertilizer which possesses N and K-levels in the amounts to meet its fertilizer value as per Wisconsin Department of Agriculture (James, 1971).

Summary

Distillery ancillary of sugar industry produces large volumes of waste water everyday containing tremendous pollutional load. In India, sugarcane molasses based distilleries produce over 10,000 million litres of waste water every year.

Distilleries situated near Karad produce about 300-600m^3 waste water/day/distillery which is distinctly acidic in nature having a pH value around 4.2 and carrying a big organic load, its BOD values being in the range of 30,000 to 52,000 mg/L and dark brown in colour.

In our investigation mixed microbial seed culture was developed using distillery waste acclimatized isolates of yeast (*Schizosaccharomyces pombe* and *Leucosporidium scottii*) and bacteria (*Bacillus macerans. lactobacillus agilis* and *Citrobacter freundii*). This mixed seed culture was employed aerobically to the treatment of anaerobically digested distillery waste in the laboratory scale (1-10L) treatment plants.

It was found that the sludge accumulated in the aerobic treatment process contains large amount of microbial biomass (coming from yeast and bacterial seed) and some organic waste solids. The % dry solids found in the sludge were 6-6.5%. The dry sludge solids were found to

contain 4-5% nitrogen and 0.3-0.4% potassium which indicated that this sludge produced after treatment of anaerobically digested distillery waste using developed mixed seed culture can be used as a biofertilizer to ameliorate the soils.

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Authors

G.R. Pathade and S.C. Kale
P.G. Department of Microbiology
Y.C. College of Science
Vidyanagar, Karad-415 124, Maharashtra

31

Significance of *Bacillus* and *Pseudomonas* in Decolourization and Degradation of Dye Effluent

Introduction

Under textile industry for the production of 1 kg of finished product needs with 150 to 200 litres of water, which are let into the nearby river basin. These wastewater slowly percolated deep in soil and affected the aquifers, underground water and later it affects the surface soil and crop productivity. There are several attempts made in last two decades with certain physical and chemical processes to decolourize the wastewater from textile and dye factory, but the resultant fluid contains with high EC, soluble salts and renders them unusable for either to crop irrigation of for the industrial reuse. Hence the present investigation is being attempted to alleviate the impact of dye colour from the dye factory effluent with least way of sludge and waste accumulation several microbial cultures were employed with static and shake culture along with and without the use of enrichment techniques and then assessed the decolourization and analyzed the physico-chemical properties of the treated samles.

Material and Methods

The dye factory effluents were collected from the three locations in and around Coimbatore textile city and were ascertained for their various physico-chemical properties (colour,pH, EC, TSS, TDS, SAR, RSC, BOD, COD, DO and availability of Na, K, Mg, Cl, SO_4, CO_3, HCO_3) in the laboratory analysis with standard procedures. To compare the effective decolourization with microbial system, elite aerobic bacteria and filamentous fungi were isolated, authenticated and test verified from the samples collected from the dye effluent drenched soils. Based on the test attempts, two bacteria (*Bacillus* and *Pseudomonas*) and three fungi (*Aspergillus*, *Tremetus* and *Phaenerocheates*) were taken for further decolourization experiment. Based on its biogrowth and protein value the bacterial and fungal cultures were inoculated at various dose levels (1:10

to 1:100) and incubated in static and shake culture for 24 hr to 120 hr of incubation. The colour reduction was observed with least cumulation of sludge was noticed. Later on to improve the efficacy these cultures were tends to enriched with nutrients *viz.*, carbon at 0.1 per cent in the form of glucose and nitrogen at 0.01 per cent in the form of urea were ascertained with various levels of test verification and the same were incubated under shake and static incubation.

Results and Discussion

The nature of dye effluents collected from the dye factory were analysed and tabulated in Table-1. The results on the selection of microbes and its natures of colour reduction and physico-chemical nature of biotreated samples (dye effluent) are presented in Tables 2-4.

Table 1

Physicochemical nature of raw dye factory effluent.

S.No.	*Characters*	*Values, treated effluent*	
		Raw	*Chemical Process*
1.	Colour	Variable	Pure white
2.	pH	9.1	8.9
3.	EC	2.1	6.8
4.	Na (mg/L)	265	129
5.	K (mg/L)	11.7	13
6.	Mg (mg/L)	15.3	12
7.	Ca (mg/L)	6.5	8
8.	Cl (mg/L)	51	36
9.	SO_4(mg/L)	195	101
10.	CO_3 (mg/L)	81	34
11.	HCO_3 (mg/L)	99	53
12.	DO (mg/L)	13	10
13.	BOD (mg/L)	3200	1010
14.	COD (mg/L)	2590	690
15.	SSP	39	45
16.	PSP	16	14
17.	SAR	32	18
18.	RSC	23	15

Physico-Chemical Properties of Dye Effluent

The results indicated that the raw effluent have high pH, EC, soluble sodic salts, with high BOD and COD values with low OD requirement. The main impact will be the sodicity and colouring pigments which need with effective treatment process. The physico-chemical of the dye effluent were also reported earlier (Agarwal and Agarwal, 1986; Kothandaraman, *et al.*, 1996). These effluents found to cause the severe impact on the growth establishment of crops (Dyana, 1987; Mahapatra, *et al.*, 1990 and Sandya Rani and Ramasami, 1996). To eliminate

the illeffect of the dye effluent several physico-chemical process were reported (McKay, 1979; Genadi, 1991). But this will not give up the better resolution on the reuse of treated effluent for either crop or of industrial recycling process.

Table 2
Types of microbes involved and methods of adoptation

1. ***Microorganisms involved:***

Bacteria	***Fungi***
Bacillus	*Aspergillus*
Pseudomonas	*Tremetus*
Derxia	*Phaenerocheates*

2. ***Enrichment Techniques:***

   Nitrogen: 0.01 per cent as urea.
   Carbon: 0.1 per cent as glucose with and without aeration

3. ***Incubation and inoculation rate:***

   24 hr to 120 hrs.
   Dose: 1:50 to 1:100 (culture/effluent mix)

4. ***Assessment after incubation:***

   a. Change in colour reduction in percentage over control
   b. Assessing the physio-chemical character of treated effluent
   c. Ascertaining the population density in treated fluid.

Isolation Authentication and Screening of Microbial Cultures for Decolourisation

To attain with effective method of decolourization we have to make use of certain microbial system that can able to do better cleavage of these organic and inorganic dye constituent by means of its metabolic activities *viz.* acid production, enzymatic reactions. The dye effluent drenched soils used for isolating various microbes and based on the preliminary screening two bacteria (*Bacillus* and *Pseudomonas*) and *fungi* (*Tremetus, Aspergillus* and *Phaenerocheates*) were selected for further investigation. These microbes were inoculated on the dye effluents and incubated under static and shake culture method. Waterman, *et al.* (1980) investigated the possible rate of degradation by microbes.

Role of Biosystem on Dye Effluent Degradation

The microbial inoculation of bacteria recorded 54-59 per cent colour reduction under static condition whereas in shake culture recorded at 52-60 per cent. Among the two bacteria efficacy was observed with *Bacillus* followed by *Pseudomonas*. The effect of fungi under static condition recorded 41 to 52 per cent under normal inoculation whereas with enrichment the same microbes

Table 3
Rate of Decolourization of dye factory effluent by microbial inoculation

Microbial Consortia	*(Percentage of colour reduction over control)*			
	Without Static	*Enrichment Aerated*	*With Static*	*Enrichment Aerated*
Raw Effluent	-	-	-	-
Bacteria:				
Bacillus	50	59	64	74
Pseudomonas	46	54	60	67
Fungi:				
Tremetus	43	62	58	69
Aspergillus	52	59	60	66
Phaenerochetes	41	54	54	58

(OD values observed at 485 nm)

Table 4
Physio-chemical nature of treated dye effluent

	Physio-chemical Characters	*Raw Effluent*	*Biotreted*	
			Bacterial	*Fungal*
1.	Colour	Dark blue	Dull white	Dull white
2.	pH	9.2	7.8	7.3
3.	EC (DSM^1)	3.1	1.4	1.6
4.	Na	279	32	39
5.	K	12	15	17
6.	Mg	16	11	14
7.	Ca	29	32	41
8.	So_4	220	98	67
9.	Cl	35	10	12
10.	CO_3	94	68	76
11.	HCO_3	123	76	92
12.	BOD	2500	440	520
13.	COD	3500	310	367
14.	SSP	29	11	13

All the values are in mg/L except colour and pH, otherwise stated.

recorded 52 to 60 per cent in static condition. The effect was found more when bacteria and fungi were given with 0.1 per cent nitrogen registered with 58 to 74 per cent decolourization. Eaton, *et al.* (1980) reported fungi can decolourize the dye effluent. Mochi, *et al.* (1991) revealed the use of microbial system for effective docolourization. Bajapai *et al.* (1993) and Chao and Lek (1994) narrated the role of white rot fungi dye decolourization. Nizzam *et al.* (1996) confirmed the microbial processes for effective decolourization of azo, diazo and reactive dye components. De Angelo and Rodriquiz (1984) indicated the use of yeast in dye decolourization. Similar to the present investigation the scientists elsewhere also recommended bacteria (Horishu *et al.*, 1977 – *Bacillus subtilis*; Hu, 1974 – *Pseudomonas*) and several filamentous white rot fungi (Rye and Wecon, 1992 for-*Aspergillus*; Archibald *et al.*, 1990- for *Coriaria versicolor*, Gleem and Gold, 1985; Collen *et al.*, 1990; Parzccoski and Crawford, 1991; Capalesh and Sharma, 1992 for *Phaenerocheate*) for colour reduction.

Comparative Performance Treated Eflluent (Biotreated Dye Effluent)

The biologically treated effluent found to be safer when compared to raw or chemically treated one with specific to reduced pH, EC and requirement of BOD and COD values. Also by the bacterial and fungal decolourized materials found to accumulate the least level of sludges than that of chemical processes. There will be least account on the sodium concentration than that of chemical one.

From this investigation the dye factory effluent can be suitably redecolourized with the use of certain elite microbe (bacteria and fungi) under enriched situation will let out effluent as pollution reduced condition over any other treatment procedure. There will be a need in future to employ the combination treatment by inclusion of biosystem and certain chemical application.

Summary

To alluviate the ill effect of dye factory effluent and other textile wastes water with specific role on the colour reduction and its degradation, several methods were employed. To expertise the technique an attempt was made to isolate certain biosystem (fungi and bacteria) from the dye effluent drenched soil and screened the elite one for further investigation. Among the bacteria *Bacillus* and *Pseudomonas* had exhibited better effect whereas in fungi *Trametus*, *Aspergillus* and *Phanerochetes* registered with better decolourization. To hazen the effect *in vitro* studies were conducted with enrichment techniques by using carbon (0.1% and nitrogen 0.01%) recorded better decolourization in four days with 65 to 70 per cent effect as compared to normal effect of 48-55 per cent. The chemical nature of the treated effluent with biosystem recorded with low EC, BOD and COD and the accumulation of solid sludge was also reduced considerably over the conventional method.

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Authors

P. Jothimani, J. Prabhakaran and G. Rajannan
Department of Environmental Sciences
Tamil Nadu Agricultural University
Coimbatore-641 003, Tamil Nadu, India.

32

Bioutilization and Decolorization of Paper Mill Effluent Waste

Intorduction

Paper mills have their own useful contribution to the economy of country but their contribution to organic load especially on receiving stream is a matter of serious concern. Lignin and its derivatives are difficult to degrade by physical, chemical or biological methods. Therefore, conventional biological treatments are only moderately effective in decreasing effluent COD. Consequently, the paper industry cannot satisfy the effluent discharge limits for COD and BOD that have been imposed by environmental pollution control board. Many technologies are available for reducing BOD but little is known for the colour removal in paper mill effluent treatment.

Material and Methods

Paper Mill effluent was collected from Padamsi Mill, Pune and stored in bulk in plastic cans with tight lid at room temperature. Estimation of UV absorbing material and color was done by UV/visible spectroscopy.

Acid Treatment

Effluent was suitably diluted and pH was adjusted to 5.0-5. 6.0 with 1N HCl and 50% H_2SO_4. The extent of decolorization was read at 480 nm.

The effluent was supplemented with 5:0 g/L of easily metabolizable carbon source such as glucose, sucrose or glycerol (0.5-10.%),. pH was adjusted in the range of 5.0-6.0 and the liquid was suitably diluted.

The M_1 and M_2 consortium were added as fresh weigh under non-sterile conditions. The cultures were incubated at 30°C + 1°C under shaking for 0-5 days.

The effect of carbon source, pH, dilution and incubation time was standardized as evident by the maximum degree of decolorization.

Decolorization Measurements

After treatment, the clear filtrate was used for the measurement of decrease in absorbence at wavelengths 210-300nm and color at 480nm. Zero hour effluent served as the control.

Three methods were followed to study the decolorization of paper mill effluent using M_1, M_2 and $M_1 + M_2$ microbial consortium. They are:

1. sterilized, pH adjusted effluent,
2. sterilized pH unadjusted effluent,
3. unsterilized pH adjusted effluent.

In all the cases after the incubation is over, biomass of fungal mat was separated by filtering through muslin cloth. The decrease in lignin content was measured at 275nm and that in color was measured at 480nm using uninoculated sample as the control (Hiroshi More *et al.*, 1995).

Results and Discussion

The paper mill effluent has the following characteristics: The effluent is dark brown in color with a strong smell. It is highly basic with a pH 10.0 to 12.0 when fresh and 7.0 – 7.5 on storage after 3 months.

Biological Method Using Mixed Consortium

Since chemical treatment methods result in voluminous sludges whose disposal is a problem, the biological treatment with mixed microbial culture consortium was tried.

The microbial cultures used in these studies have never been used before for color or COD removal. These cultures by virtue of the presence of anti-microbial compounds in them, could be grown under non-sterile condition among the treatments with mixed cultures in sterilized and pH adjusted or unadjusted effluents, there was no effective growth of the organisms and hence no significant colour removal was achieved.

In the case of non-sterilized, pH adjusted effluent, there was some growth and color removal when M_1 and M_2 were inoculated together, the growth and color removal was very good under optimum conditions of carbon source, pH, culturing time (Figs. 1 and 2). COD and BOD removal was also >90%.

On glucose containing medium there was no growth and hence no color removal was observed.

Glycerol was found to be the best co-substrate. Table 1 gives results of the effect of glycerol on COD, BOD and color removal from the black liquor.

Almost all BOD and color was removed in presence of 1% glycerol. Table 2 shows effect of sucrose at 50% effluent concentration on color, BOD and COD removal from paper mill effluent waste.

Almost all COD, BOD and color was removed in presence of 50% black liquor concentration containing 0.5% sucrose.

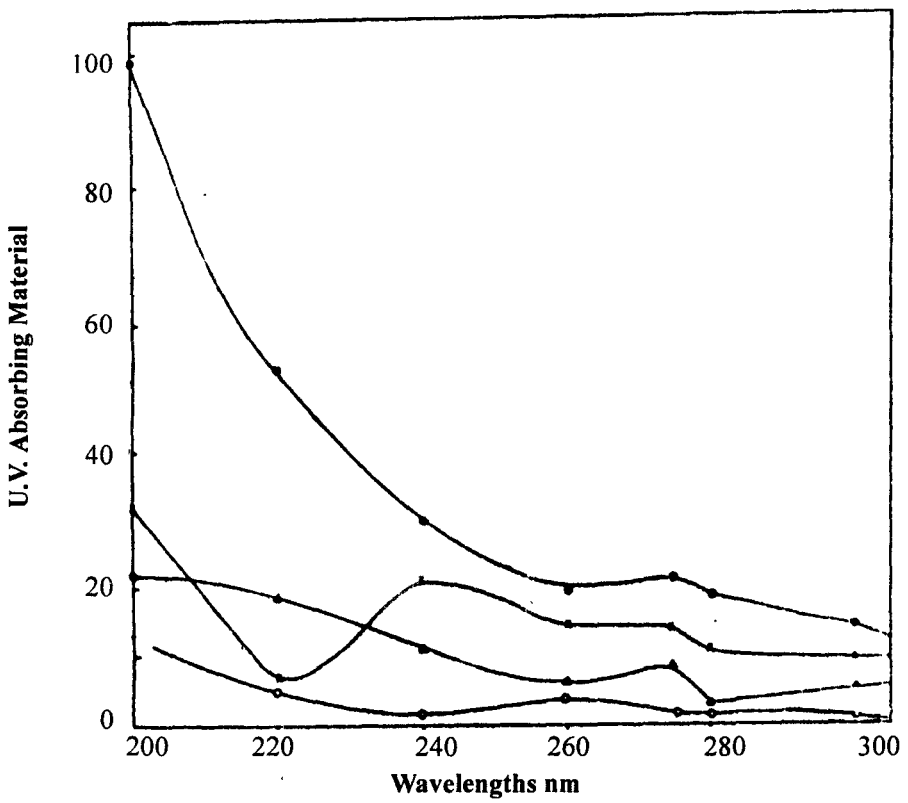

Fig. 1 Removal of U.V. absorbing material at various wavelengths in uniniculated (L) black liquor (0–0), M_1 consortium (x–x), M_2 consortium (Ô –Õ) and $M_1 + M_2$ consortium (0–0).

Table 1

Effect of glycerol on COD, BOD and colour removal of the black liquor.

Parameters	*Control*	*Experimental*
COD	24800	1168
BOD	17700	28
Colour	100	2

Table 2

Effect of Sucrose at 50% effluent on colour, COD and BOD removal.

Parameters	*Control*	*Experimental*
	1:1 (50%)	1:1(50%)
COD	24800	1400
BOD	17700	40
Colour	100	4

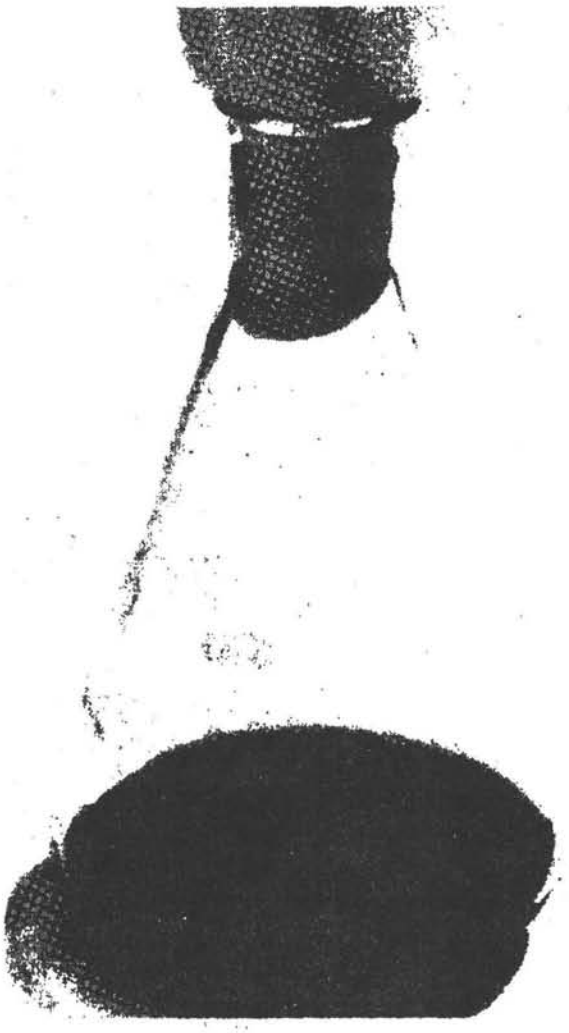

Fig. 2-A Photograph indicating complete removal of colour from the culture medium under optimum conditions such as pH 5.3, 0.5% sucrose, 50% substrate concentration etc. (Before treatment).

There is a positive correlation between growth of the organism and the degree of decolorization. Among the three treatments tested, only the effluent, unsterilized and adjusted to the pH of 5.3 with 50% sulphuric acid and containing sucrose or glycerol as a co-substrate showed maximum efficiency, indicating the specific requirements of the organisms for their survival and activity.

Important Features

1. The micro-organisms used in these studies are GRAS clear.
2. Colour was removed efficiently under non-sterile conditions.

Fig. 2-B Photograph indicating complete removal of colour from the culture medium under optimum conditions such as pH 5.3, 0.5% sucrose, 50% substrate concentration etc. (After 48h of growth).

3. The effluent was used at 50% concentration.
4. Color removal was by absortion.
5. Colorless liquid obtained after microbial treatment can be utilized for dilution at the black liquor for further treatment.
6. The biomass obtained can serve as a nitrogen rich slow release fertilizer. Thus avoiding environmental problems in the long run.

Summary

Recycling of urban and indusrial waste is an issue that is not going to go away for a long time

to come. Microbial conversion of waste is non-polluting, safe method of disposal of organic wastes. Lignin and its derivatives are in general difficult to degrade by physical, chemical or biological methods due to the presence of the complex linkages within the molecules. Therefore, conventional biological treatment methods are only moderately effective in decreasing effluent COD. Consequently, the paper industry cannot satisfy the effluent discharge limits for COD and BOD that have been imposed by environmental pollution control board. In our studies a microbial consortium containing yeasts and bacteria effectively utilizes various U.V. absorbing material from black liquor in presence of easily metabolizable carbon aerobically under non-sterile conditions, Lignin, colour and COD removal is in the range of 92-98% after 48th of growth. Evaluation of the process by extensive studies may lead to more productive approach and can help in accelerating the pollution control process.

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Authors

N.A. Sahasrabudhe
Scientist, Plant Molecular Biology Unit
Division of Biochemical Sciences
National Chemical Laboratory
Pune--411 008, Maharashtra, India.

33

Phosphate Solubilizing Soil Actinomycetes as Biofertilizers

Introduction

Next to the nitrogen, phosphorus is the limiting element of crop production. It is never found in a free state but occurs naturally in a huge amount as a calcium phosphate and in many other minerals. Mishustin (1963) reported the use of bacterial phosphatic fertilizers on agriculture land. In soil 25 to 85% of total phosphorus is present in the form of organic compounds which are not utilized directly by plants. Living plants can utilize only soluble inorganic phosphorus. The transformation of minerals or organic phosphorus into soluble inorganic phosphorus is brought about by microbial action. Badkatte (1976) demonstrated presence of phosphate solubilizing actinomycetes in the soil of Maharashtra. Konde (1978) found that 9% of the 150 cultures isolated from soil samples of Maharashtra had the ability to solubilize tricalcium phosphate (insoluble form). Banik and Dey (1982) also reported phosphate solubilizing activity in eight *Streptomyces* species isolated from soil. Present investigation deals with the same aspect *i.e.*, phosphate solubilizing activity of soil actinomycetes.

Material and Methods

112 Actinomycetes were isolated from soil samples of Solapur district area. These isolates were identified as per the criteria given in Bergey's Manual of Systematic Bacteriology, Vol. 4 by William and Sharpe. These isolates were tested for their phosphate solubilizing activity by using Pikovaskaya's medium (Modified by Sundra Rao and Sinha 1963). Isolates were spot inoculated and plants incubated at room temperature for 7 to 11 days. Plates showing clear zone surrounding the growth were considered as positive for phosphate solubilization.

Results and Discussion

Out of total 112 actinomycetes tested for phosphate solubilizing activity, only 15 (13.39%) isolates were found to have phosphate solubilizing activity (Table 1).

Table 1

% of Actinomycetes showing phosphate solubilizing activity.

Sr.	*Number of actinomycetes tested for phosphate solubilizing activity*	*Number of actinomycetes showing phosphate solubilizing activity*	*%*
1	112	15	13.39

Phosphate solubilizing activity was reported in *Streptomyces*, *Nocardia*, *Streptoverticillium*, *Thermoactinomycetes* and *Micromonospora*. (Table 2) Maximum phosphate solubilizing isolates were from *Streptomyces*. While *Streptosporangium*, *Ampullariella*; *Microbispora* were without phosphate solubilizing activity.

Table 2

Distribution of phosphate solubilizing activity of Actinomycetes in different genera.

Genus	*No. tested*	*No. showing phosphate solubilizing activity*	*% of phosphate solubilizing isolates types*	*% of phosphate solubilizing isolates in genus*
Streptomyces	79	9	60.00	11.40
Nocardia	7	2	13.33	28.60
Streptoverticillium	7	2	13.33	28.60
Thermoactinomycetes	2	1	6.67	50.00
Micromonospora	4	1	6.67	25.00
Streptosporangium/ Ampullariella/ Microbispora	13	-	-	-
Total	112	15	100	%13.39

Summary

Phosphate solubilizing activity of 112 actinomycetes isolated from soil samples of Solapur district area was tested using Pikovskaya's medium (Modified by Sundra Rao and Sinha 1963) and found that 13% isolates had the ability to solubilize tricalcium phosphate. These actinomycetes include *Streptomycesi* in majority and *Thermoactinomycetes*, *Streptoverticillium*,

Nocardia and *Micromonospora* etc. The percentage of phosphate solubilizing actinomycetes were more in black soil than in light soil. These actinomycetes can be used as biofertilizers to increase soil fertility by their phosphate solubilization activity.

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Authors

S.W. Kulkarni
Department of Microbiology
S.B. Zadbuke College
Barshi-413 401, Dist. Salapur
Maharashtra, India
A.M. Deshmukh
P.G. Department of Microbiology
Y.C. College of Science
Karad-415 124, Maharashtra, India

34

Vermicomposting of Kitchen Waste: A Case Study

Introduction

Garbage is an unavoidable consequence of prosperous, high technology and disposable economies. It is a problem not only in India but throughout the world. The word garbage reminds us of an overflowing garbage bin, with ragpickers rummaging through the garbage and animals looking for something to eat. It is very unhealthy. The daily garbage production is increased and it has become a big problem to the municipalities. The municipality machinery is helpless to manage the waste disposal.

The only solution found is to reduce the waste at source and dispose it off at source as early as possible. For this the trials were made to find out various possibilities of the solid waste disposal from the houses and apartments. The waste contains mainly the kitchen waste including vegetable cuttings, leftover foods, fruit wastes etc. which require an immediate disposal otherwise it may cause severe problems like aesthetics and spread of diseases.

It is estimated that about 500 g of biodegradable kitchen waste is generated per day in a family consisting of four members. The waste includes fruit seeds, fruit pealings and remnants, waste vegetable, roots and straw, wasted flowers, rotten food, milk, used tea wet powder, food wasted in dishes, bones, egg shells, paper, garden waste, glass, metals, used cosmetics and medicine bottles, rubber, leather, plastics and textile etc.

The present practice is to throw the entire waste in a nearby dustbin. The ill-maintained and not so regularly cleaned dustbins pose a pathetic scene to watch. It is always invariably surrounded by stray dogs, donkeys, cattle and most unfortunately rag pickers. The organic waste so strewn around gets fast degraded and starts stinking badly. The bad stink further prohibits the housewives and attendants to deposit the fresh waste into bins proper, and thus the waste starts getting spread around setting the chain of filth and unhygienic conditions to a further degree.

This problem appeared insurmountable in the past but it was successfully demonstrated at many houses in Sangli that it can be easily solved in the house itself in scientific and hygienic way by vermicomposting.

Material and Methods

The daily waste from the individual house is initially segregated at source. Two separate bins were use for garbage collection. In first bin all dry garbage like plastic, paper, bottles, tins and metallic wastes were stored and kept aside which will be collected by rag pickers. In another bin wet garbage like vegetable waste, food waste, fruit peels, egg shells, bones etc. were collected.

The vermicompost is prepared from the compost which contains a mixed microbial culture of decomposing microorganisms. The decomposed material is further subjected to digestion by earthworms. A special variety of *Isenia foetida* was used for this purpose. The culture thus prepared also contains eggs of earthworms. An earthen pot of 1 ft diameter, which is generally used for domestic flower plants, was used which was filled with specially prepared vermicompost to a height of about 4 inches. Then the waste generated daily was put into it in a properly cut and mashed form. A small porous cover was put on the top to avoid fly breeding and to maintain aerobic conditions. The watering of the material is required only during summer if the material looks dry. The watering was made so as to maintain moisture content to about 60 per cent. The normal function of the system is confirmed by smell of the contents. A foul smell indicates overloading which may warrant use of one or more pots.

Once the pot is filled, it is covered with thin layer of loose earth and is daily watered for about 10 to 15 days. The process takes about 20 to 25 days to degrade the waste completely, depending on waste quality and quantity available daily.

The chemical and microbiological analyses were made after complete degradation of the material.

Results and Discussion

The microorganisms isolated and identified from the vermicompost prepared are shown in Table 1 which shows that the normal heterotrophic organisms are responsible for the degradation of the kitchen waste. No pathogenic microorganism was detected during study. The earthworms help to speed-up the process.

The chemical analysis of the vermicompost prepared from kitchen waste is shown in Table 2. This shows that the material is pure organic and contains the nutrients required for growth of plants. The compost can be used as soil conditioner.

It is observed that one earthen pot can accommodate a waste of family including 4 members for six months. The pot is hygienic and do not pose any problem of bad smelling and house fly etc. as it can be kept aside along with other garden pots. About 30 families from Sangli are using this kind of pots for last one year and found no trouble in operation.

A seedling may be planted into the same and then can be given as a gift or can be put along with other flowering pots. It has been our experience that the plant grown in vermicompost has a better growth and health as compared to other similar plants. In effect, the waste gets converted into black gold right at the place where it is generated.

Table 1

Organisms isolated and identified from Kitchen Waste Vermicompost

Bacteria:

Aerobacter spp., *Agrobacter* spp. *Acetobacter aceti, Bacillus* spp., *Cellulomonas flavigera, Citrobacter fruindii, Pseudomonas* spp., *Zoogloea ramigera.*

Fungi

Aspergillus niger, Aspergillus flavus, Mucur pusillus, Penicillium notatum, Rhizopus nigiricans

Actinomycetes

Micromonospora purpura, Nocardia farcinica, Streptomyces bobili, Streptosporangium roseum. Thermomonospora curvata

Plate Count:	Bacteria	- 4×10^{10}
	Fungi	- 10×10^{4}
	Actinomycetes	- 10×10^{4}

Note : The common organisms observed in 6 samples are reported.

Table 2

Chemical analysis of kitchen waste vermicompost.

1.	pH (Saturated)	7.50 – 7.6
2.	Conductivity (Saturated) mmhos/cm	0.40 – 0.50
3.	Nitrogen %	2.00 – 2.50
4.	Phosphorus as P_2O_5, %	2.00 – 2.50
5.	Potassium, % as K_2O	3.20 – 3.40
6.	Organic carbon, %	46.0 – 50.0
7.	Total ash, %	31.0 – 34.0
8.	Total Volatile Solids %	66.0 – 69.0
9.	C/N Ratio	23.0 – 20.0
10.	Chlorides, %	0.30 – 0.32
11.	Iron as Fe, ppm	600 – 700
12.	Manganese as Mn, ppm	50 – 60
13.	Zinc as Zn, ppm	8 – 10
14.	Copper as Cu, ppm	15 – 18
15.	Calcium %	1.4 – 2.0
16.	Magnesium, %	0.15 – 0.28

Note : Values on dry weight basis. The average values of six samples.

Conclusion

The method is found to be excellent for the people living in apartments who have problem of disposal of daily waste, it also gives following benefits:

1. Reduction of solid waste at source which will help in lowering down the load of city solid waste disposal system.
2. The health problem will be reduced due to reduction in dust bins.
3. Stinking sites and the dirty blots on the city will be out.
4. An excellent quality manure will be made yearly which will save our money on fertilizer.
5. A better yield of flowers and fruits etc. will be plain bonus.

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Authors

S.D. Khambe
Department of Microbiology, Miraj Mahavidyalay, Miraj-416 410, Maharashtra, India
A.M. Deshmukh
Department of Microbiology, Y.C. College of Science, Vidyanagar, Karad-415 124, Maharashtra, India
M.V. Kulkarni
Maharashtra Pollution Control Board, Sangli, Maharashtra, India

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Bio-control of Fusarial Wilt of Chick Pea (*Cicer arietinum*) Variety, Chaffa in Wilt Sick Field

Introduction

Among the fungal diseases, Fusarial wilt be caused by *Fusarium udum* in pigeon pea and *Fusarium oxysporium* and *F. ciceris* in chick pea is the most destructive diseases and is observed in all the growing areas. The disease is observed through all the growth stages and becomes conspicuous at pod maturity and hence attracts attention. Padule *et al.* (1982) reported up to 90% losses due to this disease in Maharashtra. According to Kannaiyan and Nene (1981) yield losses in susceptible cultivars ranged from 21.5 to 100%. Bio-control of *Fusarium* wilt and *Erwinia carotovora* using antagonistic Ps. B-10; *Agrobacterium radiobacter* K-84 and Ps flurescens have opened up possibilities of controlling bacterial wilt; crown gall, wilt of pulses; banana and cotton (Chen *et al.*, 1992; Scher and Baker, 1980).

Material and Methods

Antagonistic property of some microbial soil and seed inoculants to control *fusarial* wilt of Chick pea under wilt sick field condition was tried at Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. Microbial inoculants were obtained from the Agricultural Microbiologist; MPKV, Pune. Chick pea variety Chaffa is sown in November 1993 and harvested in March 1994. Plot size 3 × 1.2m, replications 3; treatments-8 with randomized block design were used. Progressive wilt count and yield of gram and biomass is monitored.

Results and Discussion

Soil application of BGA (Cyanobacteria) T_3 have given the lowest wilt count *i.e.*, 16.29% followed by 19.1% in T_1 (*Rhizobium* S T) and 20.67% in T_2 (*Azotobacter* Sp). Highest wilting, *i.e.*, 38.03% was recorded in control treatment. Thus there was a reduction of 21.74% in wilting incidence (Table 1).

Table 1

Cumulative wilting in Chick pea (Chaffa) in wilt sick field wilting (% plants wilted)

	Treatment	*RI*	*RII*	*RIII*	*Mean*
T_1	*Rhizobium* sp. ST	18.3	18.2	20.8	19.01
T_2	*Azotobacter*	25.4	18.2	18.4	20.67
T_3	BGA (Soil appli.)	23.5	12.7	12.7	16.29
T_4	*Azospirillum* sp.	27.3	32	26.4	28.57
T_5	*Bacillus polymyxa*	26.6	22.1	27.5	25.41
T_6	(T1+T2+T3+T4)	40.0	28.3	21.4	29.09
T_7	Thiram (seed treat)	27.8	35.9	23.9	29.02
T_8	Control	43.2	37.6	33.3	38.03
	Mean	29.2	25.64	23.3	0

S.E. = 0.09 in Aresin Transformer value; C.D. = 0.04

The highest grain yield, 12.7 q/ha was obtained in T_1 (*Rhizobium* T) followed by 11.8 q in T_3 (BGA soil application). The seed treatment with Thiram fungicide registered the least effect. Similar trend was recorded in biomass yield. (Table 2). Cost implications are recorded with wide cost benefit ratios. (Table 3).

Table 2

Cumulative grain yield.

	Treatment	RI	RII	RIII	Mean	
					g/plot	q/ha
T_1	*Rhizobium* sp. ST	460	430	480	456	12.7
T_2	*Azotobacter*	350	340	350	346	9.6
T_3	BGA (Soil appli.)	420	410	450	427	11.8
T_4	*Azospirillum* sp.	400	380	385	388	10.8
T_5	*Bacillus polymyxa*	300	310	350	320	8.9
T_6	(T1+T2+T3+T4)	350	400	380	377	10.4
T_7	Thiram (seed treat)	300	280	310	297	8.2
T_8	Control	230	260	280	257	7.1
	Mean	351.22	351.2	373.1		

S.E. = Per plot 10.07; C.D. = 30.56

Table 3

Cost implications of different treatment to control wilt of chickpea in sick soil.

	Treatments	*Yield q/ha*		*Net gain Rs/ha*		*Cost incurred*	*Net Gain*	*Benefit per 1 rs invested*
		Grain	*biomass*	*Grain*	*biomass*			
T_1	*Rhizobium*	12.7	10.4	3920	137.5	52.5	4005	76.2
T_3	BGA	11.8	10.0	3290	127.5	92.5	3325	35.9
T_4	*Azospirillum*	10.8	7.7	2590	70.0	52.5	2607	49.6
T_7	Thiram	8.2	5.6	-	-	40	-	-
T_8	Control	7.1	4.9	Nil	Nil	Nil	-	-

Assumed rates: Chickpea grain Rs. 7.0/kg.
Chickpea biomass Rs. 1.75/kg.
Biofertilizer Rs. 5/kg.
Labourer Rs. 40/ha

Antagonistic property of *Bacillus Subtilis* against *Fusarium* are reported by Lin, Zhang and Ge (1990); Podile, Prasad, Dubey (1985). *Trichoderma* as antagonist is advocated by Cho *et al.* (1989), and Sivan & Chet (1989), and *Rhizobium* by Chao (1990).

Summary

A field trial in Wilt sick soil with 3 replications and 8 treatments was conducted in November 1993 to March 1994. *Rhizobium; Azotobacter; B.G.A. biogertilizers; Bacillus polymyxa* antagonist and Thiram fungicide were tried in different combinations. Wilt incidence; grain yield and economics were monitored. The treatment T_3 (BGA soil application) gave lowest wilt count *i.e.* 16.29% followed by 19.1% in T_1 (*Rhizobium*) and 20.6% in T_2 (*Azotobacter*). Thus there was a reduction of 21.7% in wilt incidence over control. Grain yield differences were statistically significant. Highest grain yield 12.7 q/ha was obtained in T_1 (*Rhizobium* ST) followed by 11.8 q/ha in T_3 (BGA); while in Thiram seed treatment it was 8.2 q and in control 7.1 q/ha. Cost input ranged from Rs. 52.5 to Rs. 92.5/ha in different treatments; while the net gain ranged from Rs. 2607 to Rs. 4005/ha.

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Authors

Mrunalini A. Gupte, K.G. Thakare, S.O. Kolte, and R.W. Ingle
Department of Plant Pathology
Dr. Panjabrao Deshmukh Vidyapeeth
Akola-444 104, Maharashtra, India

36

Response of Pigeonpea to *Rhizobium* and *Trichoderma viride* in Acid Soils

Introduction

Under N and P depleted soil the inoculation of nitrogen fixing microbes and a bio-control agent can able to bring out with an effective symbiosis in legumes. There are several reports have indicated the role of these microbes on legumes in the normal soil (Ganesan and Alexander, 1994; Prabakaran *et al.*, 1994 and 1996). The information on the dual inoculation of rhizobia with bio-control agent in alfisol has not been reported earlier. Hence the present investigation is proceeded with the aim to ascertain the role of nitrogen fixer in combination with a bio-control agent on enhancement of growth and yield of a legume in alfisol.

Material and Methods

During Kharif 1995 a field experiment was conducted to assess the response of Vamban-1 pigeonpea (*Cajanus cajan*) in an alfisol (pH 5.6; EC: 0.34mmho/cm^2) by the sole and dual inoculation of nitrogen fixing *Rhizobium* (VPR-1) a slow growing specific acid tolerant strain and with and without co-inoculation of a bio-control agent *Trichoderma viride*. *Rhizobium* was inoculated at the rate of 600 g/20 kg of seeds as seed inoculation whereas *Trichoderma viride* was inoculated at the rate of 4 g/kg of seed. These two biocultures were also mixed at once and N control was also maintained. Each treatment was replicated five times and conducted in RBD. The treated and control seeds were sown at 20 × 45 cm spacing in 5 × 4 m^2 plots. At 20 days after sowing, crop germinability and vigour index were calculated. At 40 days the crop growth related to biomass, root, shoot growth, nodulation and its biomass were ascertained. At harvest the yield attributes *viz.*, pod yield, tdmp, grain yield and harvest index were recorded and the data were analysed and presented in Tables 1 and 2.

Results and Discussion

The results indicated that the seed inoculation of *Rhizobium* with coinoculation of *Trichoderma* as a biocontrol agent as a single and their combination had exerted with significant increase in growth, nodulation and yield attributed in Vamban-1 pigeonpea (Tables 1 and 2).

Table 1

Response of pigeonpea to seed inoculation of *Rhizobium* and *Trichoderma viride* in alfisol – growth attributes

Treatments	*At 20 DAS*	*At 40 DAS*				
	Vigour index	*Nod. no./pl*	*Nod. biomass (mg/pl)*	*Plant biomass (g/pl)*	*Root growth (cm/pl)*	*Shoot growth (cm/pl)*
Control	650	2.6	9	1.58	5.1	14.8
N-Control	790	6.0	17	2.96	9.2	23.9
Rhizobium	850	18.0	59	2.72	7.9	21.0
Trichoderma	780	12.3	41	2.40	7.1	18.8
R + T	846	21.3	69	2.82	9.6	25.2
CD (p = 0.05)		0.06	0.3	0.06	0.4	2.1

DAS = Days after sowing; Nod. = Nodules

Table 2

Response of pigeonpea to seed inoculation of *Rhizobium* and *Trichoderma viride* in acid soil-Yield attributes

Treatment	*Pod (no/p)*	*Tdmp (t/ha)*	*Grain yield (kg/ha)*	*Percent increase over control*	*Harvest index*
Control	59	4.281	685	-	16.1
N-Control	76	4.300	810	18.0	19.6
Rhizobium	72	4.310	905	32.0	21.0
Trichoderma	65	4.900	850	24.0	17.0
R + T	79	4.363	960	39.2	22.0
CD (p = 0.05)	3.6	NS	39		

Growth Attributes

The plant biomass, nodule counts, and its biomass along with root and shoot growth were found increased in the dual inoculation of nitrogen fixing *Rhizobium* and bio-control agent

with the use of *Trichoderma* for effective controlling of root and soil invading pathogens on crops (*Rhizoctonia, Sclerotium*) had significantly augmented over the untreated control. The vigour index was enhanced the pigeonpea in the combined inocula it was due to the influence of nitrogen provision and effective arrest of invading pathogen. It is in agreement with the results reported earlier (Ganesan and Alexander, 1994; Subbarao *et al.*, 1985; Negi *et al.*, 1990; Prabakaran *et al.*, 1995 and 1996). Due to the enhanced and synergistic symbiosis of *Rhizobium* with legume biocontrol agents can able to protect the crop from invading soilborne pathogens (*Sclerotium* and *Fusarium*) which are amenable to pigeon pea. Due to these cumulative performance of these two effective microbes significantly augmented vigour index, root nodulation, biomass and plant biomass over untreated control.

Yield Attributes

Dual inoculation of *Rhizobium* and *Trichoderma* registered with better symbiosis in pigeonpea by enhancing the yield components with specific to grain yield (39.2% over control) pod counts (79/plant) over their individual inoculation of either *Rhizobium* or with *Trichoderma* on legume in alfisol. Prabakaran *et al.* (1994) revealed the dual inoculation of *Rhizobium*, Phosphobacteria along with two biocontrol agents (*Trichoderma* and *Pseudomonas*) enhanced the growth nodulation and yield of cowpea in alfisol. Also it was evident with groundnut the dual inoculation of *Rhizobium* and *Trichoderma* on JL-24 variety registered with significant enhancement in pod yield in alfisol (Prabakaran *et al.*, 1996).

The enhanced symbiosis and yield increase in pigeonpea under alfisol might be due to the following reasons:

1. Effective nitrogen fixation under symbiotic performance of slow growing rhizobia might have increased the nodulation and grain yield over control.
2. The possible control of soil and root invading pathogens (*Rhizoctonia Sclerotium* and *Fusarium*) by the root proliferation of *Trichoderma viride* can improve the germinability, growth stand and finally helps in more crop growth and yield.
3. The dual inoculation of both microbes were found to react synergistically and no where they exhibited with the suppression or antagonistic effect and they were slowly favours for the better plant stand and its yield in alfisol.

Based on these reasons, the seed bioinoculation of *Rhizobium* and *Trichoderma* registered with increased biomass nodulation and finally augmented the grain yield over their individual inoculation and control.

Summary

A field experiment was conducted during Kharif 1995 season to ascertain the growth yield of Vamban-1 pigeonpea (*Cajanus cajan*) in alfisol to the seed bacterization of slow growing *Rhizobium* (VPR-1 at the rate of 600 g for 20 kg seeds along with and without the coinoculation of a biocontrol agent *Trichoderma viride* at the rate of 4 g/kg of seed. Biogrowth were recorded when the bioinoculants were applied in the dual inoculation which affected better root

nodulation, plant biomass and finally augmented the grain yield 39.4 per cent over control (685 kg/ha) whereas the indivdual inoculation of *Rhizobium* and *Trichoderma* had affected with 32 and 24 per cent respectively over the control.

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Authors

J. Prabakaran
Department of Environmental Sciences
Tamil Nadu Agricultural University
Coimbatore-641 003, Tamil Nadu, India

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Performance of Seed Pelletization with Biofertilizers, Macro- and Micronutrients and Biocides under Different Water Holding Capacities in *Acacia leucophloea* (Roxb.)

Introduction

Acacia leucophloea (Roxb.) willd ex Del known as white barked *Acacia*, belongs to the family Mimosaceae. It is a constituent of dry tropical forest, tropical thorn forests and tropical dry evergreen forest. The temperature range in its zone is 4° to 49°C with average rainfall of 450-1500 mm per annum. It comes up on a variety of soil from shallow and gravelly on hill slopes to deep alluvial. It commonly occurs in dry regions of India. The tree attains a height of 2.90m and a girth of 15.2 cm in 5 years. The tree flowers during August-November, pods ripe in April-June. The ripe pods are beaten off the tree with a stick, on the ground previously swept clean. Pods are collected and spread in the sun to dry; and then beaten with a stick or wooden mallet to extract seeds. Seeds are dark brown, elliptical and rhomboidal in shape. For large scale afforestation programmes, aerial seeding in increasingly being adopted in India. For this purpose, the seeds should be pelleted to increase the ballistic property of seeds while aerial seeding and to withstand adverse habitat and extreme situations. Protective measures to assist individual seeds after sowing are impracticable and pelleting is the only possible mean of achieving some degree of protection (Anon, 1985). Against these backdrops, the need to intensify research on seed pelletization in *Acacia leucophloea* was felt.

Material and Methods

The acid scarified seeds of *Acacia leucophloea* were pelleted with macronutrients, micronutrients, pesticide, fungicide and biofertilizer at various combinations using gum Acacia at the rate of 30 ml kg^{-1} of seed as adhesive and gypsum at the rate of 200 g kg^{-1} of seed as the coating material. The nutrients used and their concentration and source are furnished below.

1. Macronutrient – 30 g of DAP kg^{-1} of seed to supply 0.5% of N and 1.5% of P_2O_5.
2. Micronutrient – 19.7 g of micronutrients mixture kg^{-1} of seed to supply 0.1% of Zn, Mn and Fe and 0.05% of Cu, B and Mo.
3. Biofertilizer – 50 g of *Rhizobium* kg^{-1} of seed.
4. Pesticide – 2 g of Sevin kg^{-1} of seed.
5. Fungicide – 4 g of *Trichoderma* kg^{-1} of seed.

Pelleting was done with hand operated pelletizer and the pelleted seeds of *Acacia leucophloea* were germinated in tea cups filled with black soil at different water holding capacities *viz.* 20%, 30%, 40%, 50%, 60%, 75% and 85%. The unpelleted seeds served as control. The experiment was set up in completely randomized design replicated four times. At final count day the seedlings were evaluated for germination (ISTA, 1993), dry matter production and vigour index (Abdul-Baki and Anderson, 1973).

Results and Discussion

Higher germination of 90 per cent and 87 per cent were recorded at lower water holding capacities of 30% and 40% respectively and the minimum germination of 42 per cent was noticed at higher water holding capacities of 75%. The treatments macro + micronutrient, macro + micronutrient + biofertilizer, pesticide + fungicide + macro + micronutrient and pesticide + fungicide + macro + micronutrient + biofertilizer recorded higher mean germination percentages and dry matter when compared with unpelleted seeds (Tables 1 and 2). At higher water holding capacities of 75 per cent these treatments recorded significantly higher germination and dry matter than the unpelleted which recorded 30 per cent germination. Within the treatments recorded significantly higher germination and dry matter than the unpelleted which recorded 30 per cent germination. Within the treatments significantly higher germination were registered at 30%, 40% and 50% water holding capacities and lower germination was recorded at 75% water holding capacity. Maximum vigour index was recorded at 30% water holding capacity (1066) followed by 40% water holding capacity (1011) and the minimum values at 75% water holding capacity (486). Among the treatments pesticide + fungicide + macro- + micronutrient recorded maximum (909) followed by macro- + micronutrient + biofertilizer (907) and the minimum vigour was noticed in unpelleted and pesticide + fungicide seed treatments (Table 3).

Thus 30% and 40% water holding capacity were found to be optimum for better germination and seedling vigour for both pelleted and unpelleted seeds. Though the high water holding capacity of 75% proved detrimental to both pelleted and unpelleted seeds, the pelleted seeds proved its superiority over unpelleted seeds at high water holding capacity of the soil. Similar findings were reported in sorghum and neem (Robert *et al.* 1990; Selvaraju, 1992 and Ponnuswamy, 1993). They reported that low water potential of soil reduced water absorption of pelleted seeds and delayed the emergence of radicle and coleoptile. It is an added advantage to the pelleted seeds under direct sowing, that the seeds will not germinate unless required moisture is available. However, the ability of pelleted seeds to germinate under high water holding capacity (75%) compared to lower germination of unpelleted seeds underscores the benefit of pelleting under swampy and water stagnated condition when seeds are directly sown

Table 1
Performance of pelleted seed under different water holding capacity of the soil in *Acacia leucophloea*.

Pelleting Treatments (PT)	*Germination (%)*					
	Water holding capacities of the soil (%) - (W)					
	30	*40*	*50*	*60*	*75*	*Mean*
Unpelleted (T_1)	90 (72.05)	86 (68.30)	82 (64.93)	67 (54.97)	30 (33.20)	71 (58.05)
Pesticide + fungicide (T_2)	88 (69.87)	83 (65.65)	84 (66.77)	69 (56.20)	42 (40.38)	73 (58.74)
Macro- + micronutrient (T_3)	90 (72.05)	87 (68.91)	89 (70.92)	71 (57.46)	45 (42.13)	76 (61.59)
Bio-fertilizer (T_4)	88 (69.87)	86 (68.30)	85 (67.55)	68 (55.57)	40 (39.22)	73 (59.08)
Macro- + micronutrient + Biofertilizer (T_5)	91 (72.88)	90 (72.05)	87 (68.91)	72 (58.08)	47 (43.28)	77 (61.15)
Pesticide + fungicide + Macro- + micronutrient (T_6)	93 (75.06)	90 (72.05)	89 (70.69)	71 (57.46)	45 (42.12)	78 (62.09)
Pesticide + fungicide + Bio-fertilizer (T_7)	86 (68.07)	85 (67.55)	84 (66.77)	69 (56.20)	42 (40.38)	73 (58.74)
Pesticide + fungicide + macro- + micronutrient + biofertilizer (T_8)	92 (74.10)	90 (72.05)	88 (69.87)	72 (58.08)	48 (43.85)	78 (62.09)
Mean	90 (72.05)	87 (68.91)	86 (68.30)	70 (56.75)	42 (40.57)	

	SEd	C.D. (P = 0.05)
W	0.734	1.453
PT	0.928	1.838
W × PT	2.076	4.152

(Figures in parantheses indicate are sine transformation)

Table 2

Performance of pelleted seed under different water holding capacity of the soil in *Acacia leucophloea*

Pelleting Treatments (PT)	*Dry matter production (mg seedling $^{-1}$)*					
	Water holding capacities of the soil (%) – (W)					
	30	*40*	*50*	*60*	*75*	*Mean*
Unpelleted (T_1)	11.8	11.6	11.7	11.5	11.3	11.6
Pesticide + fungicide (T_2)	11.8	11.6	11.7	11.6	11.4	11.6
Macro- + micronutrient (T_3)	12.0	11.7	11.7	11.6	11.5	11.7
Bio-fertilizer (T_4)	11.8	11.5	11.6	11.6	11.4	11.6
Macro- + micronutrient + Biofertilizer (T_5)	12.0	11.5	11.8	11.7	11.6	11.7
Pesticide + fungicide + Macro- + micronutrient (T_6)	11.9	11.5	11.8	11.9	11.5	11.7
Pesticide + fungicide + Bio-fertilizer (T_7)	11.9	11.7	11.7	11.6	11.4	11.6
Pesticide + fungicide + macro- + micronutrient + biofertilizer (T_8)	11.9	11.7	11.7	11.6	11.5	11.7
Mean	11.9	11.6	11.7	11.6	11.5	

	SEd	C.D. (P = 0.05)
W	0.12	0.24
PT	0.15	NS
W × PT	0.34	NS

during rainy season. Hence pelleting of seeds could be recommended for aerial seeding over the small hillocks with rocks and inaccessible places at low elevations to promote better establishment and survival percentage.

Summary

Acacia leucophloea seeds were pelleted with different combinations of *Rhizobium* (50 g.kg^{-1} of seed), Diammonium phosphate (30 g.kg^{-1} of seed), micronutrients mixture (19.7 g kg^{-1} of seed), sevin (2 g.kg^{-1} of seed) and *Trichoderma* (4 g.kg^{-1} of seed). Gum *Acacia* (30 ml.kg^{-1} of seed) was used as an adhesive and gypsum (200 g.kg^{-1} of seed) as coating material. The pelleted seed along with unpelleted seeds were sown at different water holding capacities *viz.*, 20%, 30%, 40%, 50%, 60%, 75% and 85% of black soil. The pelleted and non-pelleted seeds

Table 3

Performance of palleted seed under different water holding capacity of the soil in *Acacia leucophloea*

Pelleting Treatments (PT)	*Vigour Index*					
	Water holding capacities of the soil (%) – (W)					
	30	*40*	*50*	*60*	*75*	*Mean*
Unpelleted (T_1)	1062	998	960	771	339	826
Pesticide + fungicide (T_2)	1038	963	980	799	479	852
Macro- + micronutrient (T_3)	1076	1018	1045	824	519	896
Bio-fertilizer (T_4)	1042	989	998	790	456	855
Macro- + micronutrient + Biofertilizer (T_5)	1089	1035	1026	842	545	907
Pesticide + fungicide + Macro- + micronutrient (T_6)	1105	1035	1049	841	517	909
Pesticide + fungicide + Bio-fertilizer (T_7)	1021	995	985	799	479	856
Pesticide + fungicide + macro- + micronutrient + biofertilizer (T_8)	1096	1053	1036	836	554	915
Mean	1066	1011	1010	813	486	

	SEd	C.D. (P = 0.05)
W	13.0766	25.8914
PT	16.5408	32.7504
W × PT	36.9862	73.2320

failed to register significant differences in germination, dry matter production and seedling vigour at low water holding capacities (30% and 40%) than at higher water holding capacities (60% and 75%). Though high water holding capacity of 75% proved detrimental to both pelleted and unpelleted seeds, the pelleted seeds proved its superiority over unpelleted seeds. The study underscores the benefit of pelleting under swampy and water stagnated conditions when seeds are directly sown during rainy seasons.

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Authors

G. Mani, A.S. Ponnuswamy and K. Vanangamudi
Department of Seed Technology
Tamil Nadu Agricultural University
Coimbatore-641 003, Tamil Nadu, India

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Performance of Seed Pelletization with Biofertilizers, Macro- and Micronutrients and Biocides under Different Soil Conditions in *Acacia nilotica* (Linn.)

Introduction

Acacia nilotica is an evergreen tree confined to dry tropical thorn forests. It serves as an excellent fodder cum firewood tree besides its wood being valued for its gum and agricultural implements making. The tree grown on varied ecological conditions (Mathur *et al.* 1985) with an average rainfall ranges from 200 to 1270mm and temperature from 0.5°C to 47°C (Dwivedi, 1993). It is being planted under the scheme "Farm Forestry" and planted as tank for shore plantations. For large scale afforestation programmes, aerial seeding is increasingly being adopted in India. For this purpose, the seeds should be pelleted to increase the ballistic property of seeds while aerial seeding and to withstand adverse habitat and extreme situations. Protective measures to assist individual seeds after sowing are impracticable and pelleting is the only possible mean of achieving some degree of protection (Anon, 1985). Against these back drops, the need to intensify research on seed pelletization in *Acacia nilotica* was felt.

Material and Methods

The acid scarified seeds of *Acacia nilotica* were pelleted with macronutrients, micronutrients, pesticide, fungicide and biofertilizer at various combinations using gum *Acacia* at the rate of 30 ml kg^{-1} of seed as adhesive and gypsum at the rate of 200 g kg^{-1} of seed as the coating material. The nutrients used and their concentration and source are furnished below.

1. Macronutrient – 30 g of DAP kg^{-1} of seed to supply 0.5% of N and 1.5% of P_2O_5.
2. Micronutrient – 19.7 g of micronutrients mixture kg^{-1} of seed to supply 0.1% of Zn, Mn and Fe and 0.05% of Cu, B and Mo.

3. Biofertilizer – 50 g of *Rhizobium* kg^{-1} of seed.
4. Pesticide – 2 g of Sevin kg^{-1} of seed.
5. Fungicide – 4 g of *Trichoderma* kg^{-1} of seed.

Pelleting was done with hand operated pelletizer and the pelleted seeds of *Acacia nilotica* were tested for its performance in different soils like sandy loam, acidic, sodic and calcareous soils. The design used was completely randomized design with four replications. In each replication, 10 seeds were sown in tea cups. At the final count day, germination and dry matter of the seedling were evaluated. Vigour index of the seedling was computed using the formula suggested by Abdul-Baki and Anderson (1973).

Results and Discussion

Maximum germination was observed under sandy loam soil (66%) followed by the calcareous soil (59%) and the minimum germination under acidic soil (51%). Among the treatments macro + micronutrients, macro- + micronutrients + biofertilizer, and pesticide + fungicide + macro- + micronutrient + biofertilizer recorded maximum germination (76%) while the minimum germination (66%) was noticed in unpelleted ones (Table 1). Similar results were also recorded in dry matter production of seedlings (Table 2). The vigour index was maximum in sandy loam soil (3237) followed by calcareous soil (2801) and minimum values in acidic soil (2053). Among the treatments macro- + micronutrient treatment exhibited maximum vigour index and the minimum in unpelleted seeds (2252). Under the sandy loam soils macro- + micronutrient treatment recorded maximum vigour index (3365) followed by pesticide + fungicide + macro- + micronutrient + biofertilizer (3351) and the minimum in unpelleted seeds (3003) (Table 3).

Thus pelleted seeds registered significantly higher germination and seedling vigour than the unpelleted seeds under all soil types. Maximum germination and seedling vigour was noticed in sandy loam soil but the minimum germination, dry matter production and vigour index were recorded in acidic soil. In acidic soil also pelleted seeds. This is in conformity with the results of Selvaraju (1992) and Ponnuswamy (1993). Under these soil types the pelleting treatments like macro + micronutrient, macro + micronutrient + biofertilizer, pesticide + fungicide + macro + micronutrient and pesticide +fungicide + macro + micronutrient + biofertilizer had a profound influence on seed germination, dry matter production and vigour index. Olsen and Elkin (1977) reported that acidity inhibited multiplication of rhizobia in the rhizosphere of developing seedlings might be overcome by lime pelleting of seed. Hence pelleting with macro, micronutrients, biocides and biofertilizer can be recommended for augmenting germination and better seedling growth and also to withstand swampy condition and deleterious effect of soils like acidic soil.

Summary

In the present investigation, *Acacia nilotica* seeds were pelleted with *Rhizobium* (50 $g.kg^{-1}$ of seed), Diammonium phosphate (30 $g.kg^{-1}$ of seed), micronutrients mixture (19.7 $g.kg^{-1}$ of seed), sevin (2 $g.kg^{-1}$ of seed) and *Trichoderma* (4 $g.kg^{-1}$ of seed). Gum *Acacia* (30 $ml.kg^{-1}$ of

Table 1

Performance of pelleted seed under different soil types in *Acacia nilotica*

Pelleting Treatments (PT)	*Germination (%)*				
	Different soil conditions (S)				
	Calcareous soil	*Sandy loam*	*Acidic soil*	*Sodic soil*	*Mean*
Unpelleted (T_1)	68 (55.57)	79 (62.74)	34 (35.66)	65 (53.77)	66 (51.94)
Pesticide + fungicide (T_2)	72 (58.08)	81 (64.24)	64 (53.15)	74 (59.36)	73 (58.71)
Macro- + micronutrient (T_3)	75 (60.05)	85 (67.55)	68 (55.57)	77 (61.40)	76 (61.14)
Biofertilizer (T_4)	74 (59.36)	83 (65.81)	58 (49.62)	72 (58.08)	72 (58.22)
Macro- + micronutrient + Biofertilizer (T_5)	76 (60.71)	84 (66.77)	66 (54.38)	76 (60.71)	76 (60.71)
Pesticide + fungicide + Macro- + micronutrient (T_6)	75 (60.05)	84 (66.77)	62 (51.98)	75 (60.05)	74 (59.71)
Pesticide + fungicide + Bio-fertilizer (T_7)	73 (58.70)	82 (65.06)	63 (53.07)	72 (58.08)	73 (58.70)
Pesticide + fungicide + macro- + micronutrient + biofertilizer (T_8)	76 (60.71)	85 (67.60)	68 (55.57)	76 (60.71)	76 (60.71)
Mean	74 (59.16)	85 (65.82)	60 (51.13)	73 (58.71)	

	SEd	C.D. (P = 0.05)
S	0.919	1.823
PT	1.299	2.578
S × PT	2.598	NS

(Figures in parantheses indicate sine transformation)

Table 2

Performance of pelleted seed under different soil types in *Acacia nilotica*

Pelleting Treatments (PT)	*Dry matter production (mg seedling $^{-1}$)*				
	Soil condition (S)				
	Calcareous soil	*Sandy loam*	*Acidic soil*	*Sodic soil*	*Mean*
Unpelleted (T_1)	37.8	38.0	33.8	35.2	36.2
Pesticide + fungicide (T_2)	38.0	39.2	34.9	36.0	37.0
Macro- + micronutrient (T_3)	38.1	39.6	35.2	36.4	37.3
Bio-fertilizer (T_4)	37.5	38.6	34.8	36.1	36.8
Macro- + micronutrient + Biofertilizer (T_5)	38.2	39.5	35.9	36.5	37.5
Pesticide + fungicide + Macro- + micronutrient (T_6)	38.4	39.4	36.1	36.4	37.6
Pesticide + fungicide + Bio-fertilizer (T_7)	38.2	38.6	34.9	36.1	37.0
Pesticide + fungicide + macro- + micronutrient + biofertilizer (T_8)	38.2	39.2	35.8	36.6	37.4
Mean	38.044	39.009	35.175	36.163	

	SEd	C.D. (P = 0.05)
S	0.16	0.32
PT	0.23	0.45
S × PT	0.45	NS

seed) was used as an adhesive and gypsum (200 g.kg^{-1} of seed) as coating material. The pelleted seeds along with unpelleted seeds were tested for its performance in sandy loam, acidic, sodic, and calcareous soils. Results on the performance of pelleted seeds in different soils indicated that the pelleted seeds of *A. nilotica* registered significantly higher germination and vigour than the unpelleted ones under all soil types. Maximum germination (66%) and seedling vigour were recorded in sandy loam soil. In the acidic soil, pelleted seeds recorded significantly higher germination and seedling vigour than the unpelleted seeds. Hence pelleting with biofertilizer along with macro- and micronutrients and biocides could be recommended for augmenting germination and better growth and also to withstand swampy condition and deleterious effect of soils like acidic soil.

Table 3

Performance of pelleted seed under different soil types in *Acacia nilotica*

Pelleting Treatments (PT)	*Vigour Index*				
	Soil condition (S)				
	Calcareous soil	*Sandy loam*	*Acidic soil*	*Sodic soil*	*Mean*
Unpelleted (T_1)	2571	3003	1148	2288	2252
Pesticide + fungicide (T_2)	2737	3176	2232	2664	2702
Macro- + micronutrient (T_3)	2858	3365	2394	2805	2855
Bio-fertilizer (T_4)	2774	3204	2019	2599	2649
Macro- + micronutrient + Biofertilizer (T_5)	2902	3318	2320	2775	2829
Pesticide + fungicide + Macro- + micronutrient (T_6)	2880	3309	2218	2730	2784
Pesticide + fungicide + Bio-fertilizer (T_7)	2785	3175	1919	2599	2620
Pesticide + fungicide + macro- + micronutrient + biofertilizer (T_8)	2904	3351	2175	2782	2803
Mean	2801	3237	2053	2655	

	SEd	C.D. (P = 0.05)
S	47.4020	94.0924
PT	67.0367	133.0668
S × PT	134.0733	266.1336

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Authors

G. Mani, A.S. Ponnuswamy and K. Vanangamundi
Department of Seed Technology
Tamil Nadu Agricultural University
Coimbatore-641 003, Tamil Nadu, India

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Bacillus thuringiensis— An Effective Bioinsecticide

Introduction

Entomopathogens (pathogens of insects) have been suggested as controlling agents of insect pests for over a century. Synthetic organic chemical insecticides in vogue today were not available until about 50 years after control of an insect pest was demonstrated by using an entomopathogen. Obviously, the development of microbial insecticides has been slow, and therefore, increased awareness of the impact of toxic, broad-spectrum chemical insecticides is essential.

Of the nearly one million species of known insects, only about 15,000 species are considered pests and only about 300 are destructive enough to warrant for control. Fortunately, most insect pests have pathogenic microorganisms associated with them. About 1500 entomopathogens belonging to bacteria, viruses, fungi or protozoa are known. Of these, bacteria and viruses have been developed into commercial products in some countries. This is because of their known effectiveness and relative lack of toxicity or pathogenicity to non-target plants and animals.

Criteria for Microbial Insecticide

An entomopathogen must satisfy certain technical criteria before it can be developed into a microbial insecticide. Three most important criteria are:

1. Availability or feasibility of a systematic continuous production technology,
2. Minimal or no toxicity or pathogenicity to man, non-target animals and plants, and
3. Proven effectiveness against intended target pest.

Control of insect pests with bacteria was probably first attempted by d'Herelle (1914). White and Dutky (1940) succeeded in demonstrating control of the Japanese beetle by

distributing spores of *Bacillus popilliae*. Undoubtedly, this success stimulated other investigators to reinvestigate bacteria and literature began appearing on the effectiveness of *Bacillus thuringiensis*. Issuance of eight patents between the years 1960 and 1963 for *B. thuringiensis* further attested to the revived interest in bacterial insecticides (Briggs, 1964).

The group of microorganisms referred to in Bergey's Manual as *Bacillus thuringiensis* is characterized primarily by the formation of one or more proteinaceous parasporal bodies or crystals intracellularly (Aungus, 1956; Burgerian, 1965; Heimpel, 1967) which is a rare event in the living system. These crystals formed by *B. thuringiensis* group of microorganisms are toxic to the larval stages of certain Lepidopterous insects has been reported.

Certain strains of *B. thuringiensis* excrete a nucleotide derivative into the medium during the vegetative and granular phase of growth. The compound itself, the B-exotoxin, is toxic for several insects (McConell and Richards 1959).

The haemolymph is the only extracellular fluid in insects and the changes in it reflect the physiological changes after infection with *B. thuringiensis*. The estimation of different haemolymph enzymes after infection provides information on the possible mode of action of different toxins. Further, the spore forming facultative insect pathogenic bacterium possesses obvious advantage to develop an insecticide than the obligate pathogen (*B. popilliae*).

Material and Methods

The insecticidal property of *B. thuringiensis* var. *thuringiensis* is studied against the insect pests of some economic plants like jawar, potato and gram crop. The bacterium was grown in glucose-yeast extract salt medium and the culture was used for the preparation of "whole culture," spore-crystal complex and crude B-exotoxin.

The fifth instar larvae of Armywarm (*Spodoptera* spp.), potato tuber moth and gram crop were used as test insect pests. These larvae were infected with whole-culture, spore-crystal complex and crude B-exotoxin separately by feeding them with the leaves treated with these components. A separate batch was maintained as control. Larvae were immobilized by ether at 0, 24, 48 and 72 hours. The haemolymph was collected, centrifuged at high speed to remove the blood cells and tissues fragments and then used for the assay of hyaluronidase (Kass and Seastone, 1944) and acetyl cholinesterase (Hestrin, 1949).

Results

The larvae treated with B-exotoxin and whole culture showed an increased activity, while after 72 hrs the hyaluronidase activity decreased in all treated cases (Fig. 1). However, after 72 hrs, the acetylcholinesterase activity increased in all the treated cases (Fig. 2).

The larvae of potato tuber moth were found to be killed within 48 hrs while those of gram crop died within 36 hrs.

Discussion

Hyaluronic acid is a cell cementing substance of insect tissues. The gut damage and subsequent leakage of gut contents in haemocoel is possible only after destruction of hyaluronic acid by increased hyaluronidase activity. Decreased acetylcholinesterase activity as a result of spore-crystal complex and whole culture caused gut paralysis followed by the general paralysis.

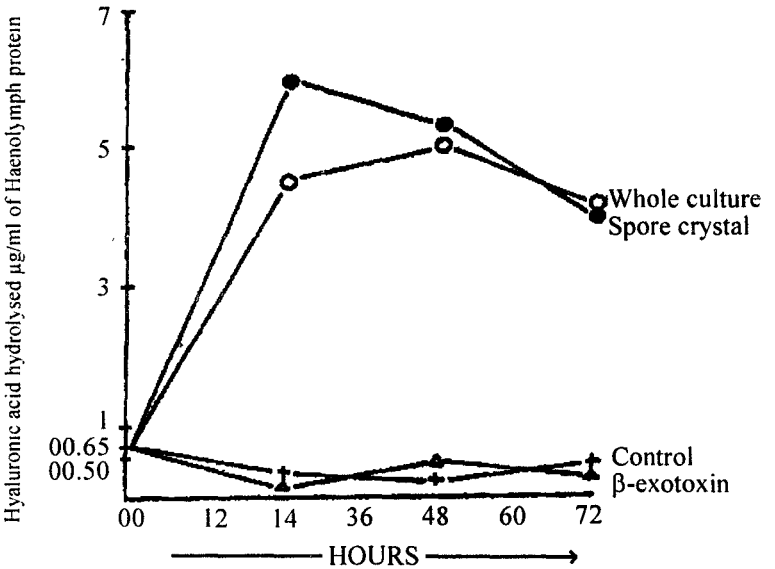

Fig. 1. Hyaluronidase activity

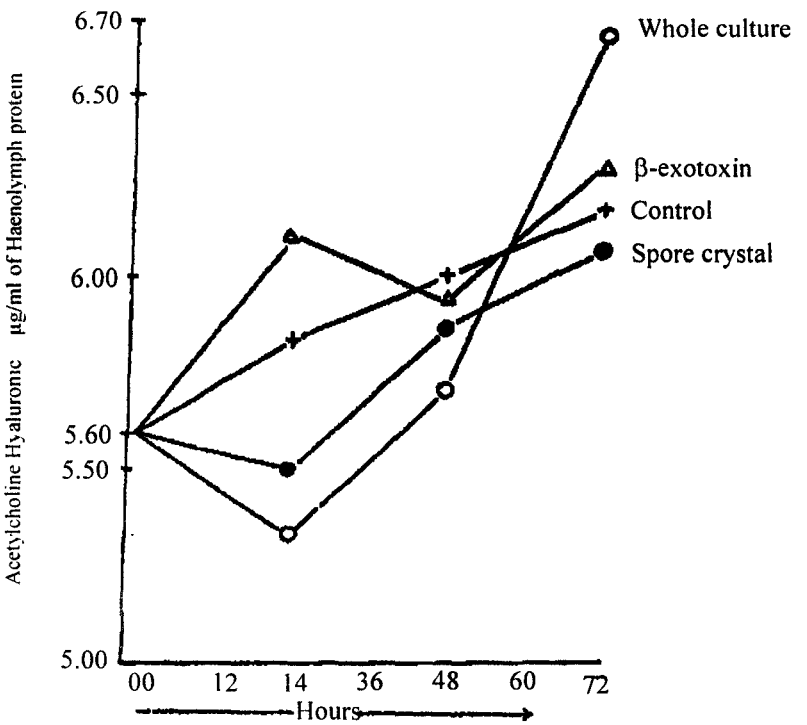

Fig. 2. Acetylcholinesterase activity

Increased hyaluronidase activity and decreased acetylcholinesterase activity in larvae by *B. thuringiensis* infection has a cumulative effect as a biological pest control agent. The death of larvae after feeding on the larvae with *B. thuringiensis* components was due to the toxic action. Thus, *Bacillus thuringiensis* can be used most effectively as a biopesticide agent.

Summary

The insecticidal properties of *Bacillus thuringiensis* were studied against the insect pests of some economic plants like jawar, potato and gram with special reference to hyaluronidase and acetylcholinesterase activity. The larvae were infected with whole-culture, spore-crystal complex and B-exotoxin prepared from *B. thuringiensis*. Increased hyaluronidase activity in larvae has a cumulative effect of *B. thuringiensis* as a biological pest control agent. The bacterium showed effectiveness against armywarm, potato tuber moth and larvae of gram crop.

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Authors

M.G. Bodhankar
Department of Microbiology
Bhartiya Vidyapeeth's A.S.C. College
Sangli-416 416, Maharashtra, India

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Field evaluation of different formulations of *Azospirillum* inoculants on Rice Crop

Introduction

Biofertilizers are eco-friendly and environmentally safe and there is a growing awareness among the farmers about their use. Biofertilizers have been recognized as a vital component of the integrated nutrient supply system and organic farming (Jeyabal *et al.*, 1999).

Azospirillum is one of the important biofertilizers, which is found to fix atmospheric nitrogen in association with crops like rice, maize, sorghum, wheat and millets. Besides nitrogen fixation, *Azospirillum* secretes plant growth regulators *viz.*, Indole - acetic acid, gibberelic acid and vitamins etc. (Tien *et al.*, 1979).

One of the vital problem in inoculant technology is the survival of the microorganisms during storage and several parameters have an influence on their survival *viz.*, the culture medium, the physiological state of the microorganisms when harvested (Chen and Alexander, 1973), the process of dehydration, rate of drying (Mary *et al.*, 1985), the temperature of storage and water activity of the inoculum (Hahn-Hagerdal, 1986). All these factors lead to the shorter shelf life of inoculants i.e., three to six months under normal storage conditions. Hence studies to increase the shelf life of inoculants or finding alternate formulations for carrier inoculants are gaining importance.

Materials and Methods

Source of Azospirillum and Phosphobacteria

Azospirillum and Phosphobacteria cultures were obtained from the Biofertilizer unit of Department of Agricultural Microbiology, Agricultural College and Research Institute, Madurai.

Studies on the induction of encystment in Azospirillum

The *Azospirillum* culture was grown in N-free malic acid broth up to the OD value of 1.45 to get a population of 10^9 cells ml^{-1}. The cells were harvested by centrifugation at 5000 rpm at 4^0C and washed three times with 100 mM potassium phophate buffer solution. Twenty-five ml of this culture was inoculated into the minimal salts medium (Neyra and Van Berkum, 1977) (Appendix) and incubated at 120 rpm at room temperature.

Regeneration of cyst cells into vegetative cells

Cyst cells of *Azospirillum* was examined in N- free malic acid medium with ammonium chloride (0.01%) as nitrogen source (N+ malate medium). Cyst inoculum was inoculated in to N+ malate medium and incubated at 120 rpm at room temperature. The morphological changes of cyst cells were observed at hourly interval with phase contrast microscope. The regeneration of cyst cells were examined by serial dilution and plating up to 12 h.

Induction of sporulation in phosphobacteria (Bacillus sp.)

Sporulation was induced by supplementary nutrient medium (Setlow and Kornberg, 1969). This media was prepared as per the composition (Appendix). After inoculation the cells are observed under phase contrast microscope at 24 h interval to calculated frequency of sporulation.

Regeneration of sporulated cells into vegetative cells

Regeneration of spores of *Bacillus* sp. was examined in nutrient broth. One ml of sporulated medium inoculated in nutrient broth and stored at room temperature. The regeneration and multiplication of cells were examined by serial dilution and plating technique (Allen, 1953) at 3 h interval up to 12h.

Studies on the addition of amendments to increase the shelf life of Azospirillum and Phosphobacteria

The rice husk ash and coir dust were powdered and sieved through 106 micron size sieve. The carrier material was mixed with amendments *viz.*, coir dust, soy meal and molasses were packed in 250 g lots polythene bags and sterilized in autoclave.

Azospirillum, Phosphobacteria and *Azophos* was inoculated into the sterile carrier material aseptically, until 35 per cent moisture was obtained. The bags were sealed and thoroughly kneaded to ensure absorbtion of the liquid culture into the carrier.

Survival of different forms of microbial inoculants on the rice seeds (MDU 5) under* in vitro *conditions

The cyst based *Azospirillum* and spore based inoculum were prepared and the adherence and survival of *Azospirillum* and *Phosphobacteria* inoculum on rice seeds (MDU 5) were observed.

Results & Discussion

Conversion of vegetative cells of Azospirillum into cyst cells in Minimal Salt Medium (MSM)

Azospirillum cells were inoculated into Minimal Salt Medium (MSM) and the conversion of vegetative cells into cyst forms were observed from 12 h to 96 h. The results revealed that the cyst conversion was 11, 25, 53, 74 and 92 per cent for 12, 24, 48, 72 and 96 h respectively.

Regeneration of cyst cells of Azospirillum into vegetative cells in N + Malate medium

The population count of *Azospirillum* revealed that N + malate medium supported rapid regeneration and multiplication.

Induction of sporulation of Bacillus sp. in Supplementary Nutrient Medium (SNM)

Supplementary Nutrient Medium (SNM) induced the conversion of vegetative cells of *Bacillus* sp. into spore cells.

Regeneration of sporulated Bacillus sp. culture

The sporulated *Bacillus* sp. was known to regenerate in nutrient broth.

Survival of Azospirillum and Phosphobacteria on Azophos inoculant treated rice seeds under in vitro condition

The population of *Azospirillum* and Phosphobacteria adhered on paddy seed coat after seed treatment with Azophos inoculant was enumerated. The results revealed that during initial stage the *Azospirillum* population ranged from 0.9×10^2 to 3.1×10^5 MPN g^{-1} for different treatments. During initial stage the Phosphobacterial population ranged from 0.7×10^1 to 4.2×10^5 cfu g^{-1} for different treatments.

Effect of different forms of microbial inoculants on rice (MDU 5) Organic carbon content

The organic carbon of field soil was analyzed during initial stage and on 30 DAT the treatment T_6 and T_2 recorded maximum organic carbon content of 0.45 per cent followed by T_4 and T_5 (0.44%), T_3 (0.42%) and T_1 (0.41%).

Soil available nitrogen, phosphorus and potassium

The initial soil available nitrogen phosphorus and potassium content was 282.2 kg ha^{-1}, 10.4 to 12.63 kg ha^{-1} and 171.2 to 178.4 kg ha^{-1} respectively. During 30 DAT the maximum nitrogen, phosphorus and potassium recorded in T_6 321.4 kg ha^{-1}, 13.78 kg ha^{-1} and 219.8 kg ha^{-1} followed by T_2, T_4, T_3, T_1 and T_5.

Effect of different forms of microbial inoculants on rice (MDU5)

The treatment T_3 recorded the maximum total nitrogen content (1.27 %), followed by T_5 (1.26

%). Treatment T_3 and T_6 recorded the maximum phosphorus content (0.40 %) and maximum potassium (0.68 %).

It was observed that the treatment T_6 (*Azophos* cyst and spore form) recorded maximum grain yield of 4.54 t ha^{-1} followed by T_2 (*Azospirillum* cyst) 4.40 t ha^{-1}. Whereas the treatment T_4 (*Azospirillum* cyst cells) recorded the grain yield of 4.34 t ha^{-1}.

Studies on the survival of Azospirillum and phosphobacteria (dormant and vegetative cells) on Azophos inoculants treated rice seeds

In the present investigation rice seeds treated with *Azophos* carried *Azospirillum* and phosphobacteria as inoculum. In *Azophos* inoculant, the survival of *Azospirillum* on rice seeds recorded maximum population of 0.5×10^3 MPN g^{-1} in treatment T_5 (*Azophos* cyst and spore + amendments + rice gruel), followed by T_3 (*Azophos* cyst and spore + rice gruel) with a population of 1.1×10^3 MPN g^{-1} after 12 h of incubation. *Azophos* having cyst and spore forms of *Azospirillum* and *Phosphobacteria* as inoculum recorded more number of survival on rice seeds.

Studies on the influence of different forms of microbial inoculants on rice (MDU 5) under field condition

Field experiment was conducted to evaluate the population dynamics of *Azospirillum* and *phosphobacteria* in the rhizosphere soil of rice.

In general, *Azospirillum and Phosphobacteria* population increased as the days progressed. During 45 DAT, maximum *Azospirillum* population (0.5×10^8 MPN g^{-1}) maximum Phosphobacterial population (1.1×10^7 cfu g^{-1}) was recorded in the treatment of T_6.

Population dynamics of Azospirillum endophyte

The population dynamics of *Azospirillum* in epidermis cells of rice roots and culm region was studied. The treatment T_6 (*Azophos* cyst and spore) recorded the highest population of 2.3×10^3 MPN g^{-1} and the lowest endophyte population was in T_3 (Phosphobacteria spore) after 15 DAT. On 30th day, the maximum population was recorded in T_2 (4.1×10^3 MPN g^{-1}) and the lowest population was recorded in T_5 in the root region.

Organic carbon content

The organic carbon content has been analysed in the soil sample collected from the field trial. The experimental data revealed that the organic carbon content in the soil increased as the days progressed up to 60 DAT. Among the treatments, T_6 (*Azophos* cyst and spore) recorded maximum organic carbon in the soil. Similar result was observed by Muralikannan (1996) who reported an increase in organic carbon content after 30 days of transplanting of rice.

Effect of different forms of microbial inoculants on soil available NPK of rice

Among the treatments, T_6 (*Azophos* cyst and spore) recorded highest level of nitrogen, followed by T_2 (*Azospirillum* cyst) and T_4 (*Azospirillum* vegetative cells). It is well known that application of *Azospirillum* enhances the soil available nitrogen and cyst form of *Azospirillum* recorded

the maximum available nitrogen indicating better survival of cyst form of *Azospirillum* and also better Nitrogen fixation.

The available phosphorus was recorded maximum during 60 DAT. Among the treatments, T_6 (*Azophos* cyst and spore) recorded highest level of phosphorus, followed by T_3 (Phosphobacterial spore) and T_5 (Phosphobacterial vegetative cells).

Ganguly *et al.* (1999) also stated that the dual inoculation of *Azospirillum brasilense* and Microphos (*Bacillus megaterium*) with low soil N and P levels helped to fix more amount of nitrogen.

The soil available potassium was maximum in the T_6 *Azophos* (cyst and spore), followed by T_3 (Phosphobacterial spore). It is inferred that application of *Phosphobacteria* also indirectly enhanced the soil available potassium.

Effect of different forms microbial inoculants on plant NPK content of rice

The *Azophos* (cyst and spore cells of *Azospirillum* and *Phosphobacteria*) recorded maximum nitrogen uptake, followed by T_2 (*Azospirillum* cyst) and T_4 (*Azospirillum* vegetative cells).

Plant phosphorus was recorded maximum during 60 DAT. Among the treatments, T_6 (*Azophos* cyst and spore) recorded highest level of phosphorus followed by T_3 (Phosphobacterial

Table 1

Effect of different forms of microbial inoculants on growth characters of rice (MDU 5)

Sl. No.	*Treatment*	*Plant height (cm)*	*No. of tillers hill^{-1}*	*No. of productive tillers hill^{-1}*	*1000 grain weight (g)*	*Grain yield (t ha^{-1})*
1.	T_1- Control	81.2	8	7	21.04	4.20
2.	T_2- *Azospirillum* (cyst)	85.3	11	9	21.65	4.40
3.	T_3- *Phosphobacteria* (spore)	82.1	10	7	20.94	4.12
4.	T_4- *Azospirillum* (log phase cells)	84.7	10	8	21.36	4.34
5.	T_5- *Phosphobacteria* (log phase cells)	81.6 86.2	9 11	7 10	21.30 22.90	4.15 4.54
6.	T_6- Azophos (cyst and spore)					
	SED	0.0856	0.07842	0.8942	0.0909	0.0921
	CD	0.1948**	1.5921**	1.9213**	0.1847**	0.3146**

** - Significant at 1 % level

spore) and T_5 (Phosphobacterial vegetative cells). Application of phosphobacteria enhanced the uptake of phosphorus and T_6 (cyst and spore form of *Azospirillum* and *Phosphobacteria*) recorded the maximum available phosphorus indicating that phosphate solubilization was more in this treatment when compared to other treatments.

Azophos (cyst and spore) and *Phosphobacteria* (spore) showed higher Phosphorus and potassium content in rice plant when compared to other treatments. Similar result was reported by Belimov *et al.* (1995) who observed enhanced absorption of Nitrogen and Phosphorus in barley plants.

Biometric observation

The *Azophos* influenced the crop growth of rice. The plant height, number of total tillers hill^{-1}, number of productive tillers hill^{-1} and 1000 grain weight were found to be higher in *Azophos* (cyst and spore) inoculated plots than uninoculated plots (control). The plant biometric observations were recorded for different treatments, the treatments T_1 (control) and T_5 (Phosphobacterial spore) resulted lowest plant height whereas the treatment T_6 (*Azophos* cyst and spore) and T_2 (*Azospirillum* cyst) recorded the highest plant height i.e. the combination of cyst form of *Azospirillum* and spore form of Phosphobacteria resulted maximum plant height, followed T_2 (*Azospirillum* cyst). The productive tillers highest in T_6 Azophos (cyst and spore), followed by T_2 (*Azospirillum* cyst). Thousand grain weight was recorded highest in the treatment of T_6 (Azophos cyst and spore), followed T_2 (*Azospirillum* cyst). The results clearly revealed that Azophos (cyst and spore) could be usefully exploited as a mixed biofertilizer. The results of the present study are similar to the finding of Watanabe and Lin (1984) who reported increased crop growth in rice inoculated with mixed cultures of *Azospirillum* and *Pseudomonas* sp.

Effect of different forms of microbial inoculants on grain and straw yield of rice

The grain and straw yield of rice were recorded after the harvest of the crop. It was observed that the treatment T_6 (*Azophos* cyst and spore form) recorded maximum grain yield of 4.54 t ha^{-1} followed by T_2 (*Azospirillum* cyst) 4.40 t ha^{-1}. Whereas the treatment T_4 (*Azospirillum* cyst cells) recorded the grain yield of 4.34 t ha^{-1}. The results clearly indicated that cyst and spore forms of *Azospirillum* and Phosphobacterial inoculants resulted maximum grain yield of rice when compared to conventional form of *Azospirillum* (vegetative cells) inoculant. The inducement of cyst and spore resulted in better survival of organisms in the soil, resulting in maximum grain yield of rice. Similar results were recorded in the straw yield of rice.

These findings are in agreement with the findings of Thamizhvendan and Subramanian (1997) who observed increased rice yield due to combined inoculation of *Azospirillum* and *Phosphobacteria*. Vijaya Nirmala and Sundaram (1996) and Kumar *et al.* (1998) also reported similar findings.

Author

R. Saravanan and G. Rajannan
Department of Environment Sciences, TNAU,
Coimbatore-641 003, Tamil Nadu.

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Effects of *Psuedomonas* on Wheat *Fusarium* Root Rot

Introduction

Fusarium root rot caused by *Gibberella zea* (anamorphe stage of *Fusarium graminearum*) and *F. culmurum*, in which *F. aminearum* is more common and an important root rot diseases of wheat in Mazandaran province, Iran. Yield losses due to this disease up to 20%.

Emergence of fungicide-resistant pathogens, health concerns for producer and consumer, and the phasing out of chemicals have prompted research into viable alternative practices to achieve more sustainable levels of agricultural production. Since the 1980s rhizobacteria and other microorganisms have been investigated as possible replacements for chemicals used to control a broad range of plant diseases (Ross *et al.*, 2000). Isolates of *Pseudomonas* have been tested due to their widespread distribution in soil, ability to colonize the rhizospheres of host plants, and produce a range of compounds antagonistic to a number of serious plant pathogens (Anjaiah *et al.*, 1998; Maurhofer *et al.*, 1991 and Thomashow *et al.*, 1997).

Biological control of wheat diseases using bacterial species (*Pseudomonas* and *Bacillus*) has been reported (Capper and Campbell, 1986; Kloepper *et al.*,1980; Ryder and Rovira,1993; Weller and Cook, 1983 and Weller, 1988). Weller and Cook (1983) reported that 10-25 % of the yield increases by the application of root-colonizing bacteria antagonistic to take-all pathogen as seed treatment at planting time.

This report is a part of the results project of "investigation of root rot diseases of barley in Mazandaran province". *P. fluorescens*. B-6 and B-11 have been demonstrated to have the ability to control of *Fusarium* root rot.

Materials and Methods

Wheat (*Triticum sativum*) seeds of Tajan facultative cultivar, which is the commercial in

the province, and *Fusarium graminearum* B-8 (the most aggressive strains of pathogen among the others), obtained from Agricultural and Natural Resources Research Center of Mazandaran.

For isolation of wheat rhizobacteria various samples were collected from different parts of wheat fields in Baykola, Dashtnaz, Kolet, Gharakheil, Firouzkandeh, Jouibar, Semeskandeh and Zirvan, Mazandaran province in Iran during 2004-2005. The samples were taken from the root zone of one month old wheat seedlings. Wheat plants were dug out and gently shaken off and then the soil closely adhering the roots were collected. Rhizosphere bacteria were isolated using Kings' B Medium (Weller & Cook, 1983). One gram of soil sample was suspended in 10 ml of sterile distilled water and was vigorously shaken for 15-20 minutes. Serial dilution was done by transferring 1 ml suspension to 9 ml sterile distilled water. A 0.1 ml suspension of 10^7 to 10^9 dilutions was spread on solidified either in Kings' B Medium. The plates then were kept for incubation at 25 to 28 °C for 24 hr the individual bacteria colonies were picked on nutrient agar (NA) slants and after incubation at 28 °C for 24 hr, the slants were kept at 4 °C for short period maintenance. All bacterial isolates were purified, coded and preserved. The code were made based on the first alphabetic of locations from which samples had been collected, such as B (Baykola), D (Dashtnaz), K (Kolet), G (Gharakheil), F (Firouzkandeh), J (Jouibar), S (Semeskandeh) and Z (Zirvan), as shown in Table 1. The bacteria were screened on the basis of their *in vitro* antagonistic activity towards *Fg* and maximum inhibition zone (Weller & Cook, 1983). Eight superior bacteria were selected for disease control in greenhouse and field conditions.

Identification of antagonist bacteria was done on the bases of the ability of isolates to produce fluorescent by plating bacteria on King's B medium, biochemical and physiological tests such as; Gram, hyper sensitive reaction in tobacco, oxidizes, nitrate reduction and fluorescence production (King *et al.*, 1954; Krieg and Hopt 1984; Holt et *al.*, 1994; Schhaad *et al.*, 2001).

Disease control

1. *In greenhouse*

Tajan wheat seeds were bacterized with 8 superior bacterial isolates. The seeds were soaked for 4 hr. in pre-grown selected bacterial isolates on nutrient broth yeast extract agar (NBYA) separately, at the rate of 10^9 colony forming unit (CFUs)/ml, approximately OD 0.2-0.3 at A620n (Weller *et al.*, 1988). Nutrient broth yeast extract agar was prepared based on 2, 2, 0.5, 2.5 and 1.5 gram of nutrient broth, yeast extract, K_2HPO_4, $K_2H_2PO_4$, glucose and agar respectively per liter distilled water (Baudoin *et al.*, 1988).

The seeds also were coated with Benlate 20% (2g/kg) as selected fungicide. This fungicide was screened following *in-vitro* inhibition assay, among the other candidate fungicides i.e., Propiconazole and Mancozeb. Fungicide was used in greenhouse and field evaluation studies, to compare the efficacy of bio-control versus chemical control against the disease.

The treated seeds then were planted on pre-inoculated potted soil with *Fg* B-8. There were three replication pods for each treatment, based on a randomized completed design (Balasubramaniyan and Palaniappan, 2004), with 11 treatments. The treatments include:

1. *P. fluorescens* B6,
2. *P. fluorescens* B11,
3. *P. fluorescens* F8,
4. *P. fluorescens* F11,
5. *P. fluorescens* N 6,
6. *P. fluorescens* S 5,
7. *P. fluorescens* Z 8,
8. *P. fluorescens* Z 48,
9. Benlate 20%,
10. Check 1 (seed treated with sterile distilled water and planted in non inoculated soil with pathogen) and
11. Check 2 (seed treated with sterile distilled water and planted in inoculated soil with pathogen).

Evaluation of the antagonists was done after disease progress in inoculated check plants with *Fg* B-8 only. Disease rating was done on the bases of 0-4 scale, where, 0 = no disease and 4 = death of plants.

2. *In field*

The experiment was carried out at Baikola Agricultural Research Station of Mazandaran on a site, previously cropped to wheat. The site was cultivated with an offset disk plough to 15 cm depth and again to a 10 cm depth on Oct.11, 2003. The soil was sterilized with methyl bromide at the rate of 40 g/sq.m on Oct.12, 2003 to removal any pre-contamination of the trial soil. Cress plant (*Lepidium sativum*) seeds were grown as bioassay test to insurance the removal of methyl bromide hazard effects on Nov. 11. 2003.

An experimental design was carried out as randomized complete block (Balasubramaniyan and Palaniappan, 2004) with 11 treatments and 3 replications as descrice in greenhouse method. Plots size was 0.9 m × 6 m. The trial received 100 kg⁻h urea, 60 kg⁻h diammonium phosphates and 50 kg⁻h potassium sulfate. The plots were pre inoculated either with 2 weeks old colonized wild oat (*Avena fatua*) seeds with strain of *Fusarium graminearum* B-8 or sterile wild oat (*Avena fatua*) only as control treatment.

The trial was sown with bacterized and fungicide coated seeds of Tajan wheat (as described above) and at the rate of 60 kg/ha on Nov.15.2004. The seeds were sown in four rows (6 m long and 30 cm of row spacing). The trial was kept weeds free by hand weeding in several times as required. Plots were visually assessed for disease after disease progress on inoculated control plants with *Fusarium graminearum* B-8 only, in the end of April 2005. Two rows of the each plot were harvested by hand and grain yields were obtained using a small thresher and analyzed on May 24, 2004.

Results & Discussion

A total of 31 wheat Fluorescen P seudomonads were isolated from the rhizosphere of different wheat fields in Mazandaran province. The antagonism of the bacteria was tested following dual culture technique. Eight out of 31 showed maximum inhibition against *Fg* B-8. The selected strains were identified as Biovar 1 *Psuedomonas fluorescens* and *Bacillus subtilis*. They could significantly ($p \leq 0.05$) affect the mycelial growth of the pathogen (Table 2).

The effective bacteria including; *P. fluorescens* B-6, *P. fluorescens* B-11, *P. fluorescens* F-8, *P. fluorescens* F-11, *P. fluorescens* N-6, *P. fluorescens* S-5, *P. fluorescens* Z-8 and *P. fluorescens* Z-48.

Effects of antagonists on infection degree and yield were summarized at Table 3. Examination of root systems of unprotected wheat plants showed extensive colonization on rotted seminal roots. There were also more disease symptoms in unprotected check (check 2), as compared with the other treatments.

Pseudomonas strains have been considered to have an attribute to biological control of some soil borne diseases (Capper and Campbell, 1986; Duffy & Weller, 1994).

In vitro antagonism experiments with *Fg*, revealed that 8 out of 31 isolates tested demonstrated detectable antifungal activity, and consistently induced reproducible zone of fungal inhibition on PDA medium. These isolates were selected for the antagonistic against the *Fusarium* root rot disease in greenhouse and in field trials. All 8 isolates could reduce disease severity and increased the grain yield in greenhouse trials. But only two isolates (*P. fluorescens*.B-6 and B-11), were the most effective in controlling the disease under natural conditions (table 3). As shown in the table, other selected bacteria did not have this ability. Because all factors in which were stabled in green house, could not controlled in the field trial. It was postulated that the 2 isolates are native and were obtained from the local area of the study. The results were in conformity with the reports of Duffy and Weller (1994) and Weller and Cook (1983). Difficulties in developing microorganisms as viable alternatives to chemical control, mentioned by Ross *et al.* (2000). They expressed that many biological control agents are found to be active only in certain soil types. Effects of factors such as soil texture, organic matter, pH, water and oxygen availability, and competition for nutrients with indigenous micro flora on dampen the biological activity of introduced inocula, also have been described by Capper *et al.*, (1993), Duffy *et al.* (1997) and Johnson *et al.* (1998).

In conclusion biological control therefore assumes special significance in being eco-friendly cost effective strategy, which can be used in the integration with other disease management systems to afford greater levels of protection and sustain crop yield.

Table 1
Areas and Code of bacterial antagonist isolates

Area	*Code*	*Number of isolates*
Baykola (Neka)	B	18
Dashtnaz (Sari)	D	18
Kolet (Neka)	K	16
Gharakheil (Ghaemshar)	G	15
Firouzkandeh (Sari)	F	24
Joibar	J	15
Semeskandeh (Sari)	S	28
Zirvan (Behshar)	Z	16

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Table 2

Mean percentage inhibition of mycelial growth of *Fg* by bacterial isolates

Isolate	*Inhibition %*	*Isolate*	*Inhibition %*
B3	2.33 ^{f}	J4	5.55^{def}
B6	15.44^{a}	J5	2.33 ^{f}
B8	6.12 ^{de}	J 6	2.33 ^{f}
B10	3.33 ^{f}	J 8	5.55^{de}
B11	10.42^{b}	J 22	2.33 ^{f}
D1	5.63^{def}	K4	2.33 ^{f}
D3	5.23^{def}	N1	6.66 ^{de}
D4	5.44^{def}	N4	3.33 ^{f}
D11	6.11 ^{de}	N6	9.66^{bc}
F1	3.33 ^{f}	N12	5.55^{def}
F5	5.55^{def}	S1	3.33 ^{f}
F8	9.76 ^{bc}	S 2	5.55^{def}
F11	9.66 ^{bc}	S3	4.44^{de}
G1	3.33 ^{f}	S5	9.66^{bc}
G8	5.15^{e}	S10	2.35 ^{f}
G11	2.33 ^{f}	S11	5.55^{def}
G22	3.33 ^{f}	S15	6.66 ^{de}
F12	2.33 ^{f}	Z1	4.44^{de}
F13	2.33 ^{f}	Z6	5.55^{def}
G1	3.33 ^{f}	Z7	3.10 ^{f}
G2	5.15^{e}	Z8	9.66^{bc}
G6	2.22 ^{f}	Z11	2.35 ^{f}
G8	3.33 ^{f}	Z14	3.39 ^{f}
G12	3.11 ^{f}	Z23	3.23 ^{f}
G14	4.15^{e}	Z25	4.22^{e}
G16	3.17 ^{f}	Z24	3.2 ^{f}
G18	3.11 ^{f}	Z27	3.10 ^{f}
G21	3.33 ^{f}	Z29	3.10 ^{f}
J1	5.22^{e}	Z31	5.1^{e}
J2	3.31 ^{f}	Z48	9.72^{bc}
J3	3.33 ^{f}	Z62	3.18 ^{f}

Table 3
Effect of bacterial isolates on disease severity and yield induced by *Fg* in greenhouse and field.

Isolate	*Disease severity (DS) and yield (Y)*			
	Greenhouse		*Field*	
	*DS**	*Y***	*DS****	*Y*****
P. fluorescens B-6	1.321^{b}	35.22^{b}	1.55^{c}	2.924^{b}
P. fluorescens B-11	1.320^{b}	35.11^{b}	1.34^{c}	2.911^{b}
P. fluorescens F-8	1.312^{b}	35.21^{b}	2.20^{b}	2.340^{c}
P. fluorescens F-11	1.318^{b}	35.4^{b}	2.15^{b}	2.328^{c}
P. fluorescens N- 6	1.318^{b}	36.10^{b}	2.19^{b}	2.332^{c}
P. fluorescens S- 5	1.320^{b}	35^{b}	2.17^{b}	2.311^{c}
P. fluorescens Z-8	1.315^{b}	35^{b}	2.20^{b}	2.312^{c}
P. fluorescens Z-48	1.314^{b}	36^{b}	2.15^{b}	2.212^{c}
Benlate 20%	1.310^{b}	35.13^{b}	1.58^{b}	2.854^{b}
Check1 (non-infected)	0.007^{c}	40.12^{a}	0.007^{d}	3.14^{a}
Check 2 (infected)	3.114^{a}	29.22^{c}	2.43^{a}	2.214^{d}
CV%	4.72	4.12	11.42	12.20

*Mean of 5 plants/pod **1000 grain weight ***Mean of 5 plants/plot, ****T/h

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Author

Foroutan, Abdolreza[a], Yasari, Esmaeil[b] and Foroutan, Aidin[c]

[a]*Agricultural & Natural Resources Research Center of Mazandaran, Sary, Iran.*

[b]*Agricultural Organization of Mazandaran Province. Sary, Iran.*

[c]*Faculty of Agricultural Sciences of Mazandaran University, Sary, Iran.*

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Effects of *Trichoderma* on Wheat Sharp Eye Spot

Introduction

Wheat is the most staple crop in Iran. Sharp eye spot of wheat plant is caused by *Rhizoctonia crealis* and *R. solani*, in which *R. crealis* is more common in Mazandran province of Iran. The disease was also reported from other parts of the world (Paulitz *et al.*, 2002; Smiley *et al.*, 2005).

The search for alternatives to chemical control of plant pathogens, such as biological control, has gained momentum in recent years. Emergence of fungicide-resistant pathogens, health concerns for producer and consumer, and the phasing out of chemicals such as methyl bromide have prompted research into viable alternative practices to achieve more sustainable levels of agricultural production.

Biological control of wheat diseases such as Take-all using *Trichoderma*, *Gliocladium* and *Aspergillus*, has been reported (Capper and Campbell, 1986; Kloepper *et al.*,1980; Ryder and Rovira,1993; Weller and Cook, 1983 and Weller, 1988). *Trichoderma* spp. was extensively explored for control of soil borne plant pathogens (Khan and Sinha, 2005).

This report is a part of the results of provincial project of investigation of crown and root rot diseases of wheat in Mazandaran province. The aim of the present study was to identify of *Trichoderma* existing on wheat plants rhizosphere and evaluation of their effectiveness in controlling wheat sharp eye spot.

Materials and Methods

Wheat (*Triticum sativum*) seeds of Tajan facultative cultivar, which is the commercial in the province, and *Rhizoctonia cerealis* D-4 obtained from Agricultural and Natural Resources Research Center of Mazandaran.

Fungal bio-agent isolates were obtained by plating of rhizosphere and soil samples on potato dextrose agar (PDA). The samples were collected from different parts of wheat fields across Mazandaran in 2003-2004 crop season. All fungal isolates were purified, coded and preserved. The codes were made based on the locations from which samples had been collected, such as G (Ghrakheil), D (Dashtnaz), S (Sary), F (Firouzkandeh), B (Baikola), N (Neka), and J (Jouibar) which is depicted in the Table 1. The fungi were screened according to *in vitro* antagonistic activity towards *Rc* D-4 with the maximum inhibition zone. Then the selected isolates were used for greenhouse and field trials.

Identification of *Trichoderma* species were done on the basis of morphoiogical characteristics such as colony growth, phialid, conidia and conidiophores (Rifai, 1969).

Table 1
Areas and Code of fungal antagonist isolates

Area	*Code*	*Number*
Gharakheil	G	8
Dashtnaz	D	10
Firouzkandeh	F	10
Baykola	B	8
Neka	N	11
Sari	S	7
Joibar	J	7

Disease control

1. *In greenhouse*

Mass cultures of the pathogens and bio-agents were prepared on oat (*Avena fatua*) grain. Two kg of natural air steam pasteurized (60°C for 30 min) soil was filled in 20 cm diameter pots. The upper surface soil of each pot was thoroughly mixed with the mass culture of pathogen at 2g/kg soil and covered with polythene sheets. After 4 days mass cultures of fungal species multiplied on oat (*Avena fatua*) grain was added to the soil at 2g/kg in each pot separately. Rovral T-S also was added to the soil at 2g/kg to compare the efficacy of bio-control versus chemical control against the disease. This fungicide was screened following *in vitro* inhibition assay, among the other candidate fungicides i.e., propiconazole, Benomyl. Fungicide was used in greenhouse and field evaluation studies, to compare the efficacy of bio-control versus chemical control against the disease.

These pots were then lightly watered. Then seeds of Tajan wheat cultivar were sown in each plot. Three replications were maintained for each treatment based on a randomized completed design (Balasubramaniyan and Palaniappan, 2004), with 15 treatments. The treatments include: 1. *T. hurzianum.* B-3, 2. *Trichoderma* sp. B-4, 3. *T. hurzianum.* B-7, 4. *T. hurzianum* D-2, 5. *T. hurzianum* D-4, 6. *Trichoderma* sp. F-6, 7. *T. hurzianum* F-7, 8. *T. virens*

S-7, 9. *T. virens* J-5, 10. *T. hurzianum* J-7, 11. *T. viridae* G-3, 12. *T. hurzianum* G-4, 13. Rovral-Ts, 14. Seed treated with sterile distilled water and planted in non inoculated soil with pathogen as control 1, 15. Seed treated with sterile distilled water and planted in inoculated soil with pathogen as control 2.

Effect of antagonists on disease severity then was done after disease progress on inoculated control plants with *Rc* D-4. Disease rating was done on the bases of 0-4 scale, where, 0 = no disease; 1 = 1-20 %; 2 = 21-40 %; 3 = 41-60 % and 4 = 61-100 % of plants showing stunting and white heads.

2. *In field*

The experiment was carried out at the Gharakheil Agricultural Research Station of Mazandaran. The site was cultivated with an offset disk plough to 15 cm depth and again to a 10 cm depth on Oct.11, 2004. The soil was sterilized with methyl bromide at the rate of 40 g/sq.m on Oct.12, 2004 to remove any pre-contamination of the trial soil. Cress plant (*Lepidium sativum*) seeds were grown as bio-assay test for confidence the removal of methyl bromide hazard effects on Nov. 11, 2004.

An experimental design was carried out as randomized complete block (Balasubramaniyan and Palaniappan, 2004) with 15 treatments and in 3 replications as described in greenhouse method. Plots size was 0.9 m × 6 m. The trial received 100 kg/h urea, 60 kg/h diammonium phosphates and 50 kg/h potassium sulfate. The soil of each plot was inoculated with the mass culture of pathogen. Simultaneously Rovral T-S fungicide and also mass culture of each fungal species were added to the soil.

The trial was sown with Tajan cultivar seeds at the rate of 60 kg/ha on Nov. 15, 2004. The seeds were sown in four rows (6 m long and 30 cm of row spacing). The trial was kept weeds free by hand weeding in several times as required. Plots were visually assessed for disease after disease progress on inoculated control plants with the *Rc* only in the end of April 2005. Two rows of the each plot were harvested by hand and grain yields were obtained using a small thresher and analyzed on May 24, 2005.

Results and Discussion

A total of 61 wheat fungal bio-agents were isolated from the rhizosphere and soil of different wheat fields in Mazandaran. The fungi were tested following dual culture technique. Twelve out of sixty-one showed maximum inhibition against *Rc*-D4. They could significantly ($p = 0.01$) affect the mycelial growth of the pathogen. The selected fungi include; *T. hurzianum*. B-3, *Trichoderma sp.* B-4, *T. hurzianum*. B-7, *T. hurzianum* D-2, *T. hurzianum* D-4, *Trichoderma* sp. F-6, *T. hurzianum* F-7, *T. virens* S-7, *T. virens* J-5, *T. hurzianum* J-7, *T. virens* G-3, *T. hurzianum* G-4.

Table 2 shows the effect of fungal isolates on disease severity and yield of wheat in greenhouse and field trials.

Trichoderma species have been considered to have an attribute to biological control of soil borne diseases (Khan and Sinha, 2005).

Examination of root systems of unprotected plants showed extensive colonization on rotted seminal roots. There were also more symptoms on leaf sheath of tillers in unprotected control (control 2), as compared with the other treatments.

Table 2
Effect of fungal isolates on disease severity (DS) and yield (Y) of *Rc* in greenhouse and field trial.

Isolate	*Greenhouse*		*Field*	
	*DS**	*Y***	*DS****	*Y*****
T. hurzianum. B-3	1.310[b]	35[b]	2[b]	2.381[c]
Trichoderma sp. B-4	1.298[b]	35.8[b]	2[b]	2.301[c]
T. hurzianum B-7	1.318[b]	35[b]	2.06[b]	2.346[c]
T. hurzianum D-2	1.318[b]	35.1[b]	2.05[b]	2.338[c]
T. hurzianum D-4	1.299[b]	36[b]	2.06[b]	2.361[c]
*Trichoderma sp.*F-6	1.318[b]	35[b]	2.05[b]	2.361[c]
T. hurzianum F-7	1.321[b]	35.20[b]	2.06[b]	2.355[c]
T. virens S-7	1.311[b]	35[b]	2.01[b]	2.328[c]
T. virens J-5	1.299[b]	36[b]	2.01[b]	2.326[c]
T. hurzianum J-7	1.318[b]	35[b]	2.03[b]	2.321[c]
T. hurzianum G-3	1.298[b]	36[b]	1.04[c]	2.915[b]
T. virens G-4	1.295[b]	36[b]	1.02[c]	2.910[b]
Rovral-Ts	1.211[b]	36.23[b]	1.57[c]	2.867[b]
Control (noninfecte)	0.007[c]	41.03[a]	0.007[d]	3.680[a]
Control (infected)	3.226[a]	28[c]	2.83[a]	2.181[d]
CV%	4.44	3.32	16.23	14.18

*Mean of. 5 plants /pod, **1000grain weight, ***Mean of 5 plants /plot, ****T/h

As shown at the Table 2, all 12 isolates could reduce the disease severity and increase the grain yield in greenhouse. Only 2 isolates (*Trichoderma hurzianum* G-3 and *T. viridae* G-4) out of twelve could reduce disease severity and increase the grain yield in field trial. The other selected bacteria did not show this ability. Because all factors in which stabled in green house, could not be controlled in the field condition. The two isolates were native which obtained from the area of the study. These strains could customize in the that field. This finding was as par with does of Duffy and Weller in 1994 and Weller and Cook, 1983. The difficulties for developing microorganisms as viable alternatives to chemical control also mentioned by Ross *et al.* in 2000. They expressed that many biological control agents are found to be active only in certain soil types. Effects of factors such as soil texture, organic matter, pH, water and oxygen availability, and competition for nutrients with indigenous micro flora on dampen the biological activity of introduced inoculum (Capper *et al.*, 1993; Duffy *et al.*, 1997; Johnson *et al.*, 1998).

Chemical protection, which are applied, for the different crop protection, are not usual in root rots control. Otherwise chemical protections may results in crops toxicity and land sterility. The constant and injudicious use of chemicals had to be curtailed owing to their hazardous

effects on non-target organisms and because of the undesirable changes they inflect on the environment. In addition chemicals create serious problems such as development of resistance to pesticides, resurgence of target pests, secondary pest outbreak, killing of non-target organisms, residual toxicity and environmental pollution (Bernet, 1995; Sharma *et al.*, 1998 and Unrech *et al.*, 2000).

In conclusion biological control assumes special significance in being eco-friendly cost effective strategy, which can be used in the integration with other disease management systems to afford greater levels of protection and sustain crop yield (Mathivanan *et al.*, 2004).

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Author

Foroutan, Abdolreza[a], Foroutan, Aidin[b] and Yasari, Esmaeil[c]

[a]*Agricultural &Natural Resources Research Center of Mazandaran, Sary, Iran.*

[b]*Faculty of Agricultural Sciences of Mazandaran University, Sary, Iran.*

[c]*Agricultural Organization of Mazandaran Province. Sary, Iran.*

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Inhibitory Effect of Siderophores Produced by *Pseudomonas* sp. on *Salmonella typhi* & its Future Biotechnological Applications

Introduction

Research in the field of siderophores began about five decades ago, which was mostly related to virulence mechanisms in microorganisms pathogenic to both animals and plants (12). Siderophores are produced from wide range of micro-orgnisms including bacteria and fungi. Certain Gram-negative genera, like the *Enterobacteria* and the genus *Vibrio*, are known to produce catecholate siderophores; whereas the Gram-positive *Streptomycetes* produce hydroxamate-type. However, there are certain genera which occasionally produce both siderophore types (3,8). *P. aeruginosa* is known to produce two siderophores: pyochelin and pyoverdin, whereas *P. fluorescens* is known to produce another siderophore pseudomonin (4, 20). These highly efficient low-molecular-weight iron chelating agents compete for and bind freely available extracellular iron [Fe(III)], forming siderophore-iron complexes, which are then recognized and internalizes by the bacteria (12). Siderophore production compromises the effective iron limitation approach of defense. The host could decrease the access of the other organism to iron if host cells could efficiently acquire and sequester iron chelated to its siderophores. Once internalized by these cells, such iron would no longer be accessible to other organisms. Since, iron is essential for a variety of functions including reduction of oxygen for synthesis of ATP, reduction of ribotide precursors of DNA, for formation of heme, and for other essential purposes. A level of at least one micromolar iron is needed for optimum growth (12). Therefore such inaccessibility of iron would lead to inhibition of growth. The two siderophores isolated from cultures Ap1 & Ap2, belonging to *Pseudomonas* sp. were tested against various pathogenic organisms.

Materials and Methods

Soil samples were collected and suspension was prepared in quarter strength Ringer's solution (7). Various dilutions were prepared and plated on N.B. agar. The cultures Ap1 & Ap2 were

isolated by their ability to produce siderophores in the iron free medium. Identification using simple biochemical tests revealed them to be members of the genus *Pseudomonas*. The siderophore production by the cultures was carried in the nutrient medium consisting succinate conc. of 0.4% (16).

After incubation for 48 hrs at room temperature on a Orbitek rotary shaker at 80 rpm, the medium was centrifuged at 1000 rpm for 20 mins at 4°C on a Remi cooling centrifuge CPR 24, and the supernatant was separated and analyzed by the Chemito UV-Visible spectrophotometer at 200-700 nm to determine maximum absorption (11). Tests were performed for detecting the nature of both siderophores. For detecting hydroxymate group 1 ml of supernatant was added to 1.5 ml of FeCl3(2%) and the absorbance was checked for a peak between 400-600 nm. For detecting catecholate nature, 1 ml of supernatant was added to 1.5 ml $FeCl_3$ (2%), the absorbance was checked for a peak at 495 nm. For detecting carboxymate nature, 3 drops of 2N NaOH and 1 drop of *phenolphthalein* were mixed, then water was added until pink colour developed. To this, 1 ml of supernatant was added and checked for the disappearance of pink colour. Another test for the same detection of carboxymate nature was performed by adding 1 ml of supernatant with 1ml $CuSO_4$ and 2 ml acetate buffer, absorbance was checked along with the complex formation. For detecting salicylic acid, colour change was observed by adding 1ml of 2M $FeCl_3$ in 10 mM HCl, to 1 ml of supernatant (20). TLC using (60%) Silica Gel-G was performed with solvent system chloroform: acetic acid: ethanol (95:5:2.5), the spots obtained were observed under U.V., eluted overnight in methanol & analyzed spectrophotometrically at 200-700 nm. The inhibitory activity of both siderophores was checked by inoculation with test cultures on Nutrient agar.

Results & Discussion

On Nutrient agar plates, culture Ap1 produced yellowish green coloured diffusible pigment, whereas culture Ap2 produced bluish-green coloured pigment. From the biochemical tests & pigmentation both the cultures were catalase positive, oxidase positive and were of gram negative nature. Hence, identified up to genus level as the members of *Pseudomonas* (15). The results for nature detection of siderophores showed the presence of hydroamate type for Ap1 siderophore, whereas catecholate type for Ap2 siderphore (Table 1).

Table 1
Test results obtained for Ap1 & Ap2 supernatants.

S. No.	*Test*	*Observation* *Ap1 supernatant*	*Ap2 supernatant*
1.	Hydroxymate test 1 ml supernatant + 1.5 ml $FeCl_3$ (2%) check absorbance at 400-600 nm	Max. absorption at 400 nm	Max. absorption at 486 nm
2.	Catecholate test 1ml supernatant + 1.5ml $FeCl_3$ (2%) check absorbance at 450-500 nm	Max. absorption at 400 nm	Max. absorption at 486 nm

3.a	Carboxymate test 3 drops of 2N NaOH+ 1 drop of phenolphthalein, add H_2O till pink colour develops + 1 ml supernatent	–	Pink colour disappeared
3.b	1 ml supernatant + 1 ml $CuSO_4$ + 2 ml acetate buffer. Absorbance at 190-250 nm.	Complex present	Complex present
4.	$FeCl_3$ test 1ml supernatant + 1ml $FeCl_3$ (2M) in 10mM HCl	Reddish-brown colour formed	–

In Fig 1a. two peaks were seen for Ap1 supernatant, one near 350 nm and the other at a lower wavelength, this is a peculiar characteristic for the pyoverdin type siderophores produced from *Pseudomonas* sp. (15), whereas culture Ap2 gave max absorbance at the lower wavelength. In Fig 1b two peaks for the Ap1 eluted fluorescent spot showed, similar wavelength range as that of Ap1 supernatant, similarly peak for Ap2 elutant corresponds to the same wavelength as that of its supernatant. Such similarity shows that the fluorescent compound lies between 250–350 nm. The inhibitory activity of both the siderophores against the bacterial cultures tested is as shown in Table 2.

Table 2
Inhibitory activity of siderophore Ap1 & Ap2 against various bacterial cultures.

Bacterial cultures	*Ap 1 supernatant*	*Ap 2 supernatant*
Bacillus thuringenesis	++	+
Escherichia coli L.E. 392	-	-
Escherichia coli H.B.101	-	-
Salmonella typhi	++++	++
Escherichia coli wild	-	-
Bacillus megaterium	++	++
Bacillus subtilis	++	-

- no effect, + only on spot, ++ moderate effect, ++++ maximum effect.

Almost all the test cultures were sensitive for Ap1, whereas only some were sensitive for the Ap2 culture. Among the sensitive cultures *S. typhi* showed most sensitivity, hence the Ap1 siderophore was considered in our further study. The results obtained so far from the supernatant tests, yellowish green pigmentation and peculiar peaks showed Ap1 siderophore to be of pyoverdin type (5). The process of iron acquisition is dependent upon proteins transferrin and

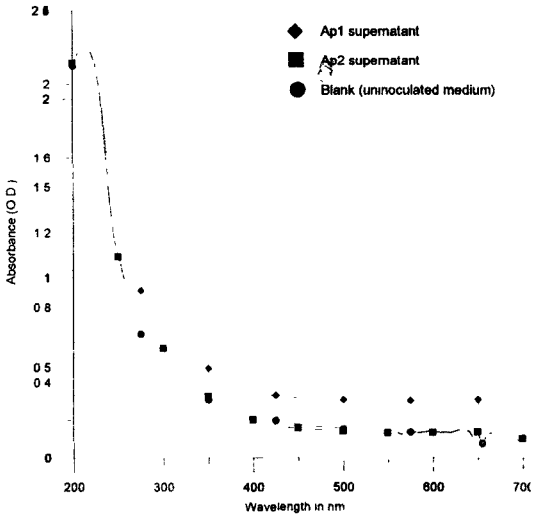

Fig 1: Spectral study of the crude siderophore samples from culture Ap1 & Ap2 cultures

(a) Absorbance of supernatants produced in succinate medium by Ap1 & Ap2 culture, culture against the uninoculated medium.

(b) Absorbance of methanol eluted fluorescent spot separated by TLC against the blank.

lactoferrin in human phagocytic cells and myeloid cell lines (2, 6, 10, 19). It has been reported that these cell lines acquire iron bound to the *P. aeruginosa*-derived siderophores pyoverdin

and pyochelin, iron acquisition from these siderophores is ATP independent, induced by multivalent cationic metals, and unaffected by inhibitors of endocytosis and pinocytosis. (1, 13, 14). Therefore it is hypothesized that cell lines would grow efficiently in the culture medium supplemented with Ap1 siderophore. The inhibitory activity of Ap1 siderophore on *S. typhi* as well as other contaminants, can be used to devise treatment in clinical applications. Plants are known to either produce compounds analogous to siderophores, named phytosiderophores, or acquire iron from microbial siderophores (17) hence the influence of Ap1 siderophore in the induction of plant growth either by suppressing the soilborne plant pathogens, by competing for iron or by some other mechanism, would provide a potential non-chemical mean for plant disease control (9). Our further study in the implementation of Ap1 siderophore in culture medium and its influence on the plant growth is in progress.

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Author

Satwekar, A.A., Birhare, S.C., Mehra, V.P., Puthan, P.N.*
Dept. of Biotechnology, Govt. Institute of Science,
Aurangabad 431 004.

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Evaluation of IPM Module against Major Pests of Cotton (*Gossypium sp.)*

Abstract

The field experiment was conducted at village Sonkhed Dist. Nanded (M.S.) during 2001-2002 to 2002-2003 to evaluate the effectiveness of IPM module for the pest of cotton cv. NHH-44. Among the module studied, IPM module could effectively manage the population of sucking pest complex and bollworms viz. Aphis gossypii,Amrasca bigutulla bigutulla, Bemicia tabaci, Thrips tabaci and Helicoverpa armigera, Earias vitella and Pectinophora gossypiella on cotton. The effectiveness of IPM module was also reflected on cotton yield, which was significantly higher in IPM module (M_1) as compared to Non-IPM module (M_2)

Introduction

Cotton (*Gossypium* spp.) the white gold of India grown as a most important commercial crop, cultivated in 9 million hectors in varied agroclimatic conditions across 9 major state Kairon *et al.* 2000. About 162 pest species are recorded feeding at various stages of cotton crop grown in India. *H. armigera* Hub. And its resistance is the limiting factor for harvesting bumper production of cotton. The loses due to pest incidence was estimated to be 50 to 60% in different parts of India, *Puri et al.*, 1998. The use of an indiscriminate pesticide, monocropping leads to heavy infestation of pest. Hence multifaceted approach (IPM) is required to cut down the load of chemical in environment. With the view, an integrated pest management module was tested in the farmer's field under Maharashtrian conditions on cotton cv. NHH-44 during 2001-2003.

Materials & methods

The field experiment was conducted during *Kharif* season of 2001 to 2003 at village level in Sonkhed dist. Nanded. The crop was raised as per the recommend IPM module develop for

cotton on an area of 5 acres and it was compared with non-IPM module as a check on an area of 5 acres which was 100 m. away from IPM plot.

Following are the 2 modules tested.

(1) M-1 IPM module:

(a) Clean-up campaign and land preparation in the month of April-May
(b) Soil application of fertilizers (15:15:15) 65 Kg before sowing per acre.
(c) Seed treatment thiomethoxam 70 WS @ 4gm/kg of seed.
(d) Layout and sowing: sowing was done as per the recommended spacing of 90 x 60 cms.
(e) Border row of maize + cowpea as trap crop and one row of *sateria* in between 10^{th} and 11^{th} row of cotton.
(f) Spraying of systemic insecticides for controlling sucking pest complex as per ETL.
(g) Release of *Trichogramma chilonis* at 45 and 55 DAS.
(h) Application of N fertilizers for top dressing in (if needed)
(i) Spraying of NSKE 5% at 50 and 60 DAS.
(j) Spraying HaNPV 250 LE /hector at 70 DAS.
(k) Spraying of Endosulphon 35 EC at 80 DAS. (If needed)

(2) M-2 Non-IPM module:

(a) No clean up campaign.
(b) Application of fertilizers (18:18:10) 100 Kg + urea 50 kg after sowing per acre.
(c) No seed treatment
(d) Layout and sowing: Sowing was done at the wider spacing of 120 × 90 cm.
(e) No border row of maize + trap crop of cowpea and red gram was grown as an intercrop along with cotton.
(f) Spraying of systemic insecticides and other insecticides in combinations for controlling sucking pest complex.
(g) Application of N fertilizers for top dressing.
(h) No bio-agents or bio-pesticides were used
(i) Rely heavily upon pesticides only for pest control.

Results and Discussion

The two years study on effectiveness of IPM module for cotton cv. NHH-44 (Tables 1 & 2) revealed that the population of sucking pests like aphids, jasids, thrips, whiteflies and bollworms like American bollworm, spotted bollworm and pink bollworm was significantly lower in IPM module as per as percent infestation of bollworms in squares and flowers and shed material is concerned cotton protected with *Trichogramma chilonis,* 5% NSKE and HaNPV has significantly lower infestation as compared to Non-IPM practices (Table-3).

Table 1
Population of sucking pest's complex in IPM and Non-IPM plots

Met. Week	*Population per plant*							
	Aphids		*Jassids*		*Thrips*		*White flies*	
	IPM	*Non-IPM*	*IPM*	*Non-IPM*	*IPM*	*Non-IPM*	*IPM*	*Non-IPM*
29	0	50.65	0.01	13.3	0.007	55.15	0	1.55
30	0.07	18.82	0.06	0.3	0.58	3.35	0.07	0.25
31	2.99	163.8	0.27	2.05	2.01	8.33	0.07	0.33
32	16.07	127.8	0.17	0.46	5.25	21.18	0.15	0.33
33	50.27	199.8	0.19	1.95	5.86	76.15	0.18	2.1
34	22.06	17.2	0.17	0.67	3.84	16.75	0.18	0.46
35	6.46	15.68	0.25	0.58	13.4	19.93	0.26	0.46
36	2.48	14.25	0.34	0.85	17.54	19.92	0.33	0.77
37	4.35	2.01	0.08	2.89	8.18	4.76	0.33	0.54
38	1.81	1.42	0.58	2	2.02	2.47	0.44	1.18
39	0.86	1.25	0.4	1.28	0.52	0.77	0.44	0.6
40	0	0	0	0	0	0	0	0
Mean	9.76	55.7	0.22	2.39	5.38	20.79	0.22	0.77

The populations of natural enemies like ladybird beetle and *Chrysopa spp.* was also recorded higher in IPM plots than Non-IPM Practices (Table-4).

The effectiveness of IPM module in managing the population of sucking pest's complex and bollworms was reflected on cotton yield. The IPM module (M1) had significantly higher cotton yield (9.47q/ha) which was recorded as 3.81q /ha. in Non-IPM practices (Table-6)

Considering the effectiveness of IPM module total rank was worked out. Looking to the values recorded on the various aspects, the IPM module (M1) stood first in rank and higher in cotton yield in comparison to Non-IPM module.

Suri et al. studied on state of art of IPM research and adoption in India. The adverse effect of continuous use of pesticides promoted the adoption of IPM methods, including surveillance, biological control and use of resistant varieties.

Table 2
Bollworm Population of IPM and Non-IPM plots

Met. Week	*Eggs IPM*	*Non-IPM*	*ABW Larvae IPM*	*Non-IPM*	*SBW Larvae IPM*	*Non-IPM*	*PBW Larvae IPM*	*Non-IPM*
30	0	0	0	0	0.01	0.05	0	0
31	0	0	0	0	0.05	0.09	0	0
32	0	0.25	0	0	0.04	0.01	0	0
33	0.41	1.1	0	0	0.07	1.5	0	0
34	0.29	0.23	0	0	0.1	0.01	0	0
35	0.18	0.26	0	0.014	0.06	0.01	0	0.01
36	0.17	1.05	0.01	0.6	0.04	0.08	0.01	0.6
37	0.23	1.05	0.007	0.01	0.06	0.1	0.01	0.01
38	0.43	1.26	0.007	0.01	0.05	0.07	0.01	0.01
39	0.2	7.77	0.01	0.06	0.07	0.06	0.01	0.06
40	0.18	0.52	0	0.05	0.03	0.02	0	0.05
41	0.11	0.49	0.08	0.08	0.03	0.03	0.08	0.08
42	0.05	0.13	0.06	0.1	0.03	0.05	0.06	0.1
43	0.02	0.04	0.07	0.18	0.03	0.01	0.07	0.18
44	0	0.07	0.09	0.21	0	0.02	0.09	0.21
45	0	0	0.01	0	0	0	0.01	0
46	0	0.01	0	0.23	0	0	0	0.23
47	0	0	0.15	0.25	0.02	0.05	0.15	0.25
48	0	0	0.13	0.25	0.01	0.05	0.13	0.25
49	0	0	0.12	0.2	0.01	0.07	0.12	0.2
50	0	0	0.18	0.15	0.03	0.06	0.18	0.15
51	0	0	0.22	0.23	0.06	0.08	0.22	0.23
52	0	0	0.22	0.26	0.05	0.08	0.22	0.26
1	0	0	0.25	0.31	0.03	0.08	0.25	0.31
2	0	0	0.22	0.23	0.06	0.1	0.22	0.23
3	0	0	0.19	0.16	0.05	0.05	0.19	0.16
4	0	0	0.19	0.21	0.02	0.03	0.19	0.21
5	0	0	0.18	0.21	0.04	0.01	0.18	0.21
6	0	0	0.19	0.2	0.05	0.05	0.19	0.2
7	0	0	0.16	0.18.	0.01	0.05	0.16	0.18
8	0	0	0.09	0.13	0	0	0.09	0.13
9	0	0	0.07	0.11	0	0	0.07	0.11
Mean	0.15		0.12	0.33	0.03	0.08	0.01	0.16

Table 3
Per cent Infestation of Bollworm Complex in IPM and Non-IPM plots

Met.	*Squares & flowers*		*Green bolls*		*Shed material*	
Week	*IPM*	*Non-IPM*	*IPM*	*Non-IPM*	*IPM*	*Non-IPM*
32	0	0	0	0	0	0
33	3.97	10.17	0	0	32	95.4
34	5.6	20.72	0	0	45	89.82
35	9.21	19.8	0.14	0	71.68	90.29
36	4.83	17.39	0.01	1.01	76.29	77.65
37	3.28	14.92	0.04	3.48	56.98	60.68
38	2.66	10.86	2.05	7.24	61.14	71.84
39	2.32	39.16	1.88	51.6	60.97	62.87
40	5	10.19	3.89	13.8	22.96	16.75
41	1.26	4.66	3.12	11.3	11.17	15.97
42	0.61	4.28	2.53	9.6	7.52	14.08
43	0.69	2.55	2.59	6.95	7.13	9.48
44	1.1	24.79	3.92	7.92	2.48	17.2
45	1.19	1.47	2.21	8.59	3.83	9.21
46	2	2.71	2.33	7.52	2.21	8.02
47	1.12	5.25	2.95	7.67	2.96	3.25
48	0.66	6.9	3.33	7.65	2.03	7.24
49	1.24	7.44	4.47	10.4	4.49	0
50	2.69	5.91	7.28	12.2	3.16	0
51	2.52	7.03	6.35	14.2	0	0
52	1.99	6.84	7.94	16.6	0	0
1	0.49	4.23	9.36	15.5	0	0
2	3.55	0.91	10.25	18.9	0	0
3	1.16	0	9.69	16.1	0	0
4	0.55	0	13.26	21.6	0	0
5	0.66	0	15.21	24.2	0	0
6	0.15	0	15.91	24.6	0	0
7	0	0	4.92	9.79	0	0
8	0	0	3.16	7.49	0	0
9	0	0	0.67	4.14	0	0
Mean	2.34	8.78	5.44	13.8	26.33	36.09

Table 4

Population of Natural Enemies in IPM and Non-IPM plots

Met.	*Lady bird beetle / plant*				*Chrysopa / plant*			
Week	*Eggs*		*Adults*		*Eggs*		*Adults*	
	IPM	*Non-IPM*	*IPM*	*Non-IPM*	*IPM*	*Non-IPM*	*IPM*	*Non-IPM*
29	0.007	0.15	0.003	0	0.003	0.1	0	0
30	0.03	0.05	0.003	0.02	0	0.007	0	0
31	0.04	0	0.02	0.08	0.03	0.01	0	0
32	0.08	0.02	0.12	0.17	0.82	0.11	0.004	0
33	0.25	0.25	0.8	3.7	0.21	0.6	0.01	0.02
34	0.45	0.05	1.95	1	0.25	0.37	0.003	0
35	0.22	0.03	7.78	1.12	1.23	0.27	0.08	0
36	0.09	0	4.7	1	0.8	0.28	0	0
37	0.028	0	4.68	0.57	0.63	0.23	0.007	0.004
38	0.01	0	8.89	0.98	0.75	0.34	0.05	0
39	0	0.06	3.55	0.62	0.48	0.1	0	0.03
40	0	0	3.65	0.97	0.17	0.2	0.01	0
41	0	0	3.88	1.22	0.22	0.14	0	0
42	0	0	3.17	0.91	0.03	0.02	0	0
43	0	0	2.92	0.92	0.06	0	0	0
44	0	0	2.52	0.88	0	0	0	0
45	0	0	2.65	0.6	0.2	0	0	0
46	0	0	2.33	0.63	0.01	0	0	0
47	0	0	2.04	0.64	0	0	0	0
48	0	0	1.63	0.57	0	0	0	0
49	0	0	1.33	0.33	0	0	0	0
50	0	0	1.16.2006 1.64	0.45	0	0	0	0
51	0	0	1.47	0.33	0	0	0	0
52	0	0	1.51	0.31	0	0	0	0
1	0	0	1.6	0.41	0	0	0	0
2	0	0	1.1	0.41	0	0	0	0
3	0	0	0.97	0.25	0	0	0	0
4	0	0	0.83	0.23	0	0	0	0
5	0	0	0.7	0.08	0	0	0	0
6	0	0	0.12	0.2	0	0	0	0
7	0	0	0.02	0.2	0	0	0	0
8	0	0		0.17	0	0	0	0
9	0	0		0.02	0	0	0	0
Mean	0.1	0.05	2.17	0.6	0.31	0.14	0.018	0.006

The population of pink bollworm larvae and its per cent locule damage was also recorded higher in non-IPM plots than IPM Practices (Table-5).

Table 5
Population and Infestation of Pink Bollworm in IPM and Non-IPM plots

Met.	*Locule damage (%)*		*PBW Larvae*	
Week	*IPM*	*Non-IPM*	*IPM*	*Non-IPM*
40	2.98	3.79	9	10
41	4.51	5.75	15	13
42	4.48	6.71	10	18
43	5	9.31	12	21
44	5.56	10.54	14	24
45	6.15	11.6	16	26
46	7.9	12.63	20	38
47	9.35	15.2	26	43
48	10.38	16.95	31	47
49	10.93	17.51	31	51
50	11.51	17.72	33	54
51	11.96	20.67	33	61
52	13.68	29.89	38	89
1	20.54	41.31	58	123
2	26.12	45.17	75	145
3	37.33	56.25	94	75
4	47.22	67.87	70	82
5	53.57	75	78	100
6	58.82	74.68	80	98
7	65.47	75.6	92	109
8	61.04	80.26	85	112
9	69.09	0	93	—
Mean	24.75	33.07	46.04	63.76

Table 6
Details of Cost of Cultivation in IPM and Non-IPM Plots

Sr. No.	*Working Details*	*Frequency of Operation*		*IPM*	*Non-IPM*
		IPM	*Non-IPM*	*Cost (Rs.)*	*Cost (Rs.)*
1	Land preparation				
	Ploughing	2	2	200	200
	Harrowing I	2	3	200	300
	Harrowing II	1	1	200	200
	Clean-up campaign	1	1	600	—
	Manures and fertilizers	1	1	1450	2300
2	Seed and sowing	1	1	300	600
3	Intercultivation	2	3	200	300
4	Weeding charges	2	3	300	500
5	Cost of cultivation	2	8	1250	2500
6	Picking charges	4	4	947	381
7	Seed cotton yield			9.47	3.81
8	Cost of cultivation			5647	7281
9	Gross income			23675	9525
10	Net profit / ha (Rs.)			18028	2244

Conclusion

The results concluded that the IPM practice was found to be promising over the traditional mode of Non-IPM practice with an eco-friendly approach. In addition to that the IPM practice resulted into curtailment of pesticide application, natural flora and fauna (predators and parasites) were conserved soil, water and air pollution due to insecticides had been avoided, leads to eco-safe environment and have a sustainable cotton production.

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Authors

Pande, A.K.; Kshirsagar, A.B; Harke S.N;
Bharose, A.A. and Deshpande, V.D.
MGM, Department of Bioinformatics &
Biotechnology, Aurangabad (M.S.)

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Bradyrhizobium japonicum for Soybean Growth

Introduction

Leguminous plants are able to take up significant amount of nitrogen through nitrogen fixation by forming nodules in their roots in symbiosis with rhizobia. The root nodule is the organ of nitrogen assimilation. Nitrogen (N_2) is reduced to NH_4^+ in bacteriods by the enzyme nitrogenase (Balestrasse *et al.*, 2003). Establishment of symbiosis inside the host root and development of nodules are complex physiological process which follows many events such as recognition and infection of host root and development of nodules. The host-symbiont specificity is governed by specific plant protein called lectin involves in recognition of symbiont (Bauer, 1981).

The formation of effective nodules in soybean when inoculated with compatible *Rhizobium* leads to fixation of atmospheric nitrogen into nitrate and nitrite. Thus, symbiotic nitrogen fixation is complex physiological process influenced by the interaction between both the symbionts. Whereas, nitrogen fixation by soybean range from 200 kg N/ha (Smith and Hume, 1987).

Materials and Methods

Seeds of soybean cv. 'Sathiya' were surface sterilized with 70% ethanol for 1 minute followed by 1% sodium hypochlorite for 4 minutes and finally washed with distilled water for 6 to 7 times. Peat culture of *Bradyrhizobium japonicum* was taken from Nepal Agriculture Research Council (NARC), Khumaltar, Lalitpur. Earthen pots (22 × 24 cm^2) were filled with unsterilized soil and sand in the ratio of 1:1 (the pH of soil was 6.7). The surface sterilized seeds were sown in the depth 2.5 to 4 cm (Upfold and Olechowski, 1994) in earthen pots. The pots were arranged in two sets: set one was inoculated with *Bradyrhizobium japonicum* and set two was uninoculated (control). Then daily tap water was provided in each pots whereas, the pots were kept in green house for 25 days at 24°C to 28°C with photoperiod 16 hours. Seedlings of soybean were

harvested on 29th, 38th and 47th day after seed sowing (DASS) at the interval of 9 days. Four replicates from each inoculated and uninoculated plants were uprooted then root length and shoot length per plant was recorded. Then dry weight of nodules, dry weight of shoot and dry weight of root per plant was recorded after drying the plant samples in hot air oven for 24 hours at 80°C ± 2°C. Total chlorophyll content in fresh leaves of soybean was estimated by the method of (Arnon, 1949) using spectrophotometer. Statistical analysis was done using standard deviation and Analysis of Variance (ANOVA) in SPSS computer program at 5% level of significance.

Result and Discussion

Result shows the inoculation of *B. japonicum* on soybean increased the total chlorophyll content in fresh leaves, nodules dry weight, shoot length, shoot dry weight and root dry weight per plant (Table 1).

Total chlorophyll content in fresh leaves was increased in inoculated plants on all days. Thus, it was found that inoculation of *Bradyrhizobium japonicum* increased the total chlorophyll content in fresh leaves of soybean as compared to uninoculated plants. The shoot length was taken in all respective DASS and found inoculated plants had higher shoot length than uninoculated plants. However, root length was found higher in uninoculated plants on all DASS. Regarding dry weight of shoot and root, it was found higher in inoculated plants. The nodules dry weight was recorded on all DASS and found that was higher in inoculated plants as compared to uninoculated plants (Table 1).

The output of ANOVA has been given in (Tables 2, 3 & 4) at 5% level of significance.

Discussion

It has been found that inoculated seed of soybean with *Bradyrhizobium japonicum* significantly increased the total chlorophyll content in fresh leaves of soybean on 29th and 47th DASS i.e. ($p<0.05$) see (Tables 2 & 4). The increased in total chlorophyll in inoculated plant might be due to higher nitrogen fixation in inoculated plant. After nitrogen fixation, the nitrogenous compounds are exported from nodules to leaves in the form of ureides (compound containing allantoin and allantonic acid) which are used for synthesis of chlorophyll. Thus, inoculated plant had higher chlorophyll content as compared to uninoculated plant (table 1) as observed by (Hoque *et al.*, 1999).

The shoot length and shoot dry weight was taken in all respective DASS i.e. 29th, 38th and 47th which was found higher in inoculated plant (Table 1). It has been found inoculated plant significantly increased the shoot length and its dry weight as compared to uninoculated plant i.e. ($p<0.05$) on 47th DASS (Table 4). The increased in shoot length in inoculated plant might be due to sufficient amount of nitrogen for normal physiology and plant growth. The dry weight of shoot was increased in inoculated plant might be due to higher photosynthesis in inoculated plant. Thus, determination of dry weight of shoot has a positive relationship to nitrogen fixation ability (Neuhausen *et al.*, 1988).

However the root length was found higher in uninoculated plant as compared to inoculated plant on 29th, 38th and 47th **DASS (Table 1). It might be due to higher number of nodules in**

Table 1
Effect of *Bradyrhizobium japonicum* on chlorophyll content, shoot length, root length, shoot dry weight, root dry weight and nodules dry weight on soybean.

Days after seed sowing	*Treat*	*Total CHL (mg/g Fresh wt. of leaves*	*SL/pl (cm)*	*RL/pl (cm)*	*RDW/pl (g)*	*SDW/pl (g)*	*NDW/pl (g)*
29	C	3.01 ± 0.10	42.5 ± 3.10	20 ± 6.05	0.16 ± 0.03	1.50 ± 0.31	0.019 ± 0.00
	I	4.06 ± 0.46	45 ± 5.94	18.7 ± 53.30	0.19 ± 0.06	1.52 ± 0.23	0.025 ± 0.00
38	C	2.74 ± 0.43	44.7 ± 5.095	37.25 ± 4.11	0.60 ± 0.05	2.14 ± 0.18	0.098 ± 0.02
	I	3.25 ± 0.39	53.25 ± 2.44	34.25 ± 7.27	0.71 ± 0.17	2.30 ± 0.36	0.18 ± 0.09
47	C	4.35 ± 0.08	48 ± 2.44	46.75 ± 7.41	0.73 ± 0.06	2.10 ± 0.50	0.16 ± 0.08
	I	5.06 ± 0.04	56.5 ± 3.69	44 ± 4.89	0.89 ± 0.07	2.97 ± 0.20	0.26 ± 0.03

± STDEV

Treat = Treatment, C = uninoculated, I = inoculated, CHL = Chlorophyll, SL/Pl = Shoot length per plant, RL/pl = Root length per plant, RDW/pl = Root dry weight per plant, SDW/pl = Shoot dry weight per plant. NDW = Nodule dry weight per plant.

Table 2
Analysis of Variance (ANOVA) of different parameters on 29th DASS.

Parameters	*D.F.*	*F cal.*	*F tab.*	*Sig.**
CHL	1,6	12.66	5.99	0.012
SL	1,6	0.65	5.99	0.450
RL	1,6	0.13	5.99	0.729
RDW	1,6	0.51	5.99	0.501
SDW	1,6	0.00	5.99	0.926
NDW	1,6	4.32	5.99	0.083

*The mean difference is significant at the 0.05 level.
D.F. = Degree of Freedom, Fcal. = F-ratio (Calculated), F tab. = F-ratio (tabulated)

Table 3
Analysis of Variance (ANOVA) of different parameters on 38th DASS.

Parameters	*D.F.*	*F cal.*	*F tab.*	*Sig.**
CHL	1,6	4.54	5.99	0.077
SL	1,6	2.39	5.99	0.172
RL	1,6	0.51	5.99	0.500
RDW	1,6	1.46	5.99	0.272
SDW	1,6	4.13	5.99	0.088
NDW	1,6	0.70	5.99	0.435

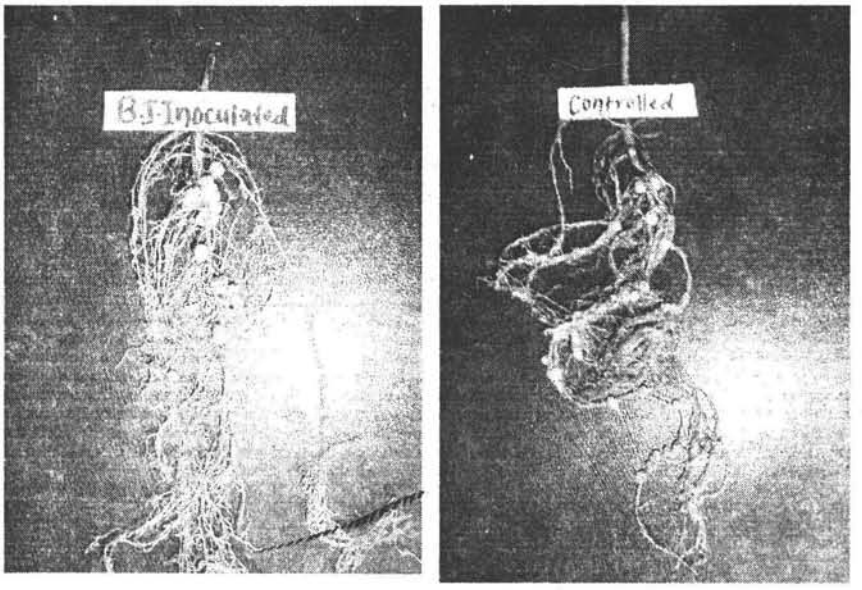
*The mean difference is significant at the 0.05 level.
D.F. = Degree of Freedom, Fcal. = F-ratio (Calculated),
F tab. = F-ratio (tabulated)

Table 4
Analysis of Variance (ANOVA) of different parameters on 47th DASS.

Parameters	*D.F.*	*F cal.*	*F tab.*	*Sig.**
CHL	1,6	207.16	5.99	0.000
SL	1,6	14.69	5.99	0.009
RL	1,6	0.38	5.99	0.559
RDW	1,6	10.30	5.99	0.018
SDW	1,6	10.40	5.99	0.018
NDW	1,6	15.61	5.99	0.008

*The mean difference is significant at the 0.05 level.
D.F. = Degree of Freedom, Fcal. = F-ratio (Calculated),
F tab. = F-ratio (tabulated)

Soybean plants are sowing in earthen pots on 50 days after seed sowing.



Root and root nodules of soybean on 50 days after seed sowing.

inoculated plants. Since, higher nodules in inoculated plant consume much more energy for nodulation which inhibit the development of root. Similar result was found by (Poudyal and Prasad, 2005) in sterilized soil. However, dry weight of root was increased in inoculated plant and statistically significant on 47th DASS ($p<0.05$). The increased in root dry weight in inoculated plant might be due to sustainable transportation of photosynthesis product like soluble sucrose to root.

Nodules dry weight was found higher in inoculated plant on 29th, 38th and 47th DASS and statistically that was found highly significant on 47th DASS (Table 4). The increased in nodule dry weight in inoculated plant might be due to higher number of effective nodules which may contain higher concentration of leghaemoglobin. The development of nodulation process depends on production of special hormone (IAA) by bacterium and seed coated with *Bradyrhizobium japonicum* strains significantly enhanced nodulation and seedling biomass as compared to control in peanut (Deshwal *et al.*, 2003). Inoculated plant had higher concentration of total chlorophyll. Chlorophyll is the site of photosynthesis thus, supply of photosynthesis product must be provided in the nodules for nitrogen fixation (Bergensen, 1970). Thus, bradyrhizobial strains play the important role in growth, dry matter yield and nodulation of soybean grown in nitrogen deficient soil (Egamberdiyeva *et al.*, 2004).

However, all parameters were not found statistically significant in all respective DASS due to the experiment was conducted in unsterilized field's soil. Thus, there was the chance of survival of *Bradyrhizobium japonicum* in uninoculated plant also.

Conclusion

The present research work concluded that inoculation of soybean seed with *Bradyrhizobium japonicum* strains enhanced to increased shoot length, shoot dry weight, root dry weight, nodule dry weight and total chlorophyll content in fresh leaves as compared to uninoculated (control) plant.

Acknowledgement

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Author

Roshan Sharma Poudyal and B.N. Prasad

Biotechnology Unit, Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

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Phenotypic and Functional Characterization of *A. caulinodans* Endophytic Symbiont of *S. rostrata*

Introduction

Sesbania rostrata is a tropical legume that exhibits stem nodulation apart from the general root nodulating feature of members of family rhizobiaceae (Biovin *et al.*, 1994). Both stem and root nodulation is due to crack entry mechanism followed by symbiotic association by a symbiotic diazotroph *Azorhizobium caulinodans* (Dreyfus *et al.*, 1988). Dreyfus *et al.* (1984) first reported this stem nodulating *rhizobia* of *Sesbania rostrata* as *Azorhizobium caulinodans* which is a type species. This rhizobia fixes nitrogen under free-living conditions and also tolerates 12μM of dissolved oxygen (Gerbhardt *et al.*, 1984) and invades the plants by a specialized process called crack entry that involves entry at region of new emerging lateral roots (Kannaiyan, 1998). The high oxygen tolerance and presence of green, photosynthetic stem nodules inhabited by azorhizobia seemed to be helpful in developing symbiosis-like system in non-legumes. Extending legume-*Rhizobium* symbiosis to non-legumes will significantly increase the amount of available nitrogen and thereby biomass of several cereal and other non-legume crops (Kalia and Gupta, 2002).

The present study was undertaken to isolate and characterize azorhizobial isolates morphologically, biochemically and functionally and further elucidation of the intrinsic antibiotic resistance spectra of the isolates.

Materials and Methods

Isolation of bacteria

Single, healthy and well-developed nodules from root and stem of *Sesbania rostrata* were detached carefully and washed with tap water. Nodules were surface sterilized by 0.1% mercuric chloride for 3 mins and washed repeatedly in excess amounts by using autoclaved sterile distilled

water. The nodules were further treated with 70% ethyl alcohol for 30 secs followed by subsequent immediate sterile distilled water washings (2x7 mins). The nodules were then crushed in sterile distilled water and 0.1 ml of 10^{-5} dilution was spread on or streaked on petri plates containing Yeast Extract Mannitol Agar (YEMA) medium (Allen, 1953) and incubated at 28^0C for 48 hours. The distinct, creamy, slimy, viscid and translucent colonies were selected that did not absorb congo red dye and then picked up and streaked repeatedly for purification on YEMA plates and finally subcultured and maintained on YEMA slants.

Reference culture

The reference cultures (*A. caulinodans* ZB-SK-5 and S_1) used in the present study were obtained from Dr. S. Kannaiyan, Vice Chancellor, Tamil Nadu Agricultural University, Coimbatore (India) and Department of Microbiology, PAU, Ludhiana, Punjab, respectively.

Characterization of isolates:

Biochemical characterization: The isolates were recorded fast and slow growers on basis of formation of colonies or appearance of growth on YEMA plates after 48 hours. The fast growing isolates formed colonies in 48 h while slow growers appeared after 3 days. The isolates were also observed for their acid/alkali production by growth on BTB supplemented YEMA (Norris, 1963). Ketolactose test (performed on lactose YE agar) was also performed to ascertain the bacterial isolates to be of rhizobiaceae family and not to be *Agrobacterium* genera (Bernaertz and Deley, 1963).

Antibiotic resistance spectra

The intrinsic antibiotic resistance spectra of isolates using commercially available antibiotic discs of seven different antibiotics viz. ampicillin, penicillin G, amikacin, chloramphemicol, gentamicin, streptomycin and oxytetracyclin were studied on lawn culture of 48h growth of all azorhizobial isolates.

Nitrogenase activity

A. caulinodans isolates were grown in YEM-broth for acetylene reduction assay. After 48 h incubation, cotton plugs of vials were replaced by subaseals and 10% of air was replaced with acetylene gas using a disposable sterile syringe. Vials were incubated for 24 h and the acetylene reduced or ethylene produced was determined by a gas chromatograph (Nucon gas chromatograph) with poropak–R column and a hydrogen flame–ionization detector (FID) (Hardy *et al.,* 1968). The protein content was estimated by Lowry method (Lowry *et al.,* 1951).

Results and Discussions

Among twelve root and stem isolates (six each) AK-SRS-4 and AK-SRS-5 and AK-SRR-6 were slow growers respectively. Both the reference cultures S_1 (stem isolate) and ZB-SK-5 were fast growers and alkali producers. All root isolates were found to give alkaline reaction except AK-SRR-1, while all stem isolate exhibited acidic reaction on BTB supplemented YEMA. The ketolactose test suggested that all the root and stem isolates did not use lactose as carbon

source and so did not produce yellow coloration in the lactose agar medium after flooding with Benedict's reagent confirming isolates to be *A. caulinodans* (Table 1).

Table 1
Morphological and Biochemical Characteristics of *Azorhizobium* isolates

Azorhizobium isolates	*Gram's staining*	*Growth Pattern on YEMA*	*Growth Pattern on CRYEMA*	*Growth Pattern on BTB supplemented YEMA*	*Growth pattern on lactose agar medium*
SRS-1	(-ve)	Fast	SLR	Acidic	NC
SRS-2	(-ve)	Fast	SLR	Acidic	NC
SRS-3	(-ve)	Fast	SLR	Acidic	NC
SRS-4	(-ve)	Slow	SDR*	Acidic	NC
SRS-5	(-ve)	Slow	SDR*	Acidic	NC
SRS-6	(-ve)	Fast	SW*	Acidic	NC
SRR-1	(-ve)	Fast	SW	Alkaline	NC
SRR-2	(-ve)	Fast	SW	Alkaline	NC
SRR-3	(-ve)	Fast	SLR	Alkaline	NC
SRR-4	(-ve)	Fast	SDR*	Alkaline	NC
SRR-5	(-ve)	Fast	SDR	Alkaline	NC
SRR-6	(-ve)	Slow	SDR	Alkaline	NC
S1	(-ve)	Fast	SDR*	Alkaline	NC
ZB-SK-5	(-ve)	Fast	SDR*	Alkaline	NC

All the cultures showed resistance to ampicillin (25mcg) and penicillin G (10mcg) (as were gram negative rods). All the isolates showed maximum susceptibility towards streptomycin (25mcg) except the root isolates AK-SRR-3, AK-SRR-4 and reference culture ZB-SK-5 followed by oxytetracyclin (30mcg) and streptomycin (30mcg) respectively.

The range of *in vitro* nitrogenase activity of isolates was recorded to be from 883.88-2718.6 nM C_2H_4 mg protein^{-1} h^{-1}. Among root isolate AK-SRR-1 gave maximum acetylene reduction activity (2253.36 nM) followed by SRR-2 (1893.0 nM). While among stem isolates maximum activity was recorded by SRS-3 (2718.6 nM) followed by SRS-2 (2229.60 nM) (Table 3).

Acknowledgement

The authors are grateful to Department of Science and Technology for providing necessary financial assistance for carrying out the present research work.

Table 2
Antibiotic resistance spectra of *Azorhizobium* isolates

Azorhiz-obium isolates	*Antibiotic discs (mcg)*						
	Ampicillin	*Penicillin G*	*Amikacin*	*Chloramp-henicol*	*Gentamicin*	*Oxytetracyclin*	*Streptomycin*
	(25)	(10)	(30)	(30)	(30)	(30)	(25)
AK-SRS-1	+/-	+/-	-(1.7)*	-(2.6)	-(1.5)	-(2.1)	-(1.6)
AK-SRS-2	+	+	-(1.6)	-(2.8)	-(1.5)	-(2.2)	-(1.6)
AK_SRS-3	+/-	+/-	-(1.5)	-(2.7)	-(1.4)	-(1.9)	-(1.6)
AK-SRS-4	+	+	-(1.6)	-(2.6)	-(1.5)	-(2.6)	-(1.5)
AK-SRS-5	+	+	-(1.55)	-(2.7)	-(1.5)	-(2.1)	-(1.4)
AK-SRS-6	+	+	-(1.8)	-(2.6)	-(1.7)	-(1.6)	-(2.0)
AK-SRR-1	+	+	-(1.5)	-(2.7)	-(1.6)	-(2.0)	-(1.4)
Ak-SRR-2	+	+	-(1.0)	-(2.0)	-(1.4)	-(1.1)	-(1.5)
AK-SRR-3	+	+	-(1.1)	+	-(1.0)	-(1.1)	-(1.1)
AK-SRR-4	+/-	+	-(1.2)	+	-(1.0)	-(1.1)	-(1.3)
AK-SRR-5	+	+	-(1.5)	-(1.2)	-(1.4)	-(2.0)	-(1.2)
AK-SRR-6	+	+	-(1.5)	-(1.0)	-(1.4)	-(1.6)	-(1.4)
S_1	+	+	-(1.0)	-(2.4)	-(0.5)	-(1.5)	-(1.0)
ZB-SK-5	+	+	-(1.5)	+	-(1.2)	-(1.1)	-(1.1)

+resistant
-susceptible
+/-doubtful
*Figures in bracket denotes the diameter of the growth inhibited by the antibiotic

Table 3
Nitrogenase activity of various *Azorhizobium* isolates under free living conditions.

Azorhizobium isolates	*Nitrogenase activity ($nM\ C_2H_4\ h^{-1}\ mg\ protein^{-1}$)*
AK-SRS-1	1718.66
AK-SRS-2	2253.36
AK-SRS-3	1691.17
AK-SRS-4	1890.53
AK-SRS-5	1317.64
AK-SRS-6	1337.0
AK-SRR-1	2718.62
AK-SRR-2	1189.85
AK-SRR-3	1473.14
AK-SRR-4	644.50
AK-SRR-5	1031.20
AK-SRR-6	889.3
S1	2229.60
ZB-SK-5	883.88

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Author

Anu Kalia and R.P. Gupta
Department of Microbiology
Punjab Agricultural University
Ludhiana, Punjab-141 004

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Effect of Different Phytoextracts on Spore Germination of *Alternaria tomato* (Cooke) G.F. Weber Causing Fruit Rot of Tomato

Introduction

Fruits and vegetables are important for human nutrition. They are also indispensible for the maintenance of human health and tomato *(Lycopersicon esculentum* Mill.) is one of them. Tomato is a crop of immense value in olericulture. It is a solanaceous fruit vegetable believed to have its origin in Tropical America. Portuguese introduced it in India in the early 18th century. Tomato is a popular fruit vegetable available throughout the year in India.

Tomato is used as a fruit as well as vegetable in our diet and preferred by all people and consumed in different forms throughout the year. It can be consumed as a fresh ripe fruit and is one of the most popular salad vegetables. It is taken after cooking or raw or is made into soups, salad, pickles, ketchups, sauces and many other products. Hence, there is a great demand for tomato in the market as a fresh fruit. A great damage is caused to the tomato fruits in the field, during transit, storage and marketing by the fungal rots followed by the bacterial rots, which are responsible for decaying the tomato fruits. Sharma (1994) reported 0.5 to 19.7 per cent damage tomato fruits annually due to post-harvest fungal rots. In field and marketing conditions, 23 to 35 per cent tomato fruit rots have been reported due to *Rhizopus stolonifer, Alternaria solani, Penicillium notatum*. Tomato fruits are attacked by many fungi, but most important are *A. solani, Alternaria alternata,Alternaria tomato, Nicotianae* spp., *Phytophthora* spp., *Fusarium roseum, Fusarium solani, Penicillium italicum, Aspergillus niger, Fusarium chlamydosporium, Penicillium digitatum, R. stolonifer, Penicillium expansion and P. oxalicum.*

Material and Methods

The spore suspension was prepared from seven days old culture of *A. tomato* grown on PDA medium in sterile distilled water. The suspension was examined under microscope in the low

power magnification (100 X) and was adjusted to about 25 spores per microscopic field. Equal volume of spore suspension and phytoextract (1:1, 1:2 and 1:4) were mixed thoroughly in watch glasses. From, this, one drop of the suspension was placed on the slide and equal quantity of spore suspension and sterile distilled water served as control. All the slides were then placed in an inverted position in moist chambers. Spore germination was recorded under microscope in ten microscopic fields after 24, 36 and 48 hrs of incubation. Observations were tabulated and analysed statistically.

Per cent spore germination was calculated by using following formula (Singh *et al.*, 1986)

$$\text{Per cent spore germination} = \frac{\text{Germinated spores}}{\text{Total number of spores}} \times 100$$

Results and Discussion

Many phytoextracts are known to have inhibitory effect on the growth and sporulation of various fungi. This information is certainly useful in exploiting inhibitory principle for developing botanical fungicides for the plant disease management. In the present investigation, five unsterilized extracts of various plant species with suitable control were screened *in vitro* to know their inhibitory effect on the spore germination of *A. tomato.*

All the phytoextracts significantly reduced the spore germination over the control, while significantly less spore germination was observed in datura leaf extract at all the concentrations tested after 24, 36 and 48 hrs of incubation (Table 1). After 48 hrs of incubation, 16.90, 15.23 and 21.29 per cent spore germination was obtained in the 1:1, 1:2 and 1:4 concentrations of datura leaf extract, respectively. Neem leaf extract was found next best in which spore germination was 19.23, 27.72 and 37.86 per cent in 1:1, 1:2 and 1:4 concentrations, respectively followed by garlic leaf extract which showed spore germination of 24.50, 38.53 and 43.26 per cent in 1:1, 1:2 and 1:4 concentrations, respectively. The spore germination was 29.12, 41.50 and 50.98 per cent in 1:1, 1:2 and 1:4 concentrations of nilgiri leaf extract, respectively. Unsterilized leaf extract of lantana was found least inhibitory. While, 66.73 per cent spore germination was observed in the control (Table 1).

Similarly, Shekhawat and Prasad (1971) observed 100 and 64 per cent inhibition of spore germination of *A. tennis* over the control (22.6 %) from the five per cent (w/v) leaf extracts of *A. cepa* and *A. sativum,* respectively, probably due to presence of allicin in *A. sativum* and protocatochuic acid and catechol in *A. cepa.* Karade and Sawant (1999) tested extracts of 12 medicinal and wild plants against A. *alternata* and observed 10 spore germination in leaf extract of A. *sativum* and minimum spore germination was inhibited with the leaf extract of *L. camara.* Thus, the present investigations are in confirmation with the findings of above research workers.

Conclusion

Bioefficacy of five phytoextracts were tested *in vitro* to study their inhibitory effect against spore germination of *A. tomato.* The unsterilized leaf extract of datura proved strongly inhibitory (10.90 %) followed by neem leaf extract (19.23 %) at 1:1 concentration after 48 hrs of incubation.

Table 1. Effect of different phytoextracts on spore germination of *A. tomato* causing fruit rot of tomato

Treatments	*Concentrations*	*Per cent spore germination after*		
		24 hrs	*36 hrs*	*48 hrs*
Datura	1:1	7.92	9.90	10.90
	1:2	8.73	11.20	15.23
	1:4	16.10	18.54	21.29
Neem	1:1	12.74	15.22	19.23
	1:2	18.69	21.77	27.72
	1:4	30.03	34.10	37.86
Garlic	1:1	17.30	19.65	24.50
	1:2	27.10	32.40	38.53
	1:4	40.19	42.57	43.26
Nilgiri	1:1	21.49	25.08	29.12
	1:2	31.37	36.94	41.50
	1:4	37.96	41.74	50.98
Lantana	1:1	29.52	35.64	44.54
	1:2	38.73	42.99	51.40
	1:4	42.45	47.83	59.80
Control		33.37	50.95	66.73
S. Em.		0.20	0.15	0.30
C.D. at 5%		0.55	0.42	0.84
C.V. %		2.32	1.49	2.51

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Effect of different phytoextracts on development of tomato fruit rot caused by *Alternaria tomato* (Cooke) G.F. Weber.

P.B. Sandipan, Ajay Singh, N.A. Patel, S.R.S. Dange, R.L. Patel and Kavita Sharma.

Authors

P.B. Sandipan, Ajay Singh, N.A. Patel, S.R.S. Dange, R.L. Patel and Kavita Sharma

Department of Plant Pathology, C.P. College of Agriculture Sardarkrushinagar Dantiwada Agricultural University Sardarkrushinagar–385 506

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Effect of Different Phytoextracts on Development of Tomato Fruit Rot Caused by *Alternaria tomato* (Cooke) G. F. Weber

Introduction

Fruits and vegetables are important for human nutrition. They are also indispensible for the maintenance of human health and tomato *(Lycopersicon esculentum* Mill.) is one of them. Tomato is a crop of immense value in olericulture. It is a solanaceous fruit vegetable believed to have its origin in Tropical America. Portuguese introduced it in India in the early 18th century. Tomato is a popular fruit vegetable available throughout the year in India.

Tomato is used as a fruit as well as vegetable in our diet and preferred by all people and consumed in different forms throughout the year. It has got importance in the diet due to its nutritive value and low price. It can be consumed as a fresh ripe fruit and is one of the most popular salad vegetables. It is taken after cooking or raw or is made into soups, salad, pickles, ketchups, sauces and many other products. Hence, there is a great demand for tomato in the market as a fresh fruit. It is a good appetizer and removes the constipation and has a pleasing taste. Despite its wide spread cultivation in our country, the availability of good quality tomato fruits are inadequate in the market because a large number of fungal, bacterial and viral pathogens affect the crop in field condition.

Material and Methods

Phytoextracts of datura *(D. stramonium),* neem (*A. indica),* nilgiri *(E. citriodora),* garlic (*A. sativum)* and lantana *(L. camara)* were tested. The fresh leaves were brought to laboratory and thoroughly washed with tap water and then with sterile distilled water and air dried. One hundred gram leaves were crushed in grinder mixer by adding 100 ml distilled water to obtain 1:1 extract. The phytoextracts, thus obtained were then, filtered through double layered sterile muslin cloth and the phytoextracts healed up to 40 °C. In case of pre-treatment, semi-ripe fruits were first dipped in the solution of the above different phytoextracts separately for five minutes, air dried and 12 hrs later, treated fruits were inoculated with. *A. tomato* by cork wounding method. In post-treatment, tomato fruits were inoculated by cork wounding method with *A.*

tomato and 12 hrs later, the inoculated fruits were dipped into different phytoextracts separately for five minutes. The untreated inoculated fruits served as control. All these fruits were placed separately in sterilized, loosely tied polythene bags with a piece of sterilized wet absorbent cotton inside each bag and bagged fruits were kept at 28 ± 2°C for seven days. Each treatment was replicated for three times and three fruits were kept in each replication. Observations on fruit rot development were recorded after three, five and seven days of incubation.

Result and Discussion

Pre-treatment Effect of Different Phytoextracts on Development of Tomato Fruit Rot

Leaf extracts of five plant species were tested against fruit rot of tomato caused by *A. tomato*. To know the efficacy of leaf extract of these plant species, semi-ripe healthy tomato fruits of equal size were first treated with different phytoextracts and after 12 hrs of incubation, they were inoculated with *A. tomato*. All the phytoextracts significantly reduced the fruit rot development over control. Significantly less fruit rot development was observed in the treatments of datura leaf extract followed by neem and garlic leaf extracts after three, five and seven days of incubation. After seven days of incubation, the lesion diameter was 24.27, 31.90, 34.03, 39.07 and 43.33 mm, while diameter of sporulated area was 14.00, 15.78, 21.43, 32.54 and 33.53 mm in leaf extracts of datura, neem, garlic, nilgiri and lantana treatments, respectively. In untreated fruits (control), the lesion diameter and diameter of sporulated area were 46.33 and 34.10 mm, respectively after seven days of incubation (Table 1).

Table 1. Pre-treatment effect of different phytoextracts at 1:1 concentration on development of tomato fruit rot caused by *A. tomato*

Treatments	*Lesion diameter*			*Diameter of*		
	3rd day	*5th day*	*7th day*	*3rd day*	*5th day*	*7th day*
Datura	4.00	8.00	24.27	1.03	3.07	14.00
Neem	7.10	14.13	31.90	1.36	5.23	15.78
Garlic	9.03	16.00	34.03	4.05	8.10	21.43
Nilgiri	10.57	23.03	39.07	4.93	10.10	32.54
Lantana	13.70	25.17	43.33	5.13	12.40	33.53
Control	14.12	27.17	46.33	6.19	13.85	34.10
S. Em. ±	0.15	0.09	0.13	0.09	0.12	0.13
C.D. at 5%	0.42	0.26	0.38	0.26	0.35	0.39
C.V. %	4.37	1.41	1.08	1.41	4.04	1.61

Post-treatment Effect of Different Phytoextracts on Development of Tomato Fruit Rot

The phytoextracts tried as pre-treatment were also tested as post-treatment against fruit rot of tomato caused by *A. tomato*. The semi-ripe healthy tomato fruits of equal size were inoculated first with *A. tomato* and after 12 hrs of incubation, they were treated with the different phytoextracts.

All the phytoextracts significantly reduced the fruit rot of tomato over control. Unsterilized leaf extract of datura was most effective followed by leaf extract of neem and garlic in controlling the fruit rot of tomato due to *A. tomato* (Table 2). After seven days of incubation, the lesion diameter was 28.00, 36.00, 37.17, 45.07 and 46.93 mm, while diameter of sporotated area was 14.00, 18.90, 21.17, 28.03 and 35.10 mm in leaf extracts of datura, neem, garlic, nilgiri and lantana treatments, respectively. While, the lesion diameter and diameter of sporulated area

were 48.46 and 36.10 mm, respectively in control after seven days, of incubation. (Table 2). Leaf extracts of neem and garlic at 1:1 concentration were equally effective in reducing fruit rot of tomato after seven days of incubation.

Patel (1991) tested phytoextracts of 19 species against *A. alternata* and reported that maximum inhibition by the turmeric rhizome (53.07 %) followed by datura leaf extract (52.24 %). Thakur *et al.* (1991) revealed that *D. metel* showed maximum antifungal activity of fungal growth of *M. roridum* and *A. alternata.* Ali *et al.* (1992) found that neem oil was as effective as thiabendazole (Tecto-6) against *A. alternata, P. italicum* and *A. niger* causing fruit rot of tomato.

The present results are in agreement with the findings of above research workers.

Table 2. Post-treatment effect of different phytoextracts at 1:1 concentration on development of tomato fruit rot caused by *A. tomato*

Treatments	*Lesion diameter (mm)*			*Diameter of sporulated area (mm)*		
	3rd day	*5th day*	*7th day*	*3rd day*	*5th day*	*7th day*
Datura	4.10	10.33	28.00	1.43	4.33	14.00
Neem	10.07	18.23	36.00	1.97	7.30	18.90
Garlic	11.43	24.00	37.17	4.10	9.53	21.17
Nilgiri	12.10	25.00	45.07	4.93	11.97	28.03
Lantana	14.23	26.03	46.93	5.93	13.10	35.10
Control	15.28	27.13	48.46	9.37	14.22	36.10
S. Em. ±	0.09	0.19	0.51	0.07	0.11	0.22
C.D. at 5%	0.26	0.53	1.46	0.20	0.30	0.63
C.V. %	2.40	2.55	3.82	4.84	3.14	2.62

Conclusion

Phytoextracts of different species proved effective in reducing fruit rot development due to *A. tomato* in tomato fruits. In both, pre- and post-treatment, datura extract was found most effective followed by leaf extracts of neem, garlic and nilgiri while, unsterilized leaf extract of lantana was found least inhibitory. Pre-treatment was found more effective than the post-treatment.

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Bioefficacy of different antagonists against fruit rot of tomato caused by *Alternaria tomato* (Cooke) G.F. Weber under *in vitro* condition.

P.B. Sandipan, Ajay Singh, N.A. Patel, S.R.S. Dange, R.L. Patel and Kavita Sharma

Authors

P.B. Sandipan, Ajay Singh, N.A. Patel, S.R.S. Dange, R.L. Patel and Kavita Sharma

Department of Plant Pathology, C.P. College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar–385 506

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Bioefficacy of Different Antagonists against Fruit Rot of Tomato Caused by *Alternaria tomato* (Cooke) G. F. Weber under *in vitro* Condition

Introduction

Fruits and vegetables are important for human nutrition. They are also indispensible for the maintenance of human health and tomato *(Lycopersicon esculentum* Mill.) is one of them. Tomato is a crop of immense value in olericulture. It is a solanaceous fruit vegetable believed to have its origin in Tropical America. Portuguese introduced it in India in the early 18th century. Tomato is a popular fruit vegetable available throughout the year in India.

Tomato is used as a fruit as well as vegetable in our diet and preferred by all people and consumed in different forms throughout the year. It has got importance in the diet due to its nutritive value and low price. It can be consumed as a fresh ripe fruit and is one of the most popular salad vegetables. It is taken after cooking or raw or is made into soups, salad, pickles, ketchups, sauces and many other products. Hence, there is a great demand for tomato in the market as a fresh fruit. Tomato is the most attractive crop for the canning industry. Despite its wide spread cultivation in our country, the availability of good quality tomato fruits are inadequate in the market because a large number of fungal, bacterial and viral pathogens affect the crop in field condition.

Material and Methods

Dual culture method was adopted to know the efficacy of different antagonists against *A. tomato.* The test organisms and pathogen were grown separately on PDA. From seven-days old culture, two mm bits of both the bio agents (*T. viride* and *T. harzianum*) and the pathogen *(A. tomato)* were cut aseptically from the periphery of the colony and placed opposite to each other approximately 60 mm apart on PDA containing Petridishes (Dennish and Webster, 1971). While, the antagonistic effect of bacteria *viz., P. fluorescens* and *B. subtilis* were tested by streaking

the bacteria at one side of the PDA containing Petridishes. A two mm bit of a pathogen from seven days old culture was placed at 60 mm apart on the opposite side on PDA containing Petridishes perpendicular to the bacterial streak.

Three replications, each with three Petridishes were kept and the Petridishes with only pathogen served as control. All Petridishes were incubated at 28 ± 2 °C temperature and after five days of incubation, the radial growth of the pathogen was measured. The per cent growth inhibition (PGI) was calculated by the following formula given by (Asalmol *et al.*, 1990).

$$PGI = \frac{C - T}{C} \times 100$$

Where,

PGI = Per cent Growth Inhibition
C = Growth in control (mm)
T = Growth in treatment (mm)

Result and Discussion

T. viride, T. harzianum, P. fluorescens and *B. subtilis* were evaluated for their antagonism against *A. tomato* by dual culture method.

All the bioagents significantly inhibited the growth of *A. tomato* over control. Maximum growth inhibition (70.85%) of *A. tomato* after five days of incubation was found in *T. viride* followed by *T. harzianum* (61.08%), while minimum growth inhibition (29.23%) was obtained in *B. subtilis* (Table 1). Effectiveness of various antagonists for *in vitro* growth inhibition of *Alternaria* spp. have been reported by many research workers. Growth inhibition of *A. solani* by *T. viride* was reported by Das and Animapal (1986). Chattannauar *et al.* (1988) found that species of *Bacillus* and *Streptomyces* showed the zone of inhibition, when tested against *A. alternata*, while *Trichoderma* and *Aspergillus* grew over the colonies of *A. alternata.*

Table 1. *In vitro* efficacy of different antagonists against *A. tomato* after five days of incubation

Treatments	*Per cent growth inhibition over control*
T. viride	70.85 (57.20)*
T. harzianum	61.08 (51.98)
P. fluorescens	40.91 (39.78)
B. subtilis	29.23 (33.28)
Control	00.00 (4.05)
S. Em. ±	0.17
C.D. at 5%	0.49
C.V. %	1.40

* Figures in the parentheses are arcsin transformed values

Hence, the present investigations are in confirmation with the findings of above research workers.

Conclusion

Trichoderma viride, T. harzianum, P. fluorescens, B. subtilis were evaluated *in vitro* for their antagonism against *A. tomato. Trichoderma viride and T. harzianum* inhibited maximum fungal growth of the pathogen and found superior, when tested by dual culture technique.

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Authors

P.B. Sandipan, Ajay Singh, N.A. Patel, S.R.S. Dange, R.L. Patel and Kavita Sharma
Department of Plant Pathology, C.P. College of Agriculture Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar–385 506

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Effect of Different Antagonists on Development of Tomato Fruit Rot Caused by *Alternaria tomato* (Cooke) G. F. Weber

Introduction

Fruits and vegetables are important for human nutrition. They are also indispensible for the maintenance of human health and tomato *(Lycopersicon esculentum* Mill.) is one of them. Tomato is a crop of immense value in olericulture. It is a solanaceous fruit vegetable believed to have its origin in Tropical America. Portuguese introduced it in India in the early 18th century. Tomato is a popular fruit vegetable available throughout the year in India.

Tomato is used as a fruit as well as vegetable in our diet and preferred by all people and consumed in different forms throughout the year. It has got importance in the diet due to its nutritive value and low price. It can be consumed as a fresh ripe fruit and is one of the most popular salad vegetables. It is taken after cooking or raw or is made into soups, salad, pickles, ketchups, sauces and many other products. Hence, there is a great demand for tomato in the market as a fresh fruit. Tomato is the most attractive crop for the canning industry.

Material and Methods

To study the effect of antagonists, the semi-ripe fruits of tomato were surface sterilized with 0.1 per cent $HgCl_2$ for two minutes followed by three washings with sterile distilled water and were air dried. Cork wounding method was used for inoculation of pathogen and antagonists *viz., T. viride, T. harzianum, P. fluorescens* and *B. subtilis* were tested.

The semi-ripe tomato fruits were first inoculated with two mm bits of seven days old culture of different antagonists separately by cork wounding method and after 12 hrs, they were inoculated on same site with two mm bits of seven days old culture *of A. tomato.* Proper controls were maintained by inoculating the semi-ripe tomato fruits only with *A. tomato.* All the fruits were placed separately in sterilized, loosely tied polythene bags with a piece of sterilized wet absorbent cotton inside each bag and bagged fruits were kept at 28 ± 2 °C. Three replications, each with three fruits were kept in each treatment. The effect of antagonists in reducing the fruit rot development was observed after five days of incubation.

Result and Discussion

Pre-treatment Effect of Different Antagonists on Development of Tomato Fruit Rot

T. viride, T. harzianum, P. fluorescens and *B. subtilis* antagonists were tried against tomato fruit rot caused by *A. tomato.* The semi-ripe tomato fruits of equal size were first inoculated

with different antagonists separately by cork wounding method and after 12 hrs of incubation, the fruits were inoculated on the same site with *A. tomato*

Of the four antagonist tested, only *P. fluorescens* and *B. subtilis* were found effective, while *T. viride* and *T. harzianum* themselves caused rotting in tomato fruits, but there was no sporulation of the *A. tomato* on the tomato fruits. After five days of incubation, minimum lesion diameter (21.64 mm) and diameter of sporulated area (10.90 mm) were observed in the treatment of *P. fluorescens* followed by *B. subtilis*. Both these treatments significantly reduced fruit rot of tomato over control (Table 1). In control, the lesion diameter and diameter of sporulated area were 28.09 and 16.47 mm, respectively after five days of incubation. *P. fluorescens* and *B. subtilis* inhibited the tomato fruit rot to some extent, while *T. viride* and *T. harzianum* themselves caused rotting in tomato fruits.

Table 1. Effect of different antagonists on development of tomato fruit rot caused by *A. tomato* after five days of incubation

Treatments	*Lesion diameter (mm)*	*Diameter of sporulated area (mm)*
T. viride	Both are causing the rotting in tomato fruits after inoculation and sporulation of *A. tomato* was not occur on tomato fruits	
T. harzianum		
P. fluorescens	21.64	10.90
B. subtilis	24.30	13.70
Control	28.89	16.47
S. Em. ±	0.14	0.09
C.D. at 5%	0.41	0.27
C.V. %	1.69	2.07

Similarly, *in vitro* dipping of citrus fruits in a suspension of cells of *B. subtilis* controlled the decay of fruits caused by *A. citri* (Singh and Deveral, 1984). Basim and Katireioglu (1990) found that isolates AB-2 and AB-27 of *B. subtilis* were most effective against *A. alternata* and *A. solani.* The present results are in confirmity with the findings of above research workers.

Conclusion

It can be said from above foregoing discussion that In pre-treatment, *P. fluorescens* and *B. subtilis* both were found in reducing the fruit rot development of tomato caused by A. *tomato* while, *T. viride* and *T. harzianum* itself caused rotting on tomato fruits.

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Author

P.B. Sandipan, Ajay Singh, N.A. Patel, S.R.S. Dange, R.L. Patel and Kavita Sharma
Department of Plant Pathology, C.P. College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar–385 506

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Chitosan Treatment for Plant Growth Regulation

Introduction

Chitin is a polysaccharide consisting predominantly the unbranched chains of b-(1-4)-linked 2-acetamido-2-deoxy-D-glucose (N-acetyl-D-glucosamine) residues. It can be regarded as derivative of cellulose, in which the C-2 hydroxyl groups have been replaced by acetamido residues. Chitin is found in huge quantity in the natural environment. Estimates of yearly natural synthesis exceed several billion tones as a structural material of all exoskeletal animals; of all members of arthropoda (crustacea, insects, spiders, etc.), mollusca (snails, squids, etc.), coelenterata (marine organisms such as hydoids and jellyfish) and nematoda (unsegmented worms) (1,2) and in cell walls of various fungi very specially mushrooms (3,4).

Chitin is typically amorphous solid that is largely insoluble in water, dilute acids, and alkali, therefore is less reactive compound compare to starch, glycogen and cellulose. These properties reduce its commercial value at industrial level. Development of pharmaceutical science and biotechnology has explored various commercial applications of chitin. It can be used significantly

(a) As adhesive in sizing process of paper,
(b) As filaments, threads, fibers, tubes, straws and seamless sausage casings in textiles and plastic fabrications,
(c) As a shrink proofing for wool, photographic product,
(d) In dewatering municipal sludge, removal of certain radioisotopes from water by percolation, removal of mercury and copper from dye house effluent carrying polymeric dyes content,
(e) As a artificial kidney membranes, preparations of immunization against parasites, biodegradable–pharmaceutical carriers, blood anti-coagulants, aggregation of leukemia cells, wound healing accelerators and microbiologic media (1, 5).

Use of chitin as a fertilizer has been disclosed by Peniston *et al.* in United States Patent No. 4/1,199,496 in 1980. The patent is concerned with the process of recovery of chemicals from shells of crustacea. As explained by Peniston, *et al.,* chitin can be used as a agro-fertilizer to release nitrogen, slowly, into the soil and thereby over a relatively long period of time increase the nitrogen content of soil (6).

Chemical hydrolysis or enzymatic degradation of chitin releases its derivatives–chitosan, chitooligosaccahrides, chitobiose and D-glucosamine and each one of them have significant commercial applications in various industries.

Chitosan is 2-amino-2-deoxy-D-glucoglycan i.e. deacetylated form of chitin (7). Chitosan compounds in a range of up to and exceeding 1×10^6 molecular weight are derived commercially from chitin. The quality of chitosan varies with the degree of substitution of the N-acetyl groups, degree of polymerization, color, manufacturing process, clarity, consistency, uniformity, and source (2).

Chitosan has many industrial, medical, pharmaceutical, cosmetics and nutritional uses, including those requiring a biodegradable, non-toxic polymer. The significant applications includes

(a) Preparation of hair dyes and other cosmetics as film forming agents, setting lotions, blow dry lotions, hair sprays, nail varnishes, humectants, and skin cosmetic agent for gels, emulsions, and substantive polymers for shampoo, hair conditioners and skin moisturizing preparations,
(b) in photography, because of its resistance to abrasion, optical characteristics, film forming ability and behavior with silver complex,
(c) it can be used as immunoadjuvents and wound healing activity and used in the production of absorbable surgical sutures and heparin covalently bounded chitosan production which display thrombo-resistant property. Its injections for staphylococcal infections are widely under chemotherapeutic uses,
(d) it is used as vehicle for sustained release drugs as the dissolution of poorly soluble drugs can be enhanced by chitosan gels,
(e) it exhibits specific biomedical properties and gastroprotective effect and also efficiently used for encapsulation for mammalian cell culture for maximum cell density development,
(f) it is significantly used as a novel agent for the immobilization of enzymes and various biostrains on its crosslinked beads formation where half life of the enzymes appeared significantly prolonged,
(g) partially depolymerised and purified squid pen chitosan can be used to make the contact lenses by spin casting technology, moreover, chitosan albumin blended membranes modified with phospholipid bilayers could act as dialysis membranes.

During working on the research project for the screening and development of potent microbial strain for chitinolytic activity fieldwork for the ascersion of natural resources as an inoculum was carried out. It comes to the observation that the farmers of coastal regions of

Saurashtra–Gujarat are very frequently and casually apply shrimp and crab waste of the seafood industries as an organic co-fertilizer in their agriculture farm and various horticultural practices. The enrichment of soil with this chitinous material proved very significant to enhance the soil fertility, vigorous growth of cultivable plant and some special effect on the health and qualitative and quantitative agroproducts.

It is postulated that the chitin and its biodegradable derivatives–chitosan may act as a fertilizer and plant growth regulator jointly or individually.

However, fertilizers differ from plant growth regulators. A fertilizer is any material which is added to soil to supply chemical elements needed for plant nutrition. Most commonly, fertilizers are designated by a three digit number which represents the respective amounts of N, P and K. A plant growth regulator, on the other hand, is an organic compound which will inhibit, accelerate or in some way influence physiological processes in plants. Where a fertilizer merely supplies needed elements for a plant to grow in normal fashion while a plant growth regulator cause some sort of change in the plant's normal growth pattern. Some of the influences of the plant growth regulators include germination enhancement, root stimulation, plant stature control, shortening or lengthening of the time to maturity of the plant, ripening control, increased yield, fruit and vegetable color control, and shortened or lengthened dormancy. Some known plant growth regulators are cytokinins and gibberlic acids.

Although fertilizers can also cause increased yields from plant, they do so at a cost. High rates of fertilizer application increase plant yield potential by creating larger plants, but such unusually large plants are susceptible to delayed maturity and a condition known as lodging. Lodging occurs when a plant is too tall and/or too heavy to support itself and is therefore easily affected by winds which cause the plant to tip and fall and lay on the ground surface. In such a lodged condition, plants are difficult to harvest because of their close proximity to the soil surface, thus resulting in reduction of crop yield. Seed damage is also likely with damp soil, with pests such as rodents and insects, which contaminate the crop and render it unmarketable.

To determine the effect of chitin and chitosan either as fertilizer and/or plant growth regulator, the experimental methodology were set up with knowledge of conventional agricultural techniques as basic tools.

Materials and Methods

Kalyan Sona (IARI) variety of wheat, which is the most popular cereal grain among the farmers of Gujarat, was selected as a test seed to determine the significant effect of chitosan as a plant growth regulator at seed germination and seedling development stage.

Healthy wheat grains were selected and treated with Andrine as a pesticide and Pentachloronitrobenzene to prevent smuts, damping-off and seed rot.

The above treated 100 grams of wheat seeds were soaked with 200 ml of treatment solution containing 25 mg of chitosan, 25.0 mg non-phytotoxic acid and 10 mg of guar gum for about 12–15 hours at 20^0C–25^0C temperature.

Chitosan treated 100 grains were selected and placed in sterile petridishes containing moisted pad of cotton and filter paper. The plates were incubated at 20°C temperature in humidified chamber for about 6 days to induce imibition and germination. Control set of untreated seeds was also placed same as above. (Table-1) During the incubation period the seeds were observed and results of germination were recorded for quantitative and qualitative aspects.

Chitosan treated 100 grains were selected and sew in a soil pot for the development of seedling for about 15 days on laboratory table near the window. Soil pot was sprayed with desired quantity of water to maintain appropriate moisture. During the cultivation, periodic

Table 1
Seed Response to Chitosan Treatment

Sr.	*Incubation*	*Untreated Seeds*			*Chitosan Treated Seeds*		
No.	*Time (hrs.)*	*Seed Response*	*Coleoptile (cms.)*	*Radical (cms.)*	*Seed Response*	*Coleoptile (cms.)*	*Radical (cms.)*
1	0	20	—	—	20	—	—
2	24	20	—	—	20	—	—
3	48	2 G.R.	—	—	8 G.R.	—	—
		18 N.G.	—	—	12 N.G.	—	—
4	72	2	0.8–1.2	1.5–2.0	4	1.3–1.5	2.5–4.0
		7	0.2–0.5	1.8–2.2	8	0.8–1.2	1.0–1.5
		2 G.R.	—	—	8 G.R.	—	—
		9 N.G.	—	—	—	—	—
5	96	7	1.0–1.5	1.0–3.0	11	2.0 2.5	3.0–5.0
		5	0.4–0.7	2.0–2.5	9	0.8–1.4	1.0–2.0
		3 G.R.	—	—	—	—	—
		5 N.G.	—	—	—	—	—
	120	8	1.0–2.0	2.8–3.2	13	2.0–3.0	3.5–6.0
6		5	0.8–1.0	2.5–3.0	7	1.0–2.0	1.0–2.0
		3 G.R.	—	—	—	—	—
		4 N.G.	—	—	—	—	—
7	144	13	1.5–3.0	3.5–4.5	15	2.0–4.0	5.0–7.0
		2	1.0–1.3	1.0–2.0	5	1.0–2.0	1.0–2.0
		3 G.R..	—	—	—	—	—
		2 N.G.	—	—	—	—	—

G.R.-Growth Retarded
N.G. - No Germination

observation was carried out to determine the progress of seedling development. Control set of untreated seeds was also placed same as above.

Results and Discussion

Soak treatment with Andrine and Pentachloronitrobenzene proved effective pesticides and germicides respectively and efficiently gives surface sterilization of seeds and prevention against various soil borne infection.

Chitosan is soluble in acidic solutions. Testing of various organic acids revealed that 0.0125% glutamic acid is best Chitosan solublizer (1:1) with non-phytotoxicity. Application of 0.005% Guar gum enhanced encapsulation of seed and prolonged adhering of adsorbed Chitosan.

0.0125% solution of Chitosan and glutamic acid with 0.005% Guar gum is found efficient for imbibition and seed germination.

The wheat seed germination response was determined for third day to six days under seed cultivation practice comparing with untreated seed as a control (Table–1).

The germinating seeds were characterized for % of germination, development of coleoptile and radical.

The treated seeds have 95%–97% seed germination within 72 hours in compare to 60%–75% of control.

The treated seeds have 30%–40% growth retarded germination in compare to 55% of control within 72 hours.

During further cultivation, treated seeds have only 1%–2% growth retarded in compare to 30%–40% of control within 96 hours.

During 120 hours of cultivation in case of treated seeds 97%–99% seeds were developed vigorous coleoptile and radical growth while untreated seeds carries about 35%–25% seeds as growth retarded or non germinated which reduced to 28%–30% in compare to about 4% to negligible seed germination failure in case of Chitosan pre-treated seeds.

The pre-treatment enhance vigorous, fast growth of radical which becomes rhizoidal within 144 hours and gives excellent sucker and hold fast system to the seedling.

Conclusion

The pre-treatment of Chitosan-glutamic acid (1:1) enhance seed germination and vigorous growth of coleoptiles and radical. Thus it can be concluded that the mixture of Chitosan-glutamic acid (1:1) is a cheaper, convenient plant growth regulator at least as compared to the conventional fertilizers.

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Authors

Nikunj H. Chhag, Kamlesh S. Pansheria, Palak R. Jadeja, Nirali J. Joshi, [1]Dr. M.D. Shukla.

Institute of Biotechnology, Raiya Road,Rajkot-360 002,
[1]M.G. Science Institute, Ahmedabad. (Gujarat)

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Screening of Various Microbial Strains Producing Antifungal Biomolecules

Introduction

In the developing countries Griseofulvin, nystine, pymaricine, and chlorotetracycline, are manufactured as fungal antibiotics for chemotherapeutic and agricultural use (1).

Water insolubility; minimize their application as an ideal drug. Most soils inhibit germination of fungal spore as well as fungal growth and known as widespread soil fungistasis. It was concluded due to nutrient deprivation hypothesis or antibiosis hypothesis (2, 3).

Somehow, plant pathogenic fungi appear to be more sensitive than saprophytic fungi (4).

The nature of fungistasis is critical as not all habitants of soil produce antifungal compounds and their inhibitory spectrum vary for different fungal species (5).

The interactions between rhizospheric bacteria and plant roots can affect plant health. Several different mechanisms were postulated for the suppression of phytopathogenic fungi in the root zone, including production of antibiotics or siderophores, competition for substrate, and niche exclusion (6).

In natural ecosystem antagonistism to fungi was observed among *Lactobacillus, Pseudomonas, Corynibacterium, Agrobacter, Bacillus and Actinomycetes* group of organisms.

The best-characterized antifungal product from an LAB species is reuterin (an equilibrium mixture of monomeric, hydrated monomeric, and cyclic dimeric forms of 3-hydroxypropionaldehyde), produced by *L. reuteri*. Reuterin is found toxic towards a wide range of Gram-negative and Gram-positive bacteria, and equally effective against lower eukaryotic genera of yeasts and fungi such as *Candida, Torulopsis*, *Saccharomyces*, *Saccharomycoides*, *Aspergillus* and *Fusarium* (7).

Proteins with antifungal activity have been isolated and characterized as the (a) cysteine-rich small defensins, (b) ribosome-inactivating proteins, (c) lipid transfer proteins,

(d) polygalacturonase inhibitor proteins, (e) nonenzymatic chitin-binding proteins, and (f) pathogenesis-related (PR) proteins (8).

Spoilage of bread and bakery products are due to fungal contamination and are belongs to *Penicillium*, *Aspergillus*, *Monilia*, *Mucor*, *Endomyces*, *Cladosporium*, *Fusarium*, and *Rhizopus* (9).

Plant root exudates stimulate rhizosphere growth of actinomycetes that are strongly antagonistic to fungal pathogens. Actinomycetes synthesize an array of biodegradative enzymes, including chitinases, glucanases, peroxidases, and enzymes involved in mycoparasity. Unfortunately it is not studied at the biochemical or mechanistic levels (10, 11).

We have attempted a screening project for the isolation, characterization and development of antifungal biomolecules from soil habitant *Bacillus* strains.

Materials and Methods

1. Various humus samples, rhizospheric soil and fertile samples were collected from agro active farm area.
2. From selected samples, suspensions were prepared aseptically in sterile distilled water.
3. The untreated above suspension was used as an inoculum to target non-spore forming antagonistic bacteria while pre treatment of pasteurization was given to the samples to target spore forming antagonistic bacteria.
4. The above prepared suspensions were individually inoculated (1.0%) into 100ml of sterilized trypton nutrient broth and trypton nutrient bile salt broth in 250 ml of flask and incubated at 32^0C at 150 rpm on environmental shaker for 8 to 10 hours, to promote the germination of spore of Gram's +ve rod shaped bacteria and primary growth of Gram's -ve bacteria respectively.
5. The positive qualified enrichment flasks were selected for the cultivation of target antagonistic bacteria by sandwich culture technique involving *A. niger* as a test fungal culture.
6. After appropriate incubation plates were studied for the observation of antagonistic reaction to test organism *A. niger* by underlined bacterial colonies.
7. Typical antifungal biomolecules producing bacterial colonies were selected and purified by subsequent sub culturing. Isolates were preserved by slant culture technique.
8. The isolates were verified for the biosynthesis of antifungal compound either as a constitutive or inducible mode of biosynthesis by means of dialysis bag cultivation technique. The constitutive low molecular and high molecular antifungal biomolecule producers were selected to carry out flask level submerged fermentation on environmental orbit shaker at 32°C at 150 rpm for 6 days.
9. During shake flask fermentation periodically samples were analyzed for the growth of inoculated bacteria by turbidometric analysis and corresponding production of antifungal biomolecules against test organism *A. niger* by cup plate technique.
10. After the completion of shake flask fermentation, the broth cultures were centrifuged individually at 10,000 rpm for 10 minutes to prepared cell free extract.

11. The prepared cell free extract were studied during secondary screening program to determine antifungal spectrum against various phytopathogenic and dermatophytic fungi by appropriate bioassay technique.
12. The cell free extracts were also studied for their various physicochemical properties by suitable techniques for their categorization.
13. Considering the physicochemical properties and spectrum of antifungal activity ideal microbial strains were selected and preserved by slant culture technique.
14. The preserved selected strains are under study for genetical modification to improve antifungal potency and to standardized various parameters for lab level submerged fermentation technology and further scaling up for appropriate commercial output.

Results and Discussion

Selected soil samples particularly humus exhibit considerable biodiversity and various antagonistic reactions to test organism *Aspergillus niger.* During primary screening through crowd plate technique various microbial colonies were selected for antifungal biomolecules production.

The secondary screening program by sandwich culture technique with isolates and test organism *A. niger* had yield pure isolates of antagonistic microbes.

The shake flask cultivation of 9 microbial strains synthesized extracellular antifungal biomolecules having different degrees of antifungal potency against selected fungal community (Table 1- a, b).

Attempts are in progress to induce the development of hyper antifungal potency by mutagenesis and selection process. The efficient downstream process and characterization of antifungal biomolecules will be determined to elucidate the nature, mechanism of biosynthesis

Table 1a.
Comparative study of isolated organisms over various fungal species.

Code	*Saccharomyces*	*Aspergillus*	*Penicillium*	*Mucor*	*Rhizopus*	*Cunning-hmella*
Bact +ve a	+++	++	++	++	+++	+++
Bact +ve b	+++	++	++	+++	+++	+++
Bact +ve c	++	++	++	++	++	++
Bact +ve d	+++	+++	+++	+++	+++	+++
Bact +ve e	+++	++	++	+++	+++	+++
Bact -ve f	++	++	++	++	++	++
Bact -ve g	+++	++	++	++	++	++
Actino h	+++	+++	+++	+++	+++	+++
Actino i	+++	+++	+++	++	++	++

+++ 15–20 mm, ++ 7–15 mm, + 2–5 mm

Table 1b.
Comparative study of isolated organisms over various fungal species.

Code No.	*Pythium*	*Phytopthora*	*Candida*	*Coccoido mycetes*	*Helminth osporium*	*Alternaria*
Bact +ve a	+++	++	+++	+++	+	+
Bact +ve b	+++	+++	+++	+++	+	+
Bact +ve c	++	++	++	++	+++	+++
Bact +ve d	++	++	++	++	+	+
Bact +ve e	++	++	+	+	½ +	½ +
Bact -ve f	++	++	++	++	+	+
Bact -ve g	++	++	+++	+++	++	++
Actino h	+++	+++	+++	+++	+++	+++
Actino i	+	½ +	+++	+++	+++	+++

+++ 15–20 mm, ++ 7–15 mm, + 2–5 mm

and physicochemical properties. The attempts will be made for the optimization of various parameters for the mass production at laboratory level and further scaling up for commercialization.

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Author

Kamlesh Pansheria, Nikunj Chhag, Dharmesh Shah, Jaydeep Joshi
Institute of Biotechnology, Chudasama Main Road,
Near Amrapali Crossing, Rajkot-360 002, Gujarat.

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Effect of Fertilizer and Bio-fertilizer on Pearl Millet with and without Intercropping under Rainfed Conditions

Introduction

The present day intensive agriculture require high input of nitrogenous fertilizers and this need is met by manufacture of ammonia form gaseous nitrogen through homer boach process (Barris 1989). The cost of producing nitrogenous fertilizers has been increasing constantly over the yields. Emphasis is now being put on the use of nitrogenous fertilizers along with biofertilizers and organic mannure in integrated nutrient supply system. As a useful biofertilizer for cereals, oil seeds, vegetable and economically important non leguminous plant. Azotobacter has occupied on important place over the year in many countries including India (Mishustin and Shihnikava 1969), Subha Rao 1979 Pandey and kumar 1989).

Material and Methods

A field experiments was conducted during kharif season of 2000, 2002 and 2004 in split plot design with four replications of medium black soil. The main plot treatments consisted of two cropping system viz. C_1 – sole pearl millet and C_2 – pearl millet + pigeonpea intercropping (2:1) row proportion. The sub plot treatment consisted of seven fertilizer and biofertilizer treatments viz. F1 – control, F2 Biotfertilizer, (Azotobacter + PSB), F3- 20 kg N + 15 kg P_2O_5/ ha, F4- 20 kg N + 15 kg P_2O_5/ha + biofertilizer, F5 – 40 kg N + 30- kg P_2O_5/ha, F6 – 40 kg N + 30 kg P_2O_5/ha + Biofertilizer, F7- 60 kg N + 40 kg P_2O_5/ha.The gross plot size was 5.0 × 5.4 m^2 during 2000 and 5.0 × 3.6 m^2 during 2002 and 2004 years. Where as the net plot size was 4.5 × 2.7 m^2 during the year 2000 and 4.4 × 2.7 m^2 during the year 2002 and 2003 .The variety AIMP-92901 of pearl millet sown at 45 × 10 cm^2 for sole crop. The intercrop of pigeonpea variety BSMR–736 was sown with 2:1 row proportion at 45 × 15 cm^2 spacing.

Results and Discussions

The data presented in Table-1 indicated that the grain yield and fodder yield of pearl millet under sole and intercropping as influenced by fertilizer and biofertilizer treatments.

The effect of different cropping system was evident on fodder and pearl millet grain equivalent yield whereas it was not significant in case of grain yield of pearl millet (Table-1). The sole crop of pearl millet gave significantly higher fodder yield than pearl millet+ pigeonpea intercropping. The per cent reduction in grain and fodder yield as compared to sole crop was 19.13 and 10.89 per cent respectively where as the PM + PP intercropping gave significantly higher PMEY than sole cropping of pearl millet.

The effect of different fertilizer levels on fodder, grain yield as well as PMEY was significant. The application of 40 kg N +30 P_2O_5/ha + Biofertizer and 60 kg N +40 P_2O_5/ha recorded almost similar grain yield of pearl millet which were also on par with 40 kg N + 30 kg P_2O_5/ha without biofertilizer and significant over remaining fertilizer treatments. Whereas the 60 kg N + 40 kg P_2O_5/ha was on par with 40 kg N + 30 kg P_2O_5 with biofertilizer in respect of fodder and pearl millet grain yield equivalent yield and was significant over remaining treatments respectively (Table-1). Field experiments conducted at different locations under varying agro-climatic conditions with pearl millet over five years in India revealed that the better inoculation responses were observed with 0 or 10 kg N/ha application than with 20 or 40 kg N/ha application (Tilak and Subburao, 1987).

The interaction effect of C × F was evident in case of grain, fodder yield and PMEY. Application of 40 kg N + 30kg P_2O_5/ha + biofertilizer to sole cropping recorded maximum pearl millet grain yield which was on par with 60 kg N + 40 kg P_2O_5/ha and 40 kg N + 30kg P_2O_5 kg/ha with out biofetiliser and was significant over remaining treatment combinations. Almost similar trend was observed in PN + PP intercropping in respect of grain yield of pearl millet. The application 60kg N + 40kg P_2O_5/ha to sole crop of pearl millet recorded maximum fodder yield which was on par with 40kg N + 30kg P_2O_5/ha with biofertilizer and was significant over remaining treatment combinations, whereas the application of 60kg N + 40kg P_2O_5/ha to PM + PP intercropping recorded maximum pearl millet grain equivalent yield which was on par with 40kg N + 30kg P_2O_5/ha with and without biofertilizer and was significant over remaining treatment combinations.

The intercropping of PM + PP also gave significantly higher GMR and NMR than sole cropping with maximum cost benefit ratio (average of three years) of 1:6.42 (Table-2). Similarly the application of 60kg N + 40kg P_2O_5/ha recorded maximum GMR nad NMR which was on par with 40kg N +30kg P_2O_5/hawith biofertilizer and was significant over remaining fertilizer treatments. The average cost benefit ratio was maximum with later treatments. Bhandari *et al.* (1989) computed the economics of Actobactor inoculation in maize and wheat and deducted a monetary gain of Rs. 110 to 217/ha.

The interaction effect of C × F on GMR and NMR was evident (Table-2). The application of 60kg N + 40kg P_2O_5/ha to PM + PP intercropping recorded maximum GMR and NMR which was on par with 40kg N +30kg P_2O_5/ha with and without biofertilizer and was significant over remaining treatment combinations. The results from various field experiments with azospirillum revealed that the total N, P and K assimilation by the inoculated plants was higher than the uninoculated plants. Bacterization resulted in yield increase with decrease of Number

increase in N concentration (Wani *et al.*,1988) and these effects have been attributed to effects of plant growth substances. Yield increase also accompanied by increase N concentration due to beneficial inoculation which may be attributed to enhance N_2 fixation or increased N assimilation by plants (Baldari *et al.*, 1983; Hegzi *et al.*, 1983; Kapulnik *et al.*, 1981 a,b; Kohire *et al.*, 1996; Pacovsky *et al.*, 1985; Negi *et al.*, 1991; Wani and Lee *et al.*, 1991).

Table 1
Production potential of pearl millet with and without inter-cropping as influenced by different fertilizer levels (Pooled for 3 years)

Treat-ments	*Grain yield (kg/ha)*			*Fodder yield (kg/ha)*			*PM equivalent yield (kg/ha)*		
	Sole PM	*PM+ PP*	*Mean Wt*	*Sole PM*	*PM+ PP*	*Mean*	*Sole PM*	*PM+ PP*	*Mean Wt.*
F_1	1453	1317 (820)	1385	2490	1902 (1440)	2196	1961	4129	2596
F_2	1488	1351 (934)	1419	2500	2409 (1590)	2454	2104	4552	2666
F_3	2230	1722 (1010)	1976	3409	2970 (1612)	3190	2773	5104	3429
F_4	2225	1905 (1036)	2064	3558	3354 (2124)	3456	2945	5545	3660
F_5	2465	2039 (1113)	2252	3909	3500 (1862)	3705	3260	5895	3999
F_6	2655	2193 (1204)	2424	4099	3646 (1941)	3873	3503	6372	4318
F_7	2648	2199 (1172)	2423	4217	3823 (2093)	4049	3477	6461	4414
Mean	2300	1860 (1041)		3463	3086 (1751)	3274	2865	5435	

	Grain Yield			*Fodder Yield*			*PMEY*		
	SE± at 5%	*CD*	*CV%*	*SE± at 5%*	*CD*	*CV%*	*SE± at 5%*	*CD*	*CV%*
C	95	NS	–	52	153	–	124	561	–
F	90	257	–	112	325	–	129	369	–
C × F	90	257	–	158	460	–	302	862	–

Table 2

Gross Monetary Returns (GMR), Net Monetary returns (NMR) & Cost Benefit Ratio (C:B) of Pearl millet with & without inter-cropping as influenced by different fertilizer levels (Pooled for three years).

Treat-ments	*Grain yield (kg/ha)*			*Fodder yield (kg/ha)*			*PM equivalent yield (kg/ha)*		
	Sole PM	*PM+ PP*	*Mean Wt*	*Sole PM*	*PM+ PP*	*Mean*	*Sole PM*	*PM+ PP*	*Mean*
F_1	10741	22180	15381	7932	19132	12457	1:2.64	1:6.02	1:4.33
F_2	11435	24322	16162	8609	21173	13198	1:2.91	1:6.15	1:4.53
F_3	16024	27465	20381	12740	24092	17039	1:3.61	1:6.15	1:4.88
F_4	16290	29824	21525	12988	26475	18157	1:3.71	1:6.81	1:5.26
F_5	17933	31766	23369	14175	27769	19471	1:3.53	1:6.56	1:5.05
F_6	19196	34342	25319	15420	30326	21426	1:3.84	1:6.79	1:5.32
F_7	18951	35738	26046	14832	31354	21797	1:3.33	1:6.47	1:4.90
Mean	15796	29377	21169	12385	25760	17649	1:3.67	1:6.42	1:4.90

	GMR		NMR	
	SE±	CD at 5%	SE±	CD at 5%
C	679	3055	707	3179
F	693	1993	697	1993
C × F	1502	4288	1508	4305

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Author

Deshmukh, L.S.[1], V.S. Shinde[2], O.D. Kohire[3] and N.P. Patil[4]

1. *National Agril. Research Station, Aurangabad, MAU, Parbhani (M.S.)*
2. *Department of the Agronomy, MAU, Parbhani (M.S.)*
3. *Plant Pathology, Agril. Research Station, Badnapur MAU, Parbhani (M.S.)*
4. *College of Agricultural Engineering, MAU, Parbhani (M.S.)*

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Survey and Identification of Different Species of Earthworms from Marathwada Region of Maharashtra

Introduction

Earthworms are very important in agriculture as they improve physical, chemical and biological status of soil and ultimately enhance the crop production (Ghanekar, 1984). The burrow prepared by earthworms leave some physical effects on soil contributed by pore space and thus infiltration and aeration in the soil. The burrows are regarded as an additional drainage system a very significant one, within the soil matrix that operates during the period of heavy rains or when soils are irrigated (Lee, 1985). Earthworms affect the chemical composition of soil and distribution of plant nutrients in various ways. The carbon content of the casts is about 1.5 to 2 times more than that of soil so that C:N ratio of casts is generally little higher than those of soil (Lee, 1985). The urine and microproteins secreted also adds to the available nitrogen in the soil and the dead earthworm tissue is about 60 to 70 per cent (Dry weight) protein and has a nitrogen content of about 12 percent (Lee,1985). Earthworm was undertaken for the first time at Rothmsted,U.K. and it was conducted that *Eisenia foetida* was the best species for vermicomposting (Edwards *et al.*, 1985). Research work on such a useful creature was not done in Marathwada.

Material and Methods

The soil samples up to a depth of 30 cm were taken with the help of kurpi and spade. These samples about 5 kgs were brought to the laboratory and the adult worms, juveniles and cocoons were separated by hand sorting. The soil samples were collected from the sites having moisture around the field capacity. The earthworm samples were maintained as culture in eathern pots containing FYM and soil mixture in 1:1 proportion for future studies. Every day watering was given to the pots to maintain proper moisture were also covered with moist gunny cloth, so as

to reduce the moisture loss and to prevent the mortality due to insufficiency of water. The pot were kept in shed and numbered.

Identification of earthworm species

The living worms were dropped in a vessel containing fresh water and anaesthetized by adding alcohol (70 per cent) drop by drop to water, gradually until worms sensed to move. Care was taken to add not more than one tenth alcohol of the total volume, otherwise the worm might have been killed before narcotization. After narcotization the worms were taken out and strained in a tray and were covered within a thin layer of cotton. Formalin 5 to 10 per cent was added to the tray and the specimen were washed in fresh water and preserved in the test tubes containing 70 to 90 per cent alcohol. The tubes were packed and sealed properly and then specimens were numbered properly and sent by registered post to the Zoological Survey of India, Kolkata for identification and got identified. Similarly the species identification was done at Pesticide Research Center, Department of Entomology as per the procedure described by Gates (1960).

Earthworm biology

FYM and soil mixture in 1:1 proportion was taken and filled in glass tubes measuring 15 cm × 8 cm. Two clitellate worms were released in each open mouth bottles, water was added twice a week to maintain the soil moisture around the field capacity. Top of the bottle was covered with muslin cloth. Observation were recorded at 5 days interval for cocoons. The cocoons were incubated on moist filter paper placed in petridishes for maintaining high humidity. The juveniles hatched were placed in bottles containing 50 per cent FYM + SOIL and observations were recorded for the clitellate form of the earthworms. Throughout the experiment moisture was maintained around the field capacity. Number of juveniles hatched was noted and average time required for cocoon formation, juvenile formation, for growth and development (clitellate form) for the three predominant species viz. *Perionys sansibaricus, Lampito mauritti* and *Eisenis foetida* was calculated.

Earthworm multiplication

Earthen pots having 30 × 30 cm size were filled with FYM + soil in 1:3, 1:1 and 3:1 proportion separately. The FYM+ soil were moistened up to field capacity. Each treatment was replicated four times. The hundred clitellate earthworms were released in each pot. The earthen pots were covered with 9 moist gunny cloth to avoid the loss of moisture and maintain adequate humidity in the pots, favorable for the earthworm multiplication. Water was sprinkled every day to maintain the moisture around the field capacity. The earthworms were released in the pots on 1.9.2003. The number of earthworms in the pots were then counted after 90 days i.e. on 30.11.2003. Another count of total number of earthworms was taken after 180 days i.e. on 28.2.2004.

Results and Discussions

It was evident from Table-1 that *perionyx sansibaricus* required about 30 days for cocoon

production after mating. *Eisenia foetida* produce cocoons in a shorter period as compared to that of *Lampito mauritti* and *perionyx sansibgricus* . Hallat, *et al.* (1990) also reported 28 days duration for coon formation after mating in *perionyx* spp. *Perionyx exavatus* produced 25 cocoons in 102 days on cow dung, 42 cocoons in 55 days on horse dung and 65 cocoons in 41 days on sheep dung after reaching to maturity (Kale *et al.*, 1982). In the present investigation the time required for cocoons formation is different than above report. These variation may be due to the variation in media used. In case of *Eisenia foetida* four days were required for cocoon formation after mating. Similar types of findings was also recorded by Venter and Reinecke (1988) in *Eisenia foetida* and hence these results are in confirmation with the results of present investigations.

It was observed that incubation period varied from 20 to 24 days. In *Perionyx sanisbaricus* cocoon period or incubation period 18 days. In *Lampito mauritti*, the average incubation period was 21 days and 22 days incubation period in *Eisenia foetida*. Similar trend was recorded by Hallat *et al.* (1990).

The results showed that number of Juveniles produced per cocoons varied as per the species. In *Perionyx sansibaricues* produced a single juvenile after hatching. The finding of Hallat, *et al.* (1990) also indicated similar results *Eisenia foetida* produced three cocoons after hatching, Reynolds (1973) in his findings reported that mean number of juveniles per cocoon were 2.6. the findings of Venter and Reinecke (1988) also indicated three juvenile/ cocoon in Africa.

The juvenile period was 30 days in *Perionyx sansibaricus* and the total duration to reach the maturity after mating of earthworm was 75 days. Kula and Kokta 1992 in her findings, it was reported that it reached to maturity in 183 days after mating when fed on cow dung when fed on sheep dung it reached to maturity in 24 days, while fed on horse dung it reached to maturity in 177 days.

This may be due to influence of medium on which they were fed Hallat *et al.* (1990) reported that Juvenile period was 28 days in *Perionyx* spp. and it took about 74 days to form clitellate earthworms reached to maturity in a period of 73 days after mating. Venter and Reinecke (1988) studied biology of *Eisenia foetida* in which, it was reported that the juvenile period in *Eisenia foetida* was about 40 to 60 days.

The results of mass multiplication in case of *Eisenia foetida* showed that the FYM:soil in 1:1 proportion was significantly superior overall other media at 90 and 180 days. Studies and may be used for mass multiplication of earthworms. The highest multiplication rate of earthworms was observed in the media containing FYM + soil in 1:1 proportion as compare to control. Patil Mazgaonkar (1991) showed that there was 2, 6.2 and 3.04 times increase in earthworm population in FYM: soil 1:3, 1:1, and 3:1 media respectively indicating thereby that FYM: IN 1:1 was best media for multiplication of earthworms. Similar type finding were reported by Ghatnekar (1984) observed that in mixtures of weed compost manure and soil in 1:2, 3:4 proportion, the number of earthworms doubled in a period of 4-5 days.

Table 1
Days to cocoon formation after mating in different earthworms species.

Species	*Mean*
Perionyx sansibaricus	30
Lampto mauritti	7
Eisenia foetida	6

Table 2
Incubation period in days in different earthworm species.

Species	*Mean*
Perionyx sansibaricus	20
Lampto mauritti	23
Eisenia foetida	24

Table 3
Number of Juveniles hatched per cocoon in different earthworms Species.

Species	*Mean*
Perionyx sansibaricus	1
Lampto mauritti	2
Eisenia foetida	2

Table 4
Juvenile period in days in different earthworm species.

Species	*Mean*
Perionyx sansibaricus	30
Lampto mauritti	32
Eisenia foetida	44

Table 5
Multiplication count of earthworm after 90 and 180 fays in different media of Eisenia foetida

Treatments	*No. of worms released*	*Mean at 90 day*	*Mean at 180 days*
FYM : Soil			
1:3	100	415	1680
1:1	100	810	2602
3:1	100	645	2194
1:0	100	575	1984
0:1	100	400	1210
SE±		2.92	84.5
CD at 5 %		7.05	253.0

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Author

Kohire O.D[1]., L.S. Deshmukh[2], Kulkarni, A.M[3]., Kohirepatil, V.O[4]. Khapre[5] and N.P. Patil[6]

1 *Agril. Research Station, Badnapur, MAU, Parbhani* (M S.)
2 *National Agril. Research Station, Aurangabad, MAU, Parbhani* (M S.)
3 *Agril. Research Station, Badnapur, MAU, Parbhani* (M . S)
4 *Agril. Research Station, Badnapur, MAU, Parbhani* (M . S.)
5 *Agril. Research Station, Badnapur, MAU, Parbhani* (M . S.)
6. *College of Agricultural Engineering, MAU, Parbhani.* (M . S.)

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Life of *Perionyx sansibaricus* during Vermicomposting of Different Wastes

Introduction

Recycling of organic waste trough biological agents such as eathworms and microbes are well establishes practices. The recycling with the help of earthworms is called vercomposting. It is practiced by culturing some selected species of earthworms on organic wastes (Bano and Kale, 1992). In India farmers has started to adopt earthworms farming to reduce their independancy on chemical fertilizers for obtaining sustainable agriculture production. Hence production of vermicompost using different wastes and various species of earthworms is gaining importance in the field of agriculture. The organic wastes such as pigeon pea husk, soyabean husk, chickpea husk and mungbean husk are available in Marathwada region of Maharashtra state. While earthworm species like *Perionyx sansibaricus*, *Eisenia foetida* and *Lampito mauritti* (Kulkarni, 1993) are harbouring their population in the soil of Marathwada region. The farmers of this region has started export quality agricultural production by using various technologies and enquiring about suitability of specific species of the earthworm for vermicom post production in the region. Hence attempts were made to study the survival of *Perionyx sansibaricus*, during vermicompsting of different wastes in Marathwada region.

Materials and Methods

A pot culture experiment was conducted using organic wastes + soil (3:1) mixture. 10 kg mixture was taken in the earthen pots and moistening up to field capacity. 100 eathworms having about 10 weeks age were inoculated in these pots. *Perionyx sansibaricus* earthworms were used for experimentation. Earthen pots were covered with gunny bags and kept moist by watering twice in a day. Survival of earthworms was measured at 90 and 180 days by direct count. Second experiment was carried out in earthen pots using 1 kg mixture of organic wastes

+ soil (3:1)10 earthworms of 10 weeks age were inoculated in the pots and moisture level was maintained by covering the pots with gunny bags and watering twice in a day. The cocoons produced were counted at weekly interval by removing inoculated earthworms and inoculating into fresh pot. Cocoons were separated from left material using moisted filter paper for spreading the material. The cocoon count was recorded by hand sortiag and saeving. Total cocoon count was considered was at 90 days and 180 days for comparison. Decomposition being essential a microbial activity, microbial analysis of organic wastes and earthworm treated wastes was also undertaken. These experiments were repeated five times for conformation of the results.

Results and Discussion

The results of the present investigation revealed that the survival of *Perionyx sansibaricus* is very much affected due to different food sources (Table-1). Maximum survival was noticed in soybean husk followed by pigeon pea husk. Least survival was recorded in FYM among the organic waste used in the experimentation. The increase in the count of earthworms was from 6.1 to 9.8 times at 90 days after inoculation of *Perionyx sansibaricus* in the mixture of organic wastes and soil (3:1). The observation proved that earthworms are very much sensitive towards food material. Nutritious food material along with heavy microbial load stimulate their health and reproduction rate. Hence highest count of earthworms i.e. 982 was recorded during studied at 90 days after inoculation. Mungbean also content succulent and easily decomposable material and harbouring more earthworms compared to FYM, pigeon pea husk and chick pea husk. Patil and Mazgaonkar (1991) reported 2 to 62 times increase in the earthworm population in various proportion of FYM + soil mixtures. At 180 days after inoculation of 100 adult *Perionyx sansibaricus* in mixture, the survival was recorded from 1130 to 2250. Highest count was recorded in the soybean husk + soil mixture followed by pigeon pea husk – soil and chick pea husk + soil, while mungbean husk + soil mixture was having more count (1170 compared to FYM + soil mixture (1130). These type of results are reported by Kale *et al.* (1982) by using various food sources like cow dung, horse dung and sheep dung. The multiplication of earthworms was recorded from 10.2 to 18.5 times in six months i.e. 180 days period.

The observations on cocoon production indicated that the 50 to 55 cocoons were produced at 90 days and 100 to 130 cocoons were produced at 180 days from five pairs i.e. 10 earthworms of *Perionyx sansibaricus* species. The maximum cocoons were collected from soybean husk + soil mixture followed by pigeon pea husk + soil and chick pea husk + soil mixture. Least count of cocoons i.e. 50 and 100 (Table-2) was recorded at 90 and 180 days after introduction *Perionyx sansibaricus* in FYM + soil mixture (3:1). Experimental evidence supported that the rate of proliferation in earthworms can be enhanced by feeding them on various organic wastes like soybean husk, pigeon pea husk, chick pea husk and mungbean husk. Kale *et al.* (1982) in *Perionyx excavatus* recorded 25 cocoons in cow dung, 42 cocoons in horse dung and 65 cocoons in sheep dung. She stated that this variation are due to variation in nutrient content in the medias. Secondly the condition of the media which provide space for reproduction also affect the rate of cocoon laying. Organic wastes like soybean husk and pigeonpea husk are better to provide congenial conditions for reproductive activities. These type of observations are recorded by Parlekar *et al.* (1993) while studying the breeding and reproduction of earthworms for vermicomposting.

The experimental results concluded that the survival of *Perinyx sansibaricus* depends upon the media in which they are growing. Soybean husk + soil and pigeon pea husk + soil (3:1) are most suitable media for cocoon production and their survival. They can multiply the number from 5.1 to 9.6 times within 90 days and from 11.2 to 19.2 times at 180 days stage after their inoculation in the feed mixtures. Similar trend in cocoon production with increase from 40 to 45 within 90 days and 90 to 120 cocoons with 180 days from five pairs of *Pheritima posthuma* has been confirmed.

Microbial analysis

There was a considerable increase in total viable count. Total bacterial count actinomycetes and nitrifying bacterial in worm treated husk were higher than those of control. It supports the increased availability of nutrients. Acceleration of decomposition of husk may be due to the increased count of actinomycetes and nitrogen fixing bacteria. Actinomycetes are helpful in completing the decomposition of partially digested lignin and convert it into ligno proteins. Nitrogen fixing bacteria indirectly help in decreasing C:N ratio by making available more nitrogen from added organic matter. Similar results were recorded by Jambhekar (1991) and Lee (1985).

Table 1
Survival of *Perionyx sansibaricus* at 90 and 180 days in different organic wastes media

Treatments	*No. of worms released*	*Earthworm count at*	
		90 days	*180 days*
FYM + soil (3:1)	100	580	1130
Pigeon pea husk + soil (3:1)	100	650	1285
Chick pea husk + soil (3:1)	100	620	1170
Mungbean husk + soil (3:1)	100	600	1140
Soybean husk + soil (3:1)	100	982	2250

Table 2
Effect of food sources on cocoon production of *Perionyx sansibaricus* at 90 and 180 days in different organic wastes media

Treatments	*No. of worms released*	*Earthworm count at*	
		90 days	*180 days*
FYM + soil (3:1)	100	50	100
Pigeon pea husk + soil (3:1)	100	54	126
Chick pea husk + soil (3:1)	100	52	118
Mungbean husk + soil (3:1)	100	51	110
Soybean husk + soil (3:1)	100	55	130

Table 3
Effect of food sources on microbial count by *Perionyx sansibaricus* at various interval.

Treatments	*Microbial count*	
	90 days	*180 days*
FYM + soil (3:1)	8×10^3	11×10^3
Pigeon pea husk + soil (3:1)	18×10^5	25×10^6
Chick pea husk + soil (3:1)	10×10^4	14×10^4
Mungbean husk + soil (3:1)	9.5×10^3	12×10^5
Soybean husk + soil (3:1)	20×10^5	30×10^6

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Author

Kohire, O.D[1]., L.S. Deshmukh[2], R.S. Raut[3] and Kohire Patil V.O.[4]

1. *Plant Pathology, ARS, Badnapur, MAU, Parbhani (M.S.)*
2. *Agronomy, NARP, Aurangabad, MAU, Parbhani (M.S.)*
3. *Agril. School, Jalana, MAU, Parbhani (M.S.)*
4. *Agril. School, Jalana, MAU, Parbhani (M.S.)*

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Positive Effect of Dual Inoculum of GPPB and AM Fungi on Growth of *Anogeissus Latifolia* Wall

Introduction

Anogeissus latifolia Wall is a moist deciduous tree with a long clean bole and full crown, widely distributed in Indian forests, found on a variety of soils. The tree has a deep root system and very suitable for the preparation of nursery seedling stock.

The role of biofertilizers has already been proved useful on agricultural and horticultural crops. But the information regarding its application in perennials is scanty. However it is AM fungi known to increase the biomass production on many forest trees. Very little or no information is available on plant growth promoting rhizobacteria and *Pseudomonas striata* on growth of *Anogeissus latifolia.* Therefore greenhouse experiments were carried out to find out the influence of GPRB, AMF and *Pseudomonas striata.* The role of tripartite association between these organisms in seedling growth, biomass production and N, P and K uptake in shoots is investigated in this paper.

Materials and Methods

The experiments were conducted in earthen pots under glass house using sterile red sandy loamy soil to understand the effect of biofertilizers on the growth and yield of *Annougeissus latifolia* plants. 5 × 25 Seeds of plant were collected from 15 year old tree. The experimental soil sand loam: pure sand (1:1) was sterilized with 5% methyl bromide and filled in 15 × 15 cm pots and its physico-chemical characteristics were analysed as outlined by Jackson (1973), shown in Table 1.

The inoculum consists of 3g root bits plus 12g rhizospheric soil of host plant with hyphae and sporocarps (114 Chalmydospore/50g soil approximately). Growth promoting rhizopbacteria and *Pseudomona striata* 1×10^9 was isolated from the rhizosphere of *A. latifolia* and applied at

Table 1
Physico-Chemical properties of soil used for pot experiments.

Soil Characteristics	*Values*
Soil type	Sandy loamy
Soil moisture	27.03
P^{H}	6.70
Electric conductivity (mmhos/cm at 25°C)	0.57
Organic carbon	0.27
Available Phosphorus (%)	0.14
Available Potassium (%)	22.71
Available Nitrogen (%)	17.92
Iron (%)	14.18
Zinc (%)	4.92
Copper (%)	2.59
Magnesium (%)	2.79
Molybdenum (%)	0.02

Table 2
Effect of growth promoting rhizobacteria, AMF and *Pseudomona striata* on plant height, root length, dry weight of shoot and root in plants for 90-days.

Treatment	*Plant height (cm)*	*Root length (cm)*	*Shoot dry weight (g)*	*Root dry weight (g)*
Uninoculated (Control)	11.3	18.5	3.9	1.3
GPRB	19.7	20.0	8.7	2.4
AMF (*G. fasciculatum*)	25.1	31.2	11.2	3.6
Pseudomonas striata	17.1	39.4	8.9	3.1
GPRB + AMF	34.5	47.2	14.3	4.5
AMF + *Pseudomonas striata*	41.5	55.2	22.5	7.1
GPRB + AMF + *Pseudomonas striata*	52.2	63.1	34.0	8.2
L.S.D. at 0.05%	15.00	09.10	3.11	0.07

the rate of 10g inoculants (1×10^9 X cells/g lignite) before sowing the seeds. The plants were harvested intervals of 90 and 180 days (days after sowing) and the parameters like plant height, dry weight of shoot and "P" uptake were recorded. The spore count was carried out by wet sieving and decanting method (Gerdemann and Nicoloson, 1963). The percent of colonisation was determined according to Phillips and Hayman (1970). The percentage of colonisation was calculated by the following formula.

$$\text{Percentage of root colonisation} = \frac{\text{No. of root bits shows colonisation}}{\text{Total number of root bits observed}} \times 100$$

The plant height was measured from ground level to tip of the plant and expressed in centimetres. The uptake of 'P' in shoots was determined according to Jackson, 1973. For each harvest experimental plant shoot and root were oven dried at 70°C until a constant weight was obtained to determine the dry weight and was expressed in terms of grams and also mycorrhizal efficiency was calculated based on the total dry weight of the plant using the following formula (Mohan Singh and Tilak 1990; Plenchette *et al.*, 1983).

$$\text{Mycorrhizal efficiency} = 100 \times 1 \frac{\text{Non-mycorrhizal plant height}}{\text{(Mycorrhizal plant height)}}$$

Results and Discussion

Many experiments to raise fuel plantation due to high mortality and poor establishment. Healthy and quality of seedlings, through difficult to grow are a pre requite to the successful establishment of hard wood plants with exception of a few species *Anogeissus latifolia* a deciduous tree with AMF, growth promoting rhizobacteria and *Pseudomonas striata* not fully recorded. In the tropics, where phosphorus fertilizers expensive and phosphorus solubilising bacteria in need of the hour. AM fungi growth promoting rhizobacteria can play an important role in tree productivity. It was observed that significance differences were recorded for plant height, root length, shoot dry weight and root dry weight between 90 to 180 days intervals (Tables 2 and 3) in *Anogeissus latifolia*. VA mycorrhizal colonisation in rhizospheric soil was varied with time depending on host root growth and N, P and K uptake in (Tables 4 and 5). Growth promoting rhizobacteria significantly influenced on growth of roots but do not influence on root colonisation. Per cent root colonisation, average number of vesicles/cm of root bits increased with increase in days from 90 to 180, when plants inoculated with AMF plus *Pseudomonas striata* (68.4%) or AMF + GPRB (76.2%) respectively. These findings are in consistent with early workers contribution (Lakshman, H.C. 1999; Bhowmik, S.N. and Sing, C.S. 2004). Dual Inoculation brought a significant inmcrease in increased plant height and biomass production with AMF + *Pseudomonas striata* over the control plants. The uptake N, P and K by the plants was higher in dual inoculated plants and was maximum in triple inoculation. A similar results were obtained by (Rangarajan, M. and Shantakrishnana P. 1995; Singh C.S. 2005). The increased biomass of *Anogeissus latifolia* must have been result of enhanced photosynthesis and transpiration (Lakshman, H.C. *et al.*, 2003) coupled with effective synergism of all the three microbial

Table 3.
Effect of growth promoting rhizobacteria, AMF and *Pseudomonas striata* on plant height, root length, dry weight of shoot and root in plants *Annougeissus latifolia* after 180-days.

Treatment	*Plant height (cm)*	*Root length (cm)*	*Shoot dry weight (g)*	*Root dry weight (g)*
Uninoculated (Control)	19.7	24.3	6.4	2.9
GPRB	27.9	32.5	13.5	5.2
AMF (*G. fasciculatum*)	33.4	44.2	15.3	6.8
Pseudomonas striata	28.3	51.6	13.8	5.7
GPRB + AMF	49.5	66.6	26.1	9.9
AMF + *Pseudomonas striata*	58.2	72.2	31.9	11.2
GPRB + AMF + *Pseudomonas striata*	86.7	84.7	46.4	12.1
L.S.D. at 0.05%	27.10	11.05	4.11	1.06

Table 4
Effect of growth promoting rhizobacteria, AMF and *Pseudomonas striata* on plant height, root colonisation, N, P and K uptake in shoots of *Annougeissus latifolia* after 90-days.

Treatment	*% of VAM colonisation*	*N uptake mg/plant (mg)*	*P uptake mg/plant (mg)*	*K uptake mg/plant (mg)*
Uninoculated (Control)	–	8.3	0.12	0.58
GPRB	–	10.2	0.19	0.72
AMF (*G. fasciculatum*)	46.2	10.6	0.42	0.85
Pseudomona striata	9.9	11.4	0.98	0.99
GPRB + AMF	48.5	19.3	1.10	1.06
AMF + *Pseudomonas striata*	47.3	21.6	1.11	1.12
GPRB + AMF + *Pseudomonas striata*	53.4	29.1	1.25	1.18
L.S.D. at 0.05%	11.5	09.02	3.00	4.07

inoculants. Plants inoculated with GPRB + AMF + *P. striata* produced increase root length biomass production. This observation has been attributed to the collective effect of both organisms. However, the effect was more pronounced in the presense of three tire epeated organisms (AMF + GPRB + *P. striata*). The effective synergism of all the three microbial inoculants. Plants inoculated with GPRB + AMF + *P. striata* produced increase root length, biomass production.

This study clearly brings out tripartite inoculation of GPRB + AMF + *P. striata* in *Anogeissus*

Table 5
Effect of growth promoting rhizobacteria, AMF and *Pseudomonas striata* on plant percentage, root colonisation, N, P and K uptake in shoots of *Annougeissus latifolia* after 180-days.

Treatment	*% of VAM colonisation*	*N uptake mg/plant (mg)*	*P uptake mg/plant (mg)*	*K uptake mg/plant (mg)*
Uninoculated (Control)	–	10.2	0.17	0.70
GPRB	–	12.5	0.28	1.11
AMF (*G. fasciculatum*)	52.5	12.8	0.69	1.16
Pseudomonas striata	14.8	13.4	1.10	1.10
GPRB + AMF	66.1	21.2	1.24	1.98
AMF + *Pseudomona striata*	68.4	29.3	2.12	2.08
GPRB + AMF + *Pseudomonas striata*	76.2	37.5	2.56	2.36
L.S.D. at 0.05%	19.11	10.03	4.01	5.10

latifolia is better and obtain plant height, shoot and root weight, percentage of AMF root colonization and N, P, K uptake. Therefore all the three inoculants found to be superior to dual or single inoculants or controls.

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Author

H.C. Lakshman and Laxman B. Kadam
P.G. Department. of Botany (Microbiology Lab.) Karnatak University,
Dharwad-580 003, India.

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Use of Press Mud for the Production of Vermicompost

Introduction

Composting is a natural process whereby organic material, such as kitchen scraps & yard waste decomposes into a dark nutrient rich soil amendment called humus. Vermicomposting or composting with worms, is an excellent technique for recycling food waste in the apartment as well as composting yard wastes in the backyard.

The use of worms speeds up the process of decomposition to produce a richer end product. To produce proper compost ordinary field worms are not used. Instead, the red wrigglers; *Lumbricus rubellus* or brandling worms: *Eisenia foetida* which commonly live in barnyard manure piles and feed on fresh organic material are used. Field worms are better at digesting things that are already well decomposed and are not likely to survive in a worm bin on a diet of kitchen scraps. Redworms or brandling worms, however, prefer the compost or manure environment. Passing through the gut of the earthworm, recycled organic wastes are excreted as castings, or worm manure, an organic material rich in nutrients that looks like fine-textured soil. The location and construction of worm bins, bedding materials, adding food waste and controlling temperature, moisture in the bins are the important steps during vermicomposting.

Varieties of fertilizers are used from ancient times to present days which include organic compost, chemical fertilizers, biofertilizers, vermicompost etc. The continuous and overuse of chemical fertilizers resulted into highly alkaline and deadly soils. Due to this the crops yields are decreasing. Vermicompost is one of the best alternative to these fertilizers. At Warananagar, press mud, waste of sugar factory is used as a major bedding material for vermicomposting. Decomposition of it is carried out for a particular time and the humus is obtained to which the red earthworms are added to yield vermicompost.

During the process it is important to maintain the moisture and optimum temperature in the beds for the proper growth of worms and to avoid fowl odours. It is also important to allow air to circulate through the bin by leaving the air holes uncovered. The red wrigglers in the bins can tolerate a wide range of temperatures, but they should not freeze or get too hot. The worms will survive in temperatures from 5°C to 32°C but prefer room temperature. Vermicompost contains not only worm castings, but also bedding materials, organic wastes at various stages of decomposition, worms at various stages of development and other microorganisms associated with the composting processing. Earthworm castings in the home garden often contain 5 to 11 times more nitrogen, phosphorus and potassium as the surrounding soil. Secretions in the intestinal tracts of earthworms, along with soil passing through the earthworms, make nutrients more concentrated and available for plant uptake. This compost also contains variety of micronutrients as well as macronutrients with various N_2 fixing and phosphate solubilising bacteria. It has various advantages over chemical fertilizers. Nutrients in vermicompost are often much higher than traditional garden compost. Finished vermicompost should have a rich, earthy smell if properly processed by worms. It can be used in potting for house plants and top dressing for lawns. With this it can also be used for various field crops in various concentrations.

The use of pres mud in vermicomposting gives significant vermicompost yield. In the field application, it shows best result as compared to traditional fertilizers without affecting soil texture and environment.

Material and Methods

Construction of worm bin

Bins are made of iron of size 5 × 2 ft. As red wrigglers are surface feeders, bins are made with not more than 2 ft depth. Bins can be made of plastic or wood, or from recycled containers like old bathtubs, barrels, or trunks. Wooden bins have the advantage that they are more absorbent and provide better insulation. Each bin has a cover to conserve moisture and exclude light as worms prefer darkness. Bins are also provided with good air ventilation capacity.

Bedding material

Bedding material is allowed to set for several days to avoid heating up and allowed to cool before adding worms. The bedding material is thoroughly moistened before adding to worms. The bedding material includes.

1. Press mud
2. Dung
3. Garden leaves (Sugarcane dried leaves)
4. Earthworms of type *Eisenia foetida.*

The layers of garden leaves, press mud and dung are prepared by taking these materials in

various concentrations as 20%, 40% and 40% respectively. The decomposition of it is allowed for 30-45 days.

Adding the worms

The bins are filled with three quarters of bedding materials. The decomposed material (humus) is then taken into the racks to which earthworms of type *Eisenia foetida* are added. For three tones of humus 2-2.5 kg of earthworms are added. The bedding is kept continuously moist and gently lifted afterwards to create air space for the worms to breathe and to control odous. Water is sprinkled to maintain moisture (upto 70%) in the beds for 30 days.

The addition of chemicals, metals, plastics, glass, soaps, pet manures, dairy products, bones, meat etc. is avoided.

Harvesting

To obtain fine and granular vermicompost, the mixture is screened to remove the stones, gravels and other non-decomposed materials. The earthworms are also separated from the finished compost by using hopper. The worms can be added back to a new bin of bedding and food waste. The vermicompost is packed without drying and waste is recycled.

Results and Discussion

The waste of sugar factory *i.e.*, press mud from nearby area is used as a principal bedding material and with this the decomposed waste yields about 2-2.5 tones of vermicompost. Due to its nutrient rich characteristics vermicompost has various advantages over other fertilizers.

Characteristics of vermicompost

1. Vermicompost is the casting of completely decomposed waste (humus).
2. It is dark black, light with pleasant odour, granular and 2-4 times heavier than cow dung.
3. Because of the granular size (2-3 μ) it gets easily utilised by the crops.
4. It has good capacity to retain the water.

Advantages of vermicompost

1. Vermicompost reduces the use of chemical fertilizers.
2. Fertility of the soil increases due to the presence of N, P, K and other micronutrients in the vermicompost.
3. The use of vermicompost increases the quality & shelf life of fruits.
4. The pH of soil is maintained at neutral.
5. Vermicompost results in increase in number of N_2 fixing and phosphate solubilizing bacteria in the soil due to which helps in improving soil fertility.

By this study we conclude that vermicompost is a good source of fertilizers for all crops. In Warananagar it is mainly used for sugarcane, wheat, groundnut, and maize to increase the yield and resistant power of crops.

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Author

S.A. Patil and G.D. Saratale

Department of Biotechnology Engineering,
Tatyasaheb Kore Institute of Engineering and Technology,
Warananagar. Tal: Panhala, Dist: Kolhapur-416 113 (M.S.) India.