POPULATION DYNAMICS OF PLANT GROWTH PROMOTING RHIZOBACTERIA UNDER THE INFLUENCE OF AGRICULTURAL CHEMICALS

By

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THESIS

submitted in partial fulfillment of the
requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

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COLLEGE OF HORTICULTURE

KERALA AGRICULTURAL UNIVERSITY

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KERALA, INDIA

2007

DECLARATION

I, Beethi Balachandran, (2003-11-18) hereby declare that this thesis entitled **'Population dynamics of plant growth promoting rhizobacteria under the influence of agricultural chemicals'** is a bonafide record of research work done by me during the course of research and this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **'Population dynamics of plant growth promoting rhizobacteria under the influence of agricultural chemicals'** is a record of research work done independently by **Mrs Beethi Balachandran** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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AKNOWLEDGEMENT

Would it be all envisaging to offer salutation at the feet of God, who imbued the energy and enthusiasm through ramifying paths of thicks and thins of my efforts.

. It is with great respect and devotion, I place on record my deep sense of gratitude and indebtedness to my major advisor ***Dr. M.V Rajendran PIllai****, Professor and chairperson of my advisory committee, for his valuable guidance, keen interest, ever willing help and creative suggestions and above all the wholehearted support, extreme patience, understanding and constant encouragement rendered throughout the period of investigation and preparation of thesis.*

I place a deep sense of obligation to ***Dr. Koshy Abraham****, Professor and Head, Department of Plant Pathology and member of my advisory committee for his creative suggestions, for his unbounded support, valuable suggestions and whole- hearted co-operation towards the satisfactory fulfillment of this endeavor. The critical evaluations during the preparation of the thesis and for providing facilities throughout the course of these investigations are also thankfully remembered.*

I express my sincere thanks to ***Dr. Surendra Gopal , K****, Associate professor , Department of Plant Pathology and member of my advisory committee for his suggestions received during the course of this study and preparation of manuscript.*

With profound respects, I place my thanks to ***Dr. Mani Chellappan****, Assistant professor(SS), Department of Entomology, College of Horticulture, and member of my advisory committee for his kind and valuable suggestions rendered during the preparation of manuscript.*

My sincere thanks to the teachers of the Department of Plant Pathology Dr. S. Beena, Dr. Sheela Paul, Dr. Sally .K, Mathew and Mrs.Sainamol P. Kurien for their encouragement, kind help and moral support offered at different stages of the study.

I am highly thankful to Sri. S. Krishnan, Department of Agricultural Statistics for his wholehearted cooperation and immense help extended for the statistical analysis of the data.

I feel overwhelmed and strongly beholden to my friends especially Mrs. Dhanya for her timely help and mental support during the lab and field works. I have no words to express my deep sense gratitude to my dearest and intimate friends Resmi, A.R., Remya, Jyothi ,Jeli, Resmi chechi and Smitha chechi for their moral support and encouragement throughout the course of my research work.

I would like to express my sincere gratitude to my juniors Nisha, Sameera and Sai sree for providing the much needed shoulders in times of need.

I am grateful to Santhosh chettan of student's computer club for his gracious help rendered during the preparation of this manuscript.

I am greatly indebted to the staff members and labourers of the Department of Plant Pathology for their help rendered during the course of investigation.

The junior research fellowship awarded by the KAU is gratefully acknowledged.

On a personal note, no words would sustain my heartfelt thanks to my loving ***Amma*** *and* ***Achan*** *who only inspired me to overcome the innumerable odds, I faced during the course of this work.*

I feel inadequacy of diction to express my eternal gratitude to my brother for his mental support and encouragement through out my academic career.

I owe all my success to my husband Mr. ***Bipin*** *and my son* ***Shravan*** *, who shared my long journey of research step by step and showered on me their love and affection without whose sacrifice the thesis work , would have never come to its final shape.*

I submit my respectful thanks to my in laws and my cousins for their warm blessings.

(Beethi Balachandran)

Dedicated to

My beloved family

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Introduction

Review of literature

Materials and methods

Results

Discussion

Summary

References

Appendices

1. INTRODUCTION

A lot of bacteria exist in soil which has the ability to improve plant growth by the production of growth regulators (Kloepper and Schroth, 1981), such as gibberellins, cytokinins and indole acetic acid (Suslow and Schroth, 1982). They are commonly called as Plant Growth Promoting Rhizobacteria (PGPR). Such PGPR were originally defined as root colonizing bacteria (rhizobacteria) that cause either plant growth promotion or control plant diseases by biological processes (Kloepper and Schroth, 1981). Thus, PGPR plays a major role in the modern ecofriendly agriculture.

A number of PGPR are commercially available now. Some major ones are *Azospirillum* sp.*, P. fluorescens* and *B. subtilis*. Among them, *Azospirillum* sp. is an associative microaerophilic nitrogen fixing bacteria found in association with several grasses, cereals and millets. Apart from nitrogen fixing ability, they are known to produce growth promoting substance like indole acetic acid which is found to enhance the growth of plants.

Pseudomonas fluorescens is another PGPR which benefits plants through various mechanisms. They produce many secondary metabolites and iron chelating siderophores. *P. fluorescens* is also found to antagonise soil borne root pathogens, solubilize phosphate and induce disease resistance. *Bacillus subtilis* is also a potential plant growth promoting rhizobacteria used in agriculture as a bioinoculant. Use of PGPR is found to increase the crop yield up to about 160 percent.

In modern agriculture, biofertilizers and biopesticides attract the attention of scientists, extension workers and farmers to a great extent due to increasing concern over environmental and health hazards by the extensive use of agricultural chemicals. Now a days, majority of the farmers are using fungicides and insecticides separately or even together for the management of pests and disease. It is also a general belief that biofertilizers and biopesticides which are components of PGPR should not be mixed together with any agricultural chemicals. Often farmers are also

advised in this line. In fact, sufficient data on the compatibility aspects of PGPR and agricultural chemicals is lacking. In this situation, such a practice will definitely limit the use of beneficial microorganisms in crops where chemical pesticides are frequently applied. Hence, it is felt necessary to study the compatibility aspects of beneficial microorganisms with commonly used agricultural chemicals. With this in background, the present study was conducted on following aspects.

1. Isolation, procurement purification and authentication of *Azospirillum* sp., *Pseudomonas* sp. and *Bacillus* sp.
2. *In vitro* study on the growth of these bacteria under exposure to selected agricultural chemicals.
3. Survival of the organisms in soil exposed to agricultural chemicals.
4. Toxicity of relatively safe chemicals at higher doses.
5. Pot culture experiment to find the effect of the selected agricultural chemicals on the population of PGPR in the rhizosphere of rice.
6. Preparation of compatibility chart of PGPR and agricultural chemicals.

2. REVIEW OF LITERATURE

The effect of plant protection chemicals on beneficial microorganisms is a subject of practical importance and scientific interest. Substantial research has been carried out on this aspect over the past so many years.

Agricultural practices are known to influence the soil microflora in various ways. The effects of chemical and fertilizer application on soil and rhizosphere microorganisms were studied as early as 1934 by Eggleton as cited by Bagyaraj and Rangaswami (1967). According to them, the fertilizer Ammonium sulphate increased bacterial and fungal population whereas Super phospate increased actinomycetes. They also found that Murate of potash (MOP) had little effect on soil microbial population. In an experiment conducted by Srivastava *et al*. (1968), the Gram positive *B. subtilis* had been inhibited by tri aryl tin compounds. The effect of insecticides on the activities of soil microorganisms were known as early as 1954 by Fletcher and Bollen, as cited by Bardiya and Gaur (1968). They found that significant reduction in CO_2 evolution in soil was occurred in the presence of lindane and dieldrin and it was due to the toxicity of these insecticides towards soil microorganisms involved in organic matter decomposition.

2.1 EFFECT OF PLANT PROTECTION CHEMICALS ON *Azospirillum* sp.

Vlassak and Livens (1975) reported that the pesticides phosmidipham and oxamyl affected the mineralization of nitrogen in soil. These chemicals also inhibited the nitrification process in soil. Oxamyl decreased the rate of denitrification and both the chemicals had a harmful effect on nitrogenase activity. In another study, Vlassak *et al.* (1976) reported that nitrogen fixation in soil was highly sensitive to the pesticide 4, 6-dinitro-o-sec butylphenol (DNBP).

Mahapatra and Rao (1981) reported that hexachlorocyclohexane treated soil had significantly higher nitrogenase activity than that in untreated soils. They found that the populations of nitrogen fixing *Azospirillum* sp. were stimulated in hexachlrocyclohexane (HCH) treated soils.

In a study conducted by Alvarez and Sleiman (1983) on the effect of ten herbicides and three insecticides on *A. lipoferum* and *A. brasilense* in pure culture, it was found that none of the pesticides affected the growth rate of any of the bacterial species. Atrazine and linuron increased the rate of acetylene reduction in *A. lipoferum* but had no effect on total nitrogenase activity. Effect of the herbicide Stomp (pendimethalin) on the nitrogenase activity of *A. lipoferum* was studied by Gadkari (1987). He found that cultural conditions had a correlation with the nitrogense activity. Increase in the production of indole acetic acid was noticed upon the application of carbofuran to *Azospirillum* sp. isolates obtained from rice roots as reported by Jena *et al.* (1987).

Influence of the herbicides metamitron, metribuzin, ethiozin and paraquat on *A. lipoferum* and *A. brasilense* was studied by Gadkari (1988). He found that metanitron and ethiozin did not affect the nitrogenase activity of these bacteria. A decrease in nitrogenase activity was observed with metribuzin and ethiozin. Effect of the seed dressing fungicide captan on *Azospirillum* sp. was reported by Govindarajan and Purushothaman (1988). They reported that the fungicide treatment before inoculation of the bacteria had little effect but treatment after inoculation resulted in a decrease in *A. brasilense* population on the seeds.

Under non-nitrogen fixing conditions, an organochlorine insecticide dicofol inhibited the population of *A. lipoferum* totally at 250 and 500 ppm concentration as reported by Mano *et al*. (1988). Under nitrogen fixing conditions, increasing dicofol concentration up to 1000 ppm was found to inhibit nitrogenase activity. The interaction of four nematicides (terbufos, carbofuran, fenamiphos and aldicarb) with *A. lipoferum* was studied by Fayez (1989). Among the nematicides used, carbofuran and aldicarb inhibited the nitrogenase activity on plant roots more seriously than fenamiphos and terbufos. Soil, irrespective of treatment, regained a part of its normal nitrogenase activity as time passed. Field concentrations of all nematicides showed different inhibitory effects on nitrogenase activity of *Azospirillum* spp. in culture

medium and such effects were increased with increased doses (10 and 100 fold) and incubation periods (10 days).

In a report by Rangaswami *et al.* (1989), it was found that concentration of monocrotophos and quinalphos upto 5 kg/ha was either stimulatory or innocuous to *Azospirillum* sp. Cultures of *Azospirillum* sp. isolated from insecticide treated soils exhibited greater nitrogen fixing activity. In a detailed study, Gallori *et al. (*1992) reported that fungicides captan (0.5-2 mg/ml) and thiram (3-7 mg/ml) caused reduction in growth rate and nitrogenase activity of *A. brasilense.* Omar and Abd-Alla (1992) reported that pesticides brominal, cuprosan and fenvalerate at 10 and 50 ppm suppressed the growth and nitrogenase activity of *Azotobacter chroococcum, Azospirillum brasilense* and *Azospirillum lipoferum*.

Inhibition of the growth of *A. brasilense* was occurred with the dithiocarbamate fungicides thiram and mancozeb at lower than 10 mg/l concentrations as reported by Ciocco *et al*. (1997). They also reported blockage of nitrogenase activity of cultures after 48 h of growth. But in growth chambers, there was no effect for *A. brasilense* inoculation and thiram or mancozeb on shoot dry weight of *Setaria italica.* However, captan significantly increased the root dry weight.

Response of *A. brasilense* to the pesticides bromopropylate and methidathion was reported by Gomez *et al.* (1998). They reported that bromopropylate at 10, 50, 100, 200 and 300 mg/ml did not affect the microbial growth, the levels of ATP and dinitrogen fixation in chemically defined media and dialysed soil media. methidathion significantly reduced the dinitrogen fixation, the levels of ATP and growth in chemically defined media. The negative effects of this insecticide were not significant in dialysed soil media showing that *A. brasilense* could tolerate high concentrations (300 mg/ml) of methidathion.

Gomez *et al.* (1999) investigated the influence of 10, 50,100, 200 and 300 mg/ml concentration of profenofos and diazinon on *A. brasizense.* Results showed that diazinon did not affect the microbial growth, concentration of ATP, dinitrogen fixation and production of vitamins. However, profenofos significantly reduced the

dinitrogen fixation, intercellular concentration of ATP, production of vitamins and growth of bacterial cells grown in chemically defined medium.

Studies on the effect of plant protection chemicals on *Azospirillum* sp. conducted by Raji and Pillai (2000) revealed that the fungicide thiram caused growth reduction of this bacterium *in vitro*. Thiram in combination with insecticides *viz.* carbofuran and phorate caused the same inhibition of bacterial growth. The rhizosphere population of *Azospirillum* sp. was also reduced in thiram treated plants.

Studies conducted by Giraud *et al.* (2001), revealed that the insecticide tebufos had a slight inhibitory effect on the growth of *A. lipoferum* in solid cultures. All the insecticide *viz.* carbofuran, chlormephos, tebufos and benfuracarb decreased the survival of *A. lipoferum* when the bacteria were inoculated directly on to the granules. Nicwiadomska and Sawicka (2002) found that the fungicides carbendazim and thiram and the herbicide imazetapir affected the nitrogenase activity of microorganisms and during the initial days of application, they prohibited the multiplication of soil microorganisms. However, subsequently the population was increased subsequently. A study by Talukdar *et al.* (2003) revealed that *A. amazonense* A-10 was more resistant to ampicillin, chloramphenicol, kanamycin, streptomycin and gentamycin at concentrations of 100-300ppm, whereas the *A. brasilense* strains were found to be sensitive at relatively lower concentration of these antibiotics.

Growth of *Azospirillum* sp. strain OAD-2 in Luria agar plates was found to be inhibited in descending order by the insecticides chlorpyriphos, fenvalerate, quinalphos, monocrotophos and endosulfan. This was reported by Ravi *et al*. (2004). The inhibition zone of *Azospirillum* sp. strain OAD-2 ranged from 2.6 to 13.2 mm and 5 to 20 mm at recommended dose (2ml/l) and at double recommended dose of insecticides respectively. Thakuria *et al.* (2004) reported that among the tested *Azospirillum* isolates, the strain A-10 showed a higher level of resistance to all the tested antibiotics *viz*. ampicillin, chloramphenicol, kanamycin, gentamycin, rifampicin and streptomycin.

In an experiment conducted by Prast (2006), it was found that all herbicides and insecticides *viz.* glyphosate, nonanomic acid and dichlorprop-p and insecticides *viz*. potassium oil, malation and pyrethrin influenced nitrification.

2.2 EFFECT OF PLANT PROTECTION CHEMICALS ON *Pseudomonas fluorescens*

A study by Fuchs and Vries (1978), using different soil and water samples as inoculum and the benzimidazole fungicides, benomyl and thiabendazole as selective agents, a large number of fluorescent *Pseudomonas* strains were isolated. These were able to grow in a minimal medium with benomyl as the sole source of carbon. However, no growth was occurred with any of other benzimidazole compounds, *viz.* benzimidazole, 2-aminobenzimidazole, thiabendazole and fuberidazole. In a study to determine the effects of dicampa, dinitramine and trifluralin on strains of rhizobacteria, Gupta *et al*. (1996) found that these herbicides were inhibitory to the growth of *P. fluorescens.*

In a study Elkins and Lindow (1999), it was found that the population of *P. fluorescens* strain A 506 was reduced to 50 per cent when mixed with terramycin. The fungicide mancozeb was also found to reduce the population when mixed, even more so than terramycin. However, terramycin and mancozeb had no detrimental effect on *P. fluorescens* strain A 506 when applied at least five days before or after *P. fluorescens* strain A 506 application.

A positive regulation of *P. fluorescens* by carbendazim was reported by Guang *et al. (*1999). His studies revealed that *P. fluorescens* stain P_{32} was not sensitive to carbendazim *in vitro.* But when applied to soil, carbendazim enhanced the population of P_{32} in the soil and rhizosphere by 11.6 and 12.8 times respectively. Carbendazim was also found to enhance the population of native fluorescent pseudomonads. A study by Kaszubiak and Durska (2000) revealed that in non-rhizosphere soil, the application of seed dressing fungicide oxafun did not change the bacterial population. However, it contributed to the proliferation of these

microorganisms in the rhizospehere zone. Laila *et al*. (2000) reported that the fungicide fenpropimorph disturbed the population of *Pseudomonas* sp. in the rhizosphere of barley. A reduced doze of metalaxyl and copper oxychloride in combination with *P. fluorescens* effectively reduced damping off and root rot in tomato. These two fungicides were found compatible with *P. fluorescens* (Sabet *et al*., 2000).

Laha and Venkatraman (2001) reported that *P. fluorescens* strain Pf.9 isolated from rice field was found to be compatible with carbendazim at 500 and 1000 ppm concentrations. In another study, Laila *et al.* (2001) reported that the fungicide imazalil affected *P. fluorescens* population positively.

Influence of thiram on *P. fluorescens* was studied by Singh *et al*. (2003). They found that the *P. fluorescens* strain in combination with thiram gave significant reduction in disease incidence. A study by Thankamani *et al.* (2003) revealed that the combined use of VAM and *P. fluorescens* strain IISR6 along with phorate and copper oxychloride resulted in better establishment and growth of black pepper cuttings.

Mathew (2003) reported that *P. fluorescens* strain P_{11} was found to be compatible with mancozeb, carbendazim, imidacloprid, etofenprox, chlorpyriphos and triazophos at the recommended doses for field use.

Joseph *et al.* (2003) reported that the *P. fluorescens* strain PSi was compatible with hexaconazole and mancozeb and hence suitable for combined application. He also found that the interaction of *P. fluorescens* culture with mancozeb did not inhibit the antagonist even at the highest concentration of the fungicide.

In another experiment, Anandaraj and Sarma (2003) found that combined application of *P. fluorescens* isolate with metalaxyl increased the rejuvenating capacity of the *P. fluorescens* isolate on pepper cuttings infected with *P. capsici.* The percent rejuvenation of the infected cuttings increased when the bacteria was treated with metalaxyl.

Thakuria *et al*. (2004) reported an exceptionally higher resistance towards the antibiotics ampicillin and chloramphenicol by fluorescent bacterial isolates psd5 and psd6.

Bhavani (2004) found that potassium phosphonate and the lowest concentration of kocide did not produce any inhibition zone in *P. fluorescens* while Bordeaux mixture and copper oxychloride at all concentrations inhibited the growth of the bacterium. Study by Priya (2005) revealed that Bordeaux mixture of all concentrations completely inhibited the growth of *P. fluorescens*, while copper hydroxide and copper oxychloride at different concentrations showed varying percentage of inhibition. It was noticed that as the concentration of fungicides increased, there was an increased inhibition of *P. fluorescens.* In this study, copper hydroxide was found to be less inhibitory against *p. fluorescens* compared to copper oxychloride and Bordeaux mixture.

Diby and Sarma (2006) reported that the combined application of *P. fluorescens* strains with metalaxyl + mancozeb resulted in 100 per cent survival of *Phytophthora capsici* affected pepper plants and the combination showed an additive effect.

2.3 EFFECT OF PLANT PROTECTION CHEMICALS ON *Bacillus subtilis*

Toxicty of benzimidazole compounds on *Bacillus* spp. was reported by Fuchs and Vries (1978). They found that benzimidazole compounds were inhibitory at concentrations of 500 to 1000 g/ml, with toxicity increasing in the order when *Bacillus* spp. was grown in liquid peptone media.

A study by Kim (1988) revealed that metalaxyl had no effect on *Bacillus* strain AC-1. But when it was combined with copper fungicides, the growth was inhibited. *B. subtilis* was reported compatible with metalaxyl and the combined application at half the recommended dose was the most effective method for reducing seed and root rot in field pea (Hwang *et al*., 1996).

Kondoh *et al.* (2000) reported the effect of combined use of *B. subtlis* strain RB-14-C and flutolanil. They reported an increase in the bacterial population when flutolanil was used @ 375 mg/pot than when flutolanil was used alone. This showed the increased activity of *B. subtilis* and flutolanil when these are used in combination. A lower dose of metalaxyl and copper oxychloride in combination with *B. subtilis* effectively reduced the damping off and root rot disease in tomato as reported by Sabet *et al.* (2000).

Kalam and Mukherjee (2001) in an experiment found that the total microbial count in the soil was highly affected in presence of hexaconazole. This toxicity was persisted up to 21 days. Carbofuran and ethion were found moderately toxic to soil microflora. They also reported that hexaconazole strongly affected the intact cells of *B. subtilis* while, carbofuran inhibited the enzyme activity in *B. subtilis.*

The result of an experiment conducted by Laha and Venkataraman (2001) showed that *Bacillus* spp. B-44 was compatible with carbendazim (Bavistin) at 500 and 1000 ppm concentrations. In an experiment conducted by Grabinska *et al*. (2002), it was found that two *B. subtilis* strains M45 rec^- and 17 rec^+ degraded the herbicides 2, 4-D (Aminopielik 720) and MCPA (Chwastox Extra).

Guven *et al.* (2003) reported that the alpha-amylase test system in *B. subtilis* was inhibited by the organo metallic fungicides, maneb and mancozeb even at 0.1 ppm. They also reported that the organic insecticide endosulfan also affected the alpha-amylase enzyme. In an experiment Luz (2003), found that bioformulation of *B. subtilis* when used in combination with the fungicides, triadimenol, iprodione, thiram, difenoconazole and fluquinconazole+procloraz were significantly improved the plant stand. He also reported that the positive effects were greater when the bacteria and chemicals were mixed.

Adeleye *et al.* (2004) studied the effect of the herbicides agroxone, atramex 50 SC and 2, 4-Damine on *B. subtilis* and the results revealed that 2,4-Damine was the most toxic among the three herbicides. An initial reduction in population, followed by increased percentage survival of *B. subtilis* was observed. According to Swarnali *et al*. (2004) the plant protection chemicals monocrotophos, imidacloprid and

carbaryl were compatible with *B. subtilis*. Thakuria *et al*. (2004) found that phosphate solubilising *Bacillus* spp. was highly sensitive to antibiotics. Van Eeden and Korsten (2004) found that the combination of *B. subtilis* and copper oxychloride had a negative effect on the survival of the organism, while carbendazim had no effect.

2.4 EFFECT OF PLANT PROTECTION CHEMICALS ON OTHER BENEFICIAL MICROORGANISMS

A study by Viera and Pagel (1978) revealed that *Beijerinckia* and *Azotobacter* were stimulated up to 7 days, partly also up to 15 days, after application of the triazins. They also reported that atrazin exhibits a stronger and more lasting effect than simazin, and *Azotobacte*r were influenced more strongly than *Beijerinckia.*

Trichoderma spp. was found to have good growth at a lower and medium concentration of capron and no growth with systemic fungicides carbendazim and benomyl (Ortiz *et al.,* 1966). Sinha *et al*. (1979) reported that higher concentrations of vapam appreciably reduced the population of bacteria and actinomycetes, though later on, their population gradually increased. They also found that the numbers of *Azotobacter* in soil amended with 125, 250 and 500 ppm of vapam did not alter the population appreciably. But their population in treated soils increased over the check on the 45th day. All concentrations of vapam were detrimental to *Rhizobia.* But vapam at 125 to 500 ppm stimulated the ammonification process.

Sullia and Anusuya (1987) reported that the fungicides; agallol, blitane, blitox-50, captan, ceresan, dithane M-45, triforine and ziram were inhibitory to *Rhizobium* at 100mg/ml. They also found that fungicides containing mercury, copper and zinc were more toxic to *Rhizobium.* Bavistin, brassicol, difoltan, thiophanate methyl and vitavax were not inhibitory to *Rhizobium.* In a study by Fabra *et al*. (1998), it was found that fungicide mancozeb reduced the growth rate of *Bradyrhizobium* sp. strain USDA 3187 to 50 percent and also affected the symbiotic interaction. They also reported that mancozeb produced biochemical alterations in membrane composition, polysaccharides and polyamines.

From the experiments conducted to study the effect of amitstar (Azoxystrobin), on the growth of beneficial microbes, Osman *et al*. (1999) found that there was no effect for this fungicide on beneficial microorganism. Seed treatment with the pesticides fenpiclonil, tiramet, terrafung and imidacloprid with strains of *R. japonicum* resulted in an increase in yield of soyabean as reported by Procopovici and Guran (2000). Arruda *et al. (*2001) reported the herbicides imazaquin (0, 0.14, 0.12, 0.24, 0.36 mg ai/g), clomazone (0, 0.4, 0.8, 1.6 and 3.2 mg ai/g) and sulfentrazone (0. 0.2, 0.4, 0.8 and 1.6 mg ai/g) drastically inhibited all the *Rhizobium* strains tested.

An evaluation of the compatibility of *Rhizobium* with the fungicides metalaxyl M and fludioxonil was done by Shetty *et al. (*2001). They found that the inoculant has a high level of compatibility and performance. The effect of six pesticides on the growth of yeasts was investigated by Elena and Renata (2003). They found that the fungicide prochloraz and the insecticide trizamate inhibited the growth of yeast strains. In another study, the effect of the pesticide lindane on microbial populations was analyzed by Rodriguez and Toranzos (2003). They found any inhibitory effect of lindane was not observed on the metabolic versatility and genetic diversity in the soils, demonstrating the ability of the bacterial populations to tolerate the pressure caused by the addition of pesticides.

A study by Silva *et al.* (2004) revealed that combined application of rhizobacteria and chemical chlorothalonyl treatments in the field reduced fungicidal spraying frequency while it increased crop yields.

Gundi *et al.* (2005) reported that the insecticides monocrotophos, quinalphos and cypermethrin significantly enhanced the proliferation of bacteria and fungi and the soil dehydrogenase activity. Antagonistic interactions of the soil microflora were more pronounced when the two insecticides (monocrotophos or quinalphos + cypermethrin) were present together in the soil at the highest level where as synergistic or additive responses occurred at lower level with the same combination of insecticides in soil.

Pandey *et al.* (2006) found that complete inhibition of *Trichoderma* spp. was occurred by tebuconazole and hexaconazole showing extremely toxic nature of the fungicides.

Lopez *et al.* (2006) reported that organochlorinated insecticides (aldrin and lindane) organophosphorus insecticides (dimethoate, methidathion and methyl parathion), herbicide atrazine and fungicide captan significantly increased the phosphatase activity of bacteria after 28 days of incubation.

2.5 GROWTH ENHANCEMENT

2.5.1 **Effect of *Azospirillum* sp.**

Azospirillum sp. is an associative symbiotic diazotrophic bacterium which fixes nitrogen to about 20 to 25 kg per hectare under ideal conditions and there by reducing 25 per cent of the nitrogen fertilizer requirement of the crops. Lakshmikumari *et al.* (1976) reported the association of diazotrophs with the rhizosphere of cereals. The first report on the occurrence of associative symbiotic diazotroph, *Azospirillum* sp. in the rhizosphere soil of coconut palm was from Dobereiner (1978).

Treatment with *Azospirillum* sp. induced better root formation. Besides, it produced phytohormones, indole acetic acid, gibberlic acid, kinetin *etc*. that help the host plants in enhancing biomass production as reported by Tien *et al.* (1979). Hades and Okon (1987) found that there was significant increase in root length, shoot and root dry weight and total leaf area in *Azospirillum* sp. inoculated tomoto plants compared to noninoculated ones. According to Amalia *et al.* (1998) the higher bacterial concentration of *A. brasilense* promoted root hair development and root hairs were produced even near the root tips of *Panicum miliaceum.*

Vasugi and Thangaraj (1997) reported that combined seed treatment with plant extracts (2.0 percent leaf extract of *Prosopis* and *Calotropis)* and *Azospirillum* sp. significantly increased the field emergence and seedling vigour in coriander

compared with untreated seeds. Kumar *et al.* (1998) found that inoculation of *Azospirillum* sp. increased nut germination in cashew. According to Murthy *et al.* (1998), the seed treatment with *Azospirillum* sp. improved the seed germination and plant growth in Amla. Bashan (1999) reported that *Azospirillum* sp. strains produce IAA and many other plant growth regulators. Gibberllins produced by *Azospirillum* sp. contributed to increased root development.

Sajindrenath *et al.* (2002) reported the biofertilizer *Azospirillum* sp. treated on seeds of okra resulted in increased seedling length with better influence on root length. Kloepper (2003) in a study found that *Azospirillum* sp. inoculation on plants resulted in greatly altered root architecture with increased overall root growth, greater production of root hairs and enhanced root area. Gopal *et al.* (2006) found that the combined inoculation of *A. lipoferum* with other plant growth promoting bacteria resulted in maximum growth, fruit, seed and alkaloid yield in Ashwagandha.

2.5.2 **Effect of *P. fluorescens***

Schippers *et al.* (1987), in a study reported that the fluorescent pseudomonad strains of PGPR when applied to crop seeds were found to improve plant growth by displacing or excluding deleterious rhizosphere microorganism.

Sidorenko *et al.* (1996) reported the effect of combined inoculation of *Pseudomonas* sp. with other growth promoting bacteria. They reported increase in the plant height, biomass and tuber yield in potato by 19.7 per cent. According to Kumar (1998), the seed bacterization of chickpea, eggplant, soyabean and tomato with *P. fluorescens* isolates showed an increased seed germination, shoot height, root length, fresh weight, dry weight and yield. Madhaiyan (1999) reported that *P. fluorescens* was effective in improving shoot and root dry weight as well as the phosphorus content in vanilla.

According to Dave and Patel (2003), glucose and galactose were the best carbon source and ammonium sulphate as best nitrogen source for phosphate solubilization by *P. fluorescens*. According to them, the phosphate solubilizing

microorganisms in soil solubilize insoluble phosphate mainly by secreting organic acids, which chelate with calcium ions in addition to lowering the pH. Thomas and Prabhu (2003) isolated a number of phosphate solubilising bacteria belonging to the genera *Pseudomonas.* They reported that inoculation of this bacterium to coconut soils increased the P content indicating that bioinoculants based on these bacteria can help to reduce phosphatic fertilizer use in coconut cultivation.

Sendhilvel *et al.*(2005) revealed that the *P. fluorescens* strain $SVPF_2$ treatment on cowpea seeds increased the germination percent and vigour index compared to untreated control. According to Diby and Sarma (2006*), P. fluorescens* strains (IISR-6, IISR-8, IISR-11, IISR-13 and IISR-51) could significantly increase the root biomass and root length in treated plants. They concluded that the enhanced growth parameters upon root bacterization could be correlated with the production of the plant growth hormones IAA and gibberllic acid by these bacterial strains and also by their phosphate solubilization potential. Gopal *et al*. (2006) reported that the *P. fluorescens* strain Aps-1 in combination with other growth promoting rhizobacteria recorded the maximum growth, fruit, seed and alkaloid yield of ashwagandha.

2.5.3 **Effect of *Bacillus subtilis***

According to Vivek *et al.* (2001) inoculation of *Bacillus* significantly increased the growth and yield attributes of potato. They reported that the increase in yield and height can be ascribed to production of hormones like indole acetic acid, gibberellins and vitamins like biotin, folic acid and B group vitamins. Experiments conducted by Sood and Sharma (2001) indicated that *Bacillus subtilis* increased the potato tuber yield from 115 to 268 quintals/hectare and this was at par with 100 per cent NPK treatment. A study by Niranjan *et al*. (2003) revealed that *B. subtilis* IN937b and other *Bacillus* spp. as fresh suspension or powdered formulation significantly enhanced the germination rate of Pearl millet seeds compared with untreated controls. Ryu *et al*. (2003) reported that extracts of the volatiles produced by *B. subtilis* strain GBO3 and *B. amytoliquefaciens* strain IN937a induced plant growth promotion at a level similar to that induced by other PGPR strains. Sunaina

and Ajay (2005) reported a large and heavily branched root system in potato plants arising from *B. subtilis* treated plants. This lead to improved uptake of water and nutrients. Gopal *et al*. (2006) found that inoculation of *Bacillus* sp. APb-1 with other growth promoting rhizobacteria recorded the maximum growth and yield in ashwagandha.

2.6 MANAGEMENT OF DISEASES BY ANTAGONISTIC RHIZOBACTERIA

Baker and Cook (1974) defined biocontrol as the reduction of inoculum density or disease producing activities of a pathogen or a parasite in its active or dormant stage by one or more organisms accomplished naturally or through manipulation of the environment, host or antagonist or by mass introduction of one or more antagonists. Among various approaches of biological control, the use of rhizobacteria as an agent is emerging as a popular trend due to its additional benefits of promoting growth and yield. Plant growth promotion by beneficial rhizobacteria may be an indirect mechanism of biological control, leading to disease escape when the growth promotion results in shortening the time that a plant is in a susceptible state (Kloepper and Schroth, 1981).

Anuradha and Gnanamanickam (1990) reported the antagonistic activity of *P. fluorescens* and *Bacillus* spp. against the bacterial wilt pathogen *Ralstonia solanacearum.* According to Defago *et al*. (1990), the biological control is the after effect of production of metabolites, such as antibiotics, hydrogen cyanide (HCN), iron-chelating siderophores and cell wall degrading enzymes. Laha *et al.* (1992) found that forty four per cent of the tested *P. fluorescens* isolates suppressed the growth of cotton pathogen *Rhizoctonia solani* very strongly. The disease intensity was reduced from 52.6 per cent (with non-bacterized seeds) to zero (with bacterized seeds).

Siderophore produced by *Pseudomonas* spp. and *Bacillus* spp. have efficiently controlled the damping off of cotton caused by *Pythium ultimum* as reported by Laha *et al*. (1992). Fukui *et al.* (1994) clearly demonstrated that certain

fluorescent Pseudomonads, when applied to crop plants as root or soil inoculants, suppressed soil-borne plant pathogens and subsequently the diseases.

According to Loper *et al.* (1997) fluorescent Pseudomonads are typically among the most effective antagonists selected for suppression of both soil-borne and aerial diseases of plants. Nandakumar (1998) reported that combined use of *P. fluorescens* strains *viz*. Pf_1 and Pf_7 has given effective control of rice sheath blight disease when compared to these strains applied individually.

Jubina and Girija (1998) found that a rhizobracteral isolate B_{13} was most promising in reducing the plant mortality, foliar blightening and in providing prolonged protection against *Phytophthora capsici.* Another isolate B_7 had a dual function of disease suppression and growth promotion and these isolates were identified as endospore forming *Bacillus* spp.

A study by Ongena *et al.* (1999) found that antifungal compounds induced by cucumber roots upon inoculation with the fluorescent pseudomonas strains participate actively in the protection of cucumber plants against *Pythium aphanidermatum.*

Meena *et al.* (2000) reported that seed treatment followed by foliar application of *P. fluorescens* Pf_1 strain significantly reduced the disease by C*ercosporidium personatum* in groundnut. Yeole and Dube (2000) reported the involvement of siderophores in the antibiosis of the rhizobacteria *P. fluorescens* against soil borne pathogens in various crops. Acharya *et al.* (2001) found that *P. fluorescens* effectively controlled the blister blight causing *Exobasidium vexans* to 72 to 85 percent after three months treatment.

El habbaa *et al.* (2002) reported that *B. subtilis* effectively controlled the fungal pathogens *F. solani, M. phaseolina, Botryodiplodia theobromae, S. rolfsii* and *R. solani.* A study by Kloepper *et al.* (2004) revealed that the protection resulting from induced systemic resistance elicited by *Bacillus* spp. has been reported against leaf spotting fungal and bacterial pathogens, systemic viruses, and a crown-rotting fungal pathogen, stem-blight fungal pathogen as well as damping-off diseases.

2.7 COMPATIBILITY OF *Azospirillum* sp., *P. fluorescens* and *B. subtilis*.

Sidorenko *et al.* (1996) reported that combined inoculation of *Azotobacter, Bacillus* and *Pseudomonas* increased plant height, biomass and tuber yield in potato. An attempt was made by Joseph and Vijayan (2003) to study the role of *P. fluorescens* and *B. subtilis* in comparison with *T. harzianum* on improving seed germination, seedling growth and protection from fungal pathogens. They found that seed coating with *T. harzianum, P. fluorescens* and *B. subtilis* has resulted in increased seed germination and enhanced growth and vigour of the seedlings.

Combination of *B. subtilis* along with *Azospirillum* sp. increased the root and shoot length significantly over *Azospirillum* sp. alone (Sankar and Jeyarajan 1996). They also found that an increase in rhizosphere population occurred in the combined application. Kamble *et al.* (2000) found that individual inoculation of seeds with *Azospirillum* sp., *P. fluorescens* and phosphobacteria were more effective than combined inoculation. Anandaraj and Sarma (2003) reported that five fluorescent *Pseudomonas* strains when used in combination were having a synergistic effect in disease control.

3. MATERIALS AND METHODS

The study on 'Population dynamics of plant growth promoting rhizobacteria under the influence of agricultural chemicals' was conducted in the Department of Plant Pathology, College of Horticulture,Vellanikkara,Thrissur during the period from October 2003 to August 2007. The details of the materials used and the techniques adopted for the investigation are presented below.

3.1 COLLECTION AND PURIFICATION OF *Azospirillum* sp.

The culture of *Azospirillum* sp. available in the Department of Plant Pathology was used for the study. Purification and characterisation of the culture was carried out to avoid any possible contamination. A loopful of the culture suspension was stabbed into Nitrogen free Bromothymol blue medium (Semi solid Malate Medium, Appendix I). The tubes were incubated at 37^0 C for two days. These were observed for the presence of thin, white, subsurface pellicular growth of *Azospirillum* sp. Further purification of *Azospirillum* sp. was done by serial transfer of the subsurface pellicles in to fresh Semisolid Malate Medium taken in test tubes.

3.2 CHARACTERISATION OF *Azospirillum* sp.

Cultural, morphological and biochemical characters of the purified *Azospirillum* sp. were studied for the confirmation of the bacteria.

3.2.1 **Gram staining (**Hucker and Conn, 1923**)**

A bacterial smear was prepared on a clean glass slide and it was heat fixed by passing a few times over a flame. The smear was then flooded with Hucker's ammonium crystal violet solution (Appendix II) for one minute and then washed in a gentle stream of running tap water. It was then flooded with Gram's iodine solution (Appendix II) for one minute and again washed. Later, the smear was decolourised with 95 per cent ethyl alcohol. After washing, the smear was again stained with

saffranin for one minute and the excesses stain was washed off in running water. The smear was then blot dried and examined under light microscope for Gram reaction.

3.2.2 **Cultural and biochemical characters**.

Cultural characters of the *Azospirillum* sp. was studied by growing it on Rojo Congo (RC) medium (Appendix I). A loopful of 24 hour old culture was taken from the white pellicular growth formed on Solid Malate Media and streaked on RC medium. These plates were incubated at 37^0 C for four days and observed for the development of the colour and shape of the colonies.

3.2.2.1 ***Acid production from glucose***

Acid production from glucose by *Azospirillum* sp. was tested using the special medium containing glucose (Appendix I).

Five ml quantities of the medium were taken in test tubes and autoclaved. The sterilised medium was then stab inoculated with 0.1 ml suspension of 48 h old culture of *Azospirillum* sp. and incubated at 37^0 C for four days. These were observed for the change in colour of the medium.

After purification and characterization, the culture was streaked on Solid Malate Medium in sterile Petriplates. The colonies of *Azospirillum* sp. formed were transferred to test tube slants of the same medium. The tubes were incubated at 37^0 C for two days and subsequently stored in a refrigerator. The culture was maintained by periodic purification and sub culturing in to fresh test tube slants.

3.3 COLLECTION AND MAINTENANCE OF *Pseudomonas fluorescens*

The *P. fluorescens* culture available in the Department of Plant Pathology was used for the study. Purification and characterisation of the culture was carried out to avoid any possible contamination. The culture was streaked on King's B

medium (Appendix I). The plates were incubated for two days at room temperature of 28±2^{0}C. The culture was observed for the fluorescent colonies on King's B medium. Further purification of *P. fluorescens* was done by serial transfer and plating of single colonies of the fluorescent bacteria on King's B medium.

3.4 CHARACTERISATION OF *P. fluorescens*

Cultural, morphological and biochemical characters of the purified *P. fluorescens* culture were done for characterization and conformation.

3.4.1 **Cultural characters**

P. fluorescens culture, after 48 h of growth, was streaked on King's B medium in Petri plates. After an incubation period of 24 h, the colonies were observed for shape and margin. The colonies were also observed under ultra violet light for observing fluorescent pigment production.

3.4.2 **Morphological characters**

Morphological characters such as shape and arrangement of the cells were observed under the oil immersion objective of a light microscope after Gram staining. Culture of 24 h growth was used for the study.

3.4.3 **Biochemical characters**

3.4.3.1 ***Catalase test***

A few drops of three per cent hydrogen peroxide were put at the centre of a sterile glass slide. A loopful of bacterial culture was then mixed with hydrogen peroxide (Cappucino and Sherman, 1992) and observed for effervescence.

3.4.3.2 ***Starch hydrolysis***

Nutrient agar medium containing 0.2 per cent soluble starch was used for starch hydrolysis test. The bacterial isolate was spot inoculated on the medium in sterilised Petri plates and incubated at room temperature. After four days of incubation, the media was flooded with Lugol's iodine solution and observed for colourless zone around the bacterial growth in contrast to the blue background.

3.4.3.3 ***Arginine dihydrolase reaction***

Thornley's semi solid medium (Appendix I) was used for the test. Five ml quantities of the medium (Thornley, 1960) was dispensed in test tubes and sterilized in an autoclave at 121^0 C. The medium was stab inoculated with a loopful of bacterial growth. The surface of the medium was then sealed with paraffin oil and incubated for seven days at room temperature. Change in colour of medium to pink or red was recorded at regular intervals for a period of seven days.

3.4.3.4 ***Production of levan***

Peptone beef extract medium (Appendix I) containing five per cent sucrose was used for this test. The bacterial isolate was streaked over the medium in sterilised Petri plates. The culture on the Petri plates were observed after 48 hours for the presence of large, white domed and mucoid colonies.

3.4.3.5 ***Urease test***

Chistenson's urea agar (Chistenson, 1946 Appendix I) was used in this test. Ninety ml of the medium was dispensed in 250 ml conical flasks and autoclaved. To each flask, 10ml of 20 per cent sterilized urea solution was added. Five ml of the media was dispensed in sterilized test tubes and slants were prepared. These slants were inoculated with the isolate and observed for five days for any change in colour of the medium from yellow to pink or red.

3.4.3.6 ***KOH test***

A loopful of the culture was taken on a clear glass slide. One drop of three per cent potassium hydroxide solution was put over it and thoroughly mixed with a needle. Appearance of thin thread like growth, if any, was observed.

3.4.3.7 ***V.P test***

Five ml quantities of the methyl red broth (Appendix I) were dispersed in test tubes and sterilized by steaming for 30 minutes for three successive days. Tubes were inoculated with 98 h old cultures of *P. fluorescens* isolate. The tubes were incubated for seven days. Alpha naphthol solution (0.6 ml of 5% solution in 95% ethanol) and 0.2 ml of 40 per cent aqueous solution of KOH were added to one milli litre suspension of *Pseudomonas fluorescens* in methyl red broth. The mixture was shaken for a few minutes and allowed to stand for 2 hrs. It was observed for the development of Crimson colour.

3.4.3.8 ***Nitrate reduction test***

Nitrate agar medium (Appendix II) was dispensed in test tubes, autoclaved and inoculated with 24 h old cultures of *P. fluorescens.* The test tubes were then incubated at room temperature and tested for the reduction of nitrate at regular intervals up to 15 days. The test was performed by adding few drops of Griess Llosvay's reagent consisting of sulphanilic acid (0.8% in 5 M acetic acid) and dimethyl alpha-naphthyl amine (0.5%in 5 M acetic acid) to the nitrate broth culture. Development of pink or red colour was later observed. Absence of pink or red colour development indicated the presence of nitrate as such or reduced to ammonia and free nitrogen.

After purification and characterization, the culture was streaked on King's B medium in sterile Petri plates. Colonies of *P. fluorescens* formed were transferred to test tube slants of the same medium. The tubes were incubated at room temperature

for two days. They were then stored in a refrigerator. The culture was maintained by periodic purification and subculturing in to fresh test tube slants.

3.5 COLLECTION AND PURIFICATION OF *Bacillus subtilis*

The culture of *B. subtilis* from TNAU was used for the study. Purification and characterisation of the culture was carried out to avoid any possible contamination. A loop full of the culture was streaked on Nutrient Agar medium (Appendix I). The plates were then incubated for three days at room temperature. White creamy colonies were selected for purification

The bacterium was purified by repeated streaking on Nutrient Agar medium. Individual colonies showing typical characters of *B. subtilis* were streaked on nutrient agar slants. The tubes were incubated for three days and then maintained in a refrigerator for further work.

3.6 CHARACTERISATION OF *B. subtilis*

Morphological and biochemical characters of the purified *B. subtilis* culture were studied.

3.6.1 **Morphological and cultural characters**

B. subtilis culture, after 48 h of growth, was streaked on Nutrient Agar medium in Petri plates. After an incubation period of 24 h, the colonies were observed for shape and margin. Morphological characters such as shape and arrangement of the cells were studied under the oil immersion objective of a light microscope after Gram staining using 24 h old culture of *B. subtilis*.

3.6.2 **Endospore staining**

A smear of the isolate was prepared on a clean glass slide and heat fixed. It was then stained with malachite green and allowed to react in the cold for 30-60

seconds and then passed over the flame for 30 seconds. The smear was then rinsed with water and again stained with aqueous solution of saffranin for 30 seconds. Later, the smear was rinsed with water, blotted dried and observed under oil immersion objective of the microscope.

3.6.3 **Biochemical tests**

Catalase test, KOH test, starch hydrolysis test and V.P test were done as described earlier.

After purification and characterisation, the *B. subtilis* culture was streaked on Nutrient Agar medium in sterile Petri plates. The colonies of *B. subtilis* formed were transferred to test tube slants of the same medium. The tubes were then incubated at room temperature for two days. They were then stored in a refrigerator. The culture was maintained by periodic purification and subculturing in to fresh test tube slants.

3.7 *In vitro* EVALUATION OF MUTUAL COMPATIBILITY OF THE PGPR

The three PGPR bacteria were streaked perpendicular to each other on the Petri plates having nutrient medium. The plates were then observed daily for any lysis at the junction of the streaks.

3.8 *In vitro* SCREENING OF PGPR FOR ANTAGONISTIC PROPERTIES AGAINST *Rhizoctonia solani*

Isolates of the test bacteria *viz. Azospirillum* sp., *P. fluorescens* and *B. subtilis* were tested for their antagonistic effect against *R. solani* by dual culture method. Potato dextrose agar medium was prepared and allowed to solidify in sterilized Petri plates. Mycelial discs of 8 mm size of the pathogen were inoculated at the centre of each Petri plate. The bacterial isolates were inoculated as a line of streak on either side of the pathogen and 2.25 cm from the edge of the Petri plate. Plates with *R. solani* without bacterial streak served as control. Three replications were maintained.

The percent inhibition of the pathogen was calculated using the formula,

$$I = \frac{C-T}{C} \times 100$$

Where, C – growth of the pathogen in control (mm)

T – Growth of the pathogen in dual culture (mm)

The observations on the growth of the pathogen were taken after two days of growth.

3.9 GROWTH PROMOTING EFFECT OF PGPR

The three rhizobacteria were screened for their plant growth promoting effect by seed germination test, estimation if indole acetic acid and salicylic acid.

3.9.1 **Seed germination test**

The selected PGPR isolates were bioassayed for their effect on seed germination (Shende *et al*. 1977 as modified by Elliot and Lynch, 1984). The cowpea and sorghum seeds were used for this test. The seeds were surface sterilized with 0.1 per cent mercuric chloride for three minutes followed by successive washing with sterile distilled water till all the traces of mercuric chloride was removed. After decanting, the seeds were treated with the bacterial suspension. The suspension was prepared by dispersing the 48 h old cultures in sterile distilled water. The seeds were then soaked in the suspension for 20 minutes and decanted. Plain agar (0.8 per cent) was used for inoculation. The seeds were then placed on the medium and incubated at room temperature for three days. Ten seeds were inoculated with three replications. Control seeds were treated with sterile water. After three days, the per cent germination, root length and shoot length were recorded.

3.9.2 **Estimation of Indole Acetic Acid (IAA) production by PGPR**

A loopful of each culture was inoculated in 25 ml broth of LB medium (Luria Bertani medium, Appendix I) and incubated for 24 h at room temperature on rotary shaker. The culture was then centrifuged at 10000 rpm for 15 minutes. From the supernatant, two ml was taken in test tube. To this, two to three drops of orthophosphoric acid and four ml of reagent mixture (ferric chloride and perchloric acid mixture) were added. The tubes were incubated for 25 minutes at room temperature. The absorbance values were read at 530 nm using spectrophotometer. The quantity of IAA produced by each bacterial isolate was calculated using a standard curve prepared with IAA.

3.9.3 **Salicylic Acid production**

Each bacterial culture of 0.1 ml was inoculated in to 25 ml of Casamino acid broth (Appendix I) and incubated for 36 hours at 200 rpm on the rotary shaker at 34^0 C in the dark. The bacterial cultures were then mixed with ethyl acetate (3:1 ratio) and the extract in the ethyl acetate phase was taken. The extract was then evaporated in vacuum to one ml. To this, concentrated extract of 0.05 ml of 2M ferric chloride and three ml of distilled water were added. Then absorbance of purple iron-SA complex, which was developed in the aqueous phase, was measured at 527 nm using spectrophotometer. The value was compared with a standard curve of salicylic acid dissolved in ethyl acetate. The three PGPR bacteria were used for the SA estimation.

3.10 *In vitro* EVALUATION OF PLANT PROTECTION CHEMICALS ON THE GROWTH OF PGPR

The effect of plant protection chemicals at the recommended doses and their combinations on the growth of *Azospirillum, P. fluorescens* and *B. subtilis* (PGPR) were tested by paper disc method (Johnson and Curl,1972). The experiment was conducted as 6^3 factorial experiment in CRD with three replications.

Table 1: Chemicals used for *in vitro* evaluation

Treatments	Chemicals
Fungicides (F)	F_0-Control
	F_1-Mancozeb -0.3 per cent
	F_2-Carbendazim -0.1 per cent
	F_3-Metalaxyl -0.1 percent
	F_4-Copper oxychloride -0.3 per cent
	F_5-Tridemorph -0.1 per cent
Insecticides (I)	I_0-Control
	I_1-Lindane -1 kg ai/ha
	I_2-Chlorpyriphos -500g ai/ha
	I_3-Carbaryl -1kg ai /ha
	I_4-Lamda cyhalothrin -12.5g ai/ha
	I_5-Imidacloprid -25 g ai/ha
Herbicides (H)	H_0-Control
	H_1-Glyphosate -800g ai/ha
	H_2-2,4-D -1 kg ai/ha
	H_3-Butachlor -1.25 kg ai/ha
	H_4-Pretilachlor -750 g ai/ha
	H_5-Paraquat -600g ai/ha

The above concentrations of fungicides, insecticides and herbicides and their combinations were prepared in sterile distilled water. Sterile filter paper discs of 5 mm diameter were dipped in the chemical solutions for 20 minutes and were placed at the centre of the selective media seeded with the corresponding bacteria. The media used were Solid Malate media, King's B media and Nutrient Agar media for *Azospirillum* sp., *P. fluorescens* and *B. subtilis* respectively. Sterile filter paper discs dipped in sterile water served as control. The plates were incubated at room temperature and the diameter of the inhibition zones were recorded after two days.

3.11 COMPATIBILITY OF PGPR TO DIFFERENT DOSES OF PLANT PROTECTION CHEMICALS

The evaluation of *Azospirillum* sp*., P. fluorescens* and *B. subtilis* to different plant protection chemicals were tested by paper disc method. The experiment was conducted with the following chemicals each with lower, middle and higher doses.

Table 2: Doses of chemicals used

Chemicals		Lower dose (per 100ml)	Normal dose (per 100ml)	Higher dose (per 100ml)
Fungicides (F)	Mancozeb	0.2g	0.3g	0.4g
	Carbendazim	0.05g	0.1g	0.2g
	Metalaxyl	0.05g	0.1g	0.2g
	Copper oxychloride	0.2g	0.3g	0.4g
	Tridemorph	0.05ml	0.1ml	0.2ml
Insecticides(I)	Lindane	0.025g	0.05g	0.1g
	Chlorpyriphos	0.025ml	0.05ml	0.01ml
	Carbaryl	0.1g	0.2g	0.3g
	Lamda cyhalothrin	0.005	0.01ml	0.02ml
	Imidacloprid	0.012	0.025ml	0.05ml
	Glyphosate	0.3ml	0.4ml	0.5ml
	2,4-D	0.1g	0.2g	0.3g
	Butachlor	0.4ml	0.5ml	0.6ml
	Pretilachlor	0.2ml	0.3ml	0.4ml
	Paraquat	0.3ml	0.4ml	0.5ml

The same procedure was followed as explained in 3.10.

3.12 SURVIVAL OF PGPR IN SOIL APPLIED WITH THE PLANT PROTECTION CHEMICALS.

Survival of *Azospirillum* sp., *P. fluorescens* and *B. subtilis* inoculated in sterile soil applied with the selected plant protection chemicals were studied. The experiment details are as follows

Design – CRD

Replication -Three

Number of Treatments. 16.

Treatments were the fungicides, insecticides and herbicides listed in 3.10 at the same doses.

Fifty grams of soil was taken in Petri plates and sterilized in an autoclave for one h. The soil was then inoculated with five ml of the culture of each bacterial suspension. The bacterial count in the suspension was adjusted to 10^{8} cfu ml^{-1}. The test chemicals were applied fifteen days after the bacterial inoculation to the soil.

3.12.1 **Estimation of PGPR in the soil**

Populations of the PGPR were estimated by serial dilution and plating technique using their selective media. Ten gram soil sample was added into 90ml of sterile water in 250ml conical flask and mixed well. Mixing was done on a rotary shaker for 20 minutes. From this soil suspension, one ml was pippetted out to nine ml sterile water in test tube blank using micropipette and was shaken well for about 10 minutes. From this suspension, one ml was again pippetted to another nine ml blank. Diluting the suspension was repeated to get 10^{8} dilution. From this diluted suspension, one ml was pippetted out to sterile Petri plates using a sterile micropipette. About 10-15 ml of the corresponding media (Solid Malate medium, King' B medium and Nutrient Agar medium for *Azospirillum* sp., *P. fluorescens* and *B. subtilis* respectively) was poured and swirled to mix the soil suspension with the medium uniformly. The Petri plates were incubated for observing the colonies of the PGPR.

For estimation of the population of the three PGPR bacteria in the soil, random samples were taken from each Petri plate and mixed together to get composite samples and from these composite samples, ten gram soil was taken for estimation of the bacteria. Population was estimated on the next day after chemical application and subsequent observations were taken at fortnight intervals. Three observations were taken using the selective media for each organism.

3.13 EXPERIMENT ON THE TOXIC EFFECT OF CHEMICALS, IF ANY, ON PGPR AT HIGHER DOSE.

Based on the above experiment, carbendazim was found the least inhibitory fungicide to PGPR. This is being used in rice fields extensively**.** Among insecticides, all except carbaryl were found compatible to PGPR. However, chlorpyriphos is one which is used in rice fields to control borer and gall fly. Among herbicides 2, 4-D is being used largely in rice fields against sedges and dicot weeds. Thus, carbendazim, chlorpyriphos and 2, 4-D were selected for this study to find any possible toxic effects for these chemicals at a higher dose.

The experiment was done by paper disc method. The experiment details are as follows.

Design- CRD

Treatments -9

Replications -3

Treatments were as follows.

Table 3: Higher doses of selected chemicals used in the experiment

Chemicals	Dosage
Carbendazim	0.2,0.3.0.4 g/100ml
Chlorpyriphos	0.1,0.2,0.3 ml/100ml
2,4-D	0.3,0.4,0.5 g/100ml

Plate assay method was used for the study. The chemicals were prepared at the desired concentrations in sterile distilled water. Forty eight hour old cultures of

Azospirillum, P. fluorescens and *B. subtilis* were seeded in Solid malate, King's B and Nutrient agar medium respectively. Sterile filter paper discs of five mm diameter were dipped in the chemical solutions for 20 minutes. The discs soaked in chemical were placed at centre of the appropriate medium. Sterile filter paper dipped in sterile water served as control. The plates were then incubated at room temperature of 28 ± 2^0C for two days and the diameter of the inhibition zone was measured.

3.14 EFFECT OF SELECTED CHEMICALS ON THE POLPULATION OF PGPR IN RHIZOSPHERE OF RICE.

A pot culture experiment was laid out to evaluate the efficacy of selected chemicals on the population of PGPR applied in rhizosphere of rice. The details of the experiment are presented below.

Design- CRD

Treatments-12

Replication -5

Rice variety- ASD-16

Table 4: Details of various treatments

Treatments	
T_1	Soil application of *Azospirillum* @ 10ml/kg soil (X 10^8 cfu ml $^{-1}$) + Carbendazim (0.1 %)
T_2	Soil application of *Azospirillum* @ 10ml/kg soil (X 10^8 cfu ml^{-1}) + Chlorpyriphos (500g ai/ha)
T_3	Soil application of *Azospirillum* @ 10ml/kg soil (X 10^8 cfu ml^{-1}) + 2, 4-D (1 kg ai/ha)
T_4	Soil application of *Pseudomonas fluorescens* @ 10 ml/kg soil (X 10^8 cfu ml^{-1}) + Carbendazim (0.1 %)
T_5	Soil application of *Pseudomonas fluorescens* @ 10 ml/kg soil (X 10^8 cfu ml^{-1}) + Chlorpyriphos (500g ai/ha)
T_6	Soil application of *Pseudomonas fluorescens* @ 10 ml/kg soil (X 10^8 cfu ml^{-1}) + 2, 4-D (1 kg ai/ha)
T_7	Soil application of *Bacillus subtilis* @ 10ml/kg soil (X 10^8 cfu ml^{-1}) +

	Carbendazim (0.1 %)
T_8	Soil application of *Bacillus subtilis* @ 10ml/kg soil (X 10^8 cfu ml^{-1}) + Chlorpyriphos (500g ai/ha)
T_9	Soil application of *Bacillus subtilis* @ 10ml/kg soil (X 10^8 cfu ml^{-1}) +2, 4-D (1 kg ai/ha)
T_{10}	Soil application of *Azospirillum* @ 10ml/kg soil (X 10^8 cfu ml^{-1})
T_{11}	Soil application of *Pseudomonas fluorescens* @ 10 ml/kg soil (X 10^8 cfu ml^{-1})
T_{12}	Soil application of *Bacillus subtilis* @ 10ml/kg soil (X 10^8 cfu ml^{-1})

Soil for this experiment was collected from the paddy field at Agricultural Research Station, Mannuthy. Pots of 30cm diameter were used for the experiment. Seeds of widely sown local rice variety, ASD-16 was used for this experiment. The seeds were first sown in nursery. Twenty one days old seedlings were uprooted and washed in water to remove all soil and mud. Later, the roots were dipped in the appropriate bacterial cultures for 30 minutes. These seedlings were used for transplanting. Two seedlings were planted in each pot. The bacterial culture was also added to the soil at the rate of 10 ml/kg soil each. Fifteen days after transplanting, treatments were applied. The chemicals were applied at the recommended doses. On the next day of chemical application, the microbial count was taken by the method of serial dilution and plating technique. The biometric observations on plant height, number of leaves, number of tillers and number of productive tillers were also recorded. Subsequent observations on bacterial count and biometric measurements were taken at monthly intervals for three months.

3.14.2 **Growth parameters of rice plants**

Observations on the growth parameters of rice plants were taken at monthly intervals as detailed below.

- Plant Height - Plant height was taken from the soil level to the tip of the longest leaf.
- Number of leaves - Number of the leaves was recorded after transplanting for a period of three months up to harvest at monthly intervals.
- Numbers of tillers- Number of the tillers were recorded after tillering upto harvest at monthly intervals.

- Numbers of Productive tillers- Number of the productive tillers were recorded after panicle emergence up to harvest at monthly intervals.
- Shoot fresh weight- Fresh weight of shoots were recorded immedietly after harvest by up rooting the plants and separating the shoot portions.
- Root fresh weight-Recorded immediately after harvest by up rooting the plants and separating the root portions.
- Shoot dry weight- The samples were sun dried for two days. There after oven dried at 60^0C till the weight become constant.
- Root dry weight- The samples were sun dried for two days. There after oven dried at 60^0C till the weight become constant.

3.14.2.1 **N P K content**

Nutrients like N, P and K in the plant samples were analysed after the harvest. For this, the plants were harvested, washed off the soil particles and dried under sun for 2-3 days and then dried in a hot air oven at 60^0 C. After drying, samples were powdered and the fine powder was used for estimating N, P and K content. Methods used are given below.

Table 5: Methods and procedures used for nutrient analysis

Nutrients	Digestion procedure	Method of estimation	Reference
Nitrogen	H_2SO_4 single acid Digestion	Micro kjeldhals estimation	Jackson (1964)
Phosphorus	HNO_3: $HClO_4$ (2:1)diacid digestion	Vanadomolybdate yellow colour method using Spectrophotometer	Jackson (1964)
Potassium	HNO_3: $HClO_4$ (2:1)diacid digestion	Direct reading using Flame Photometer	Jackson (1964)

3.15 PREPARATION OF COMPATIBILITY CHART

Based on the whole study, an attempt was made to prepare a compatibility chart of chemicals against the PGPR used. The basis of preparing the chart was based on the results of the *in vitro* experiments. The various chemicals used were classified in to three categories namely, C, Q and N.

C: Completely compatible combinations of the PGPR and the concerned chemical.

Q: Questionable combination. The combination where inhibition zone was less than one cm was categorized under this group.

N: Not compatible combination where the chemical produced a zone of one centimeter and more.

3.16 STATISTICAL ANALYSIS OF DATA

Analysis of variance was performed on the data collected in the experiments using statistical package of MSTAT (Freed,1986). Multiple comparisons of the treatment means were done using DMRT.

4. RESULTS

Results of the experiments on 'Population dynamics of plant growth promoting rhizobacteria under the influence of agricultural chemicals' conducted, are presented below.

4.1 COLLECTION AND PURIFICATION OF *Azospirillum* sp.

Culture *Azospirillum* maintained in the Department of Plant Pathology was used for the various studies conducted. The cultures used were thin, white and showed subsurface pellicular growth in semisolid malate medium. This bacterial culture was used for further characterisation.

4.1.1 **Cultural and morphological characters**

The results of Gram staining revealed that the bacterium was Gram negative and rod shaped. In Rojo Congo red media, colonies formed were scarlet red, dry, round to irregular in shape, with rugose surface and undulating edges

4.1.2 **Physiological characters**

4.1.2.1 ***Acid production from glucose***

The test indicated acid production from glucose. This was revealed by the change in colour of the medium from green to yellow after incubation at 37^0 C for four days.

4.2 COLLECTION AND PURIFICATION OF *P. fluorescens*

Colonies of *P. fluorescens* culture maintained in the Department of Plant Pathology were cream in colour, slimy, smooth and fluorescent when grown in King's B medium. Such culture was used for further characterisation.

4.2.1 **Cultural and morphological characters**

The bacterium was Gram negative and short rod in shape. On King's B medium, the bacterial colonies appeared as cream, slimy, smooth and fluorescent.

4.2.2 **Biochemical characters**

The results of the biochemical characters are presented in Table 6.

4.2.2.1 ***Catalase test***

The positive reaction of catalase test was indicated by the production of effervescence.

4.2.2.2 ***Starch hydrolysis***

Absence of colourless zone in the blue back ground indicated negative results for starch hydrolysis.

4.2.2.3 ***Arginine dihydrolase reaction***

A colour change of Thornley's medium from yellow to pink with in two days indicated the positive reaction.

4.2.2.4 ***Production of levan***

Formation of large, white domed and mucoid colonies in the medium indicated the production of levan from glucose by the bacterium.

4.2.2.5 ***Urease test***

Colour change of the medium from yellow to pink, indicated the production of urease.

4.2.2.6 ***KOH test***

When the bacterial smear was thoroughly mixed with three per cent KOH with a needle the bacteria came out as thin threads which indicated the positive reaction for this test.

4.2.2.7 ***V. P. test***

Absence of crimson colour development in the nutrient broth indicated negative result for V. P. test.

4.3 COLLECTION AND PURIFICATION OF *B. subtilis*

Culture of *B. subtilis* used for the study was from TNAU. The bacterium was purified and maintained on Nutrient agar slants by periodic sub culturing.

4.3.1 **Cultural and morphological characters**

The culture of *B. subtilis* used was Gram positive and rod in shape. The isolate was also positive to endospore staining. In nutrient agar medium, the colonies formed were thick, rugose and creamy white.

4.3.2 **Biochemical reaction**

The results of the biochemical reactions were furnished in Table 6.

4.3.2.1 ***Catalase test***

When the culture was treated with few drops of three per cent hydrogen peroxide, effervescence of oxygen was observed which indicated positive reaction.

4.3.2.2 ***KOH test***

The bacteria did not come out as thin threads which indicated that KOH test was negative.

4.3.2.3 ***Starch hydrolysis***

A colourless zone around the bacterial growth in contrast to the blue background when the agar surface was flooded with Lugol's iodine solution indicated positive result for starch hydrolysis.

4.3.2.4 ***V. P test***

Presence of crimson colour of the nutrient broth indicated that V.P test was positive.

4.4 *In vitro* EVALUATION OF MUTUAL COMPATIBILITY OF THE PGPR.

The results showed no lysis at the junction of the streaks. This indicated that the three bacterial isolates *viz. Azospirillum* sp*., P. fluorescens* and *B. subtilis* were mutually compatible.

4.5 SCREENING OF PGPR FOR THEIR ANTAGONISTIC ACTIVITY AGAINST *Rhizoctonia solani*

The results of the experiments are presented in Table 7.

The PGPR *viz. Azospirillum* sp*., P. fluorescens* and *B. subtilis* showed a little inhibition of *R. solani* on the second day of incubation. This is evident from the table that the maximum inhibition was noticed with *P. fluorescens* (47.4 per cent) and the minimum was observed for *Azospirillum* sp (35.8 per cent).

Table 6: Biochemical tests of *P. fluorescens* and *B. subtilis*

Sl.No.	Test	*P. fluorescens*	*B. subtilis*
1	Catalase test	+	+
2	Starch hydrolysis	-	+
3	Arginine di hydrolase	+	
4	Production of levan	+	
5	Urease test	+	
6	KOH test	+	-
7	Pigment production	+	
8	V. P test	-	+

Table 7: Percent inhibition of the pathogen on second day of inoculation

Sl.No.	Culture streaked on both sides	Per cent inhibition (second day)
1	*Azospirillum* sp.	35.8
2	*P. fluorescens*	47.4
3	*B. subtilis*	39.7

4.6 TESTING PGPR FOR PLANT GROWTH PROMOTING EFFECTS

4.6.1 **Effect of PGPR on germination of cowpea and sorghum seeds**

The three bacterial isolates were tested for their effect on the germination and growth of cowpea and sorghum seeds. Per cent germination and the length of root and shoot are presented in the Table 8 and 9. The results revealed that the cowpea seeds treated with PGPR recorded 100 per cent germination. The treatments were at par among them but differed from control in the case of root length. Maximum root length was noticed in *Azospirillum* treated seeds (8.0cm) and the minimum was recorded in *P. fluorescens* treated seeds. Seeds treated with bacterial isolates did not show any significant difference among them in influencing shoot length. However, maximum shoot length was found with *Azospirillum* treated seeds (7.1cm) and minimum length was noticed in control (6.4cm).

Sorghum seeds also recorded 100 per cent germination (Table 9). When the seeds were treated with *P. fluorescens,* increased root length up to 3.58 cm was noticed. Among the treatments, the minimum root length was observed in *Azospirillum* treated seeds. Here also the bacterial isolates did not significantly influence the shoot length. However, maximum shoot length was observed in *B. subtilis* treated seeds (1.97 cm) followed by treatment with *P. fluorescens* (1.56 cm). The lowest shoot length was recorded in control (1.22 cm). In both cases, all treatments were better than control in improving root length.

4.6.2 **Production of indole acetic acid and salicylic acid by PGPR**

The results are presented in Table 10. It is seen that, the maximum IAA and SA were produced by *Azospirillum* sp. (139, 22.3µg ml^{-1} respectively)

Table 8: Effect of PGPR on germination of cow pea seeds

Treatments	Per cent germination	Root Length(cm)	Shoot Length(cm)
Azospirillum sp.	99	8.0^{a} $(2.9)^{a}$	7.1^{a} $(2.8)^{a}$
P. fluorescens	98	6.2^{a} $(2.6)^{a}$	6.6^{a} $(2.7)^{a}$
B. subtilis	99	7.9^{a} $(2.9)^{a}$	7.03^{a} $(2.74)^{a}$
Control	98	7.0^{ab} $(2.7)^{ab}$	6.4^{a} $(2.6)^{a}$
			NS

Mean of ten replications

NS: Not significant

Figures followed by same letter do not differ significantly according to DMRT

Values in the parentheses are $\sqrt{x} + 0.05$ transformed

Table 9: Effect of PGPR on germination of sorghum seeds

Treatments	Per cent germination	Root Length(cm)	Shoot Length (cm)
Azospirillum sp.	98	$2.55^{b}(1.7)^{b}$	$1.33^{a}(1.33)^{a}$
P. fluorescens	98	$3.58^{a}(2.01)^{a}$	$1.56^{a}(1.38)^{a}$
B. subtilis	98	$3.22^{ab}(1.92)^{ab}$	$1.97^{a}(1.55)$a
Control	97	$3.22^{ab}(1.91)^{ab}$	$1.22^{ab}(1.28)^{ab}$
			NS

Mean of ten replications

NS: Not significant

Figures followed by same letter do not differ significantly according to DMRT

Values in the parentheses are $\sqrt{x} + 0.05$ transformed

4.7 *In vitro* EFFECT OF PLANT PROTECTION CHEMICALS ON THE GROWTH OF PGPR

4.7.1 **Effect of fungicides on PGPR**

The results of the effect of the fungicides on PGPR are furnished in the Table 11. The results revealed that, copper oxychloride was highly inhibitory to *Azospirillum* sp. (Plate I a) and *P. fluorescens* (1.2cm and 1.27 cm respectively) where as tredimorph was the most inhibiting fungicide to *B. subtilis* (0.9 cm). In the case of *Azospirillum* sp. mancozeb was found next to copper oxychloride in inhibition (0.87cm) where as Metalaxyl (Plate I d) was found next to copper oxychloride in inhibiting the growth (1.0 cm) of *P. fluorescens*. It was found that tredimorph(Plate I e) was followed by copper oxychloride in inhibiting the growth of *B. subtilis* (0.73 cm). Carbendazim was found compatible with *Azospirillum* sp. Carbendazim and metalaxyl were found compatible with *B. subtilis*, showing no inhibition of growth, whereas carbendazim, mancozeb and tredimorph were least inhibitory to *P. fluorescens* (0.6 cm). However, metalaxyl was also found compatible to *B. subtilis.*

4.7.2. **Effect of insecticides on PGPR**

The effect of the insecticides on the PGPR is presented in the Table 12. The effect of insecticides showed that they are not inhibitory to *Azospirillum* sp. and *B. subtilis* at the recommended dose. Similarly all insecticides except carbaryl were highly compatible to *P. fluorescens.* Carbaryl was the only insecticide which inhibited the growth of *P. fluorescens* (0.63 cm, Plate I c).

4.7.3. **Effect of herbicides on PGPR**

The results of the *in vitro* effects of the herbicides on PGPR are given in Table 13. Among herbicides, paraquat was the only one found inhibitory to *Azospirillum* sp., inhibition zone being 3.6cm (Plate I b). In the case of *P. fluorescens,* the inhibition was 1.2 cm when treated with paraquat.

Table 10: Indole Acetic Acid and Salicylic Acid production

Bacterial culture	IAA in µg ml^{-1}	S A in µg ml^{-1}
Azospirillum sp.	139	22.3
P. fluorescens	56	10.7
B. subtilis	59	13.9

Table 11: Effects of fungicides on PGPR.

(Diameter of inhibition zone (cm)

Fungicides	*Azospirillum* sp.	*P. fluorescens*	*B. subtilis*
F_0 -Control	0	0	0
F_1- Mancozeb - 0.3 per cent	0.87	0.6	0.6
F_2-Carbendazim - 0.1 per cent	0	0.6	0
F_3-Metalaxyl - 0.1 per cent	0.77	1.0	0
F_4-Copper oxychloride- 0.3 per cent	1.2	1.27	0.73
F_5-Tridemorph - 0.1 per cent	0	0.6	0.9

Mean of three replications.

Azospirillum sp. CD = 0.035. *P. fluorescens* CD= 0.03. *B. subtilis* CD= 0.044

Table 12: Effects of insecticides on PGPR.

(Diameter of inhibition zone (cm)

Insecticides	*Azospirillum* sp.	*P. fluorescens*	*B. subtilis*
I_0 -Control	0	0	0
I_1-Lindane -1 kg ai/ha	0	0	0
I_2-Chlorpyriphos -500g ai/ha	0	0	0
I_3-Carbaryl -1kg ai /ha	0	0.63	0
I_4-Lamda cyhalothirn -12.5g ai/ha	0	0	0
I_5-Imidacloprid -25 g ai/ha	0	0	0

Mean of three replications.

Azospirillum sp. CD = 0.035. *P. fluorescens* CD= 0.03. *B. subtilis* CD= 0.044

The herbicide 2, 4-D was found compatible with *P. fluorescens* where as for *B. subtilis* 2, 4-D and paraquat were highly compatible. There was no inhibition in these cases.

4.7.4. **Interactive effect of fungicides and herbicides on PGPR**

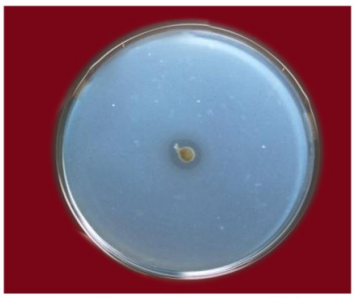
Interactive effect of fungicides and herbicides on PGPR was evaluated and the results of this study are given in the Tables 14, 15 and 16. It is evident from the results that the mancozeb in combination with paraquat was highly inhibitory to *Azospirillum* sp. producing an inhibition zone of 3.9 cm. Paraquat alone and also in combination with all fungicides were found inhibitory to the growth of *Azospirillum* sp. On the other hand, among the herbicides, 2, 4-D was found least inhibitory when used alone and in combination with all fungicides. However, the 2,4-D when combined with carbendazim was compatible to *Azospirillum* sp. There was no inhibition in this combination. In the case of *P. fluorescens,* almost all combinations were found least inhibitory. The combination of carbendazim with glyphosate, 2,4-D, butachlor, and paraquat did not affect the growth of *P. fluorescens*. Paraquat when combined with all fungicides caused high inhibition of *B. subtilis.* The combination of carbendazim and 2, 4-D did not show any inhibition of the growth of *B. subtilis*.

4.7.5. **Interactive effect of fungicides and insecticides on PGPR**

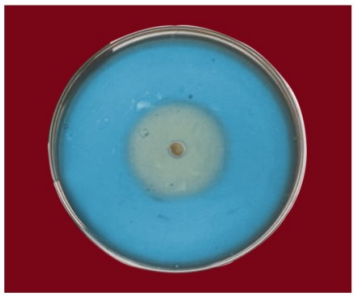
The results on the interactive effects of fungicides and insecticides on PGPR are presented in the Tables 17, 18 and 19. It is found that mancozeb (Table 17) alone and when combined with all insecticides were highly inhibitory to *Azospirillum* sp. The combinations of copper oxychloride with lamda cyhalothrin and with imidacloprid were most inhibitory to the growth of *Azospirillum* sp. Inhibition zone produced in these cases were 3.5 and 3.6 cm respectively. The fungicide carbendazim, when combined with all insecticides were compatible with *Azospirillum* sp. and the inhibition ranged from 0.1 to 0.6 cm. In the case of *P. fluorescens,* it is evident (Table 18) that the combination of the fungicide copper oxychloride with carbaryl was highly inhibitory.

PLATE - 1

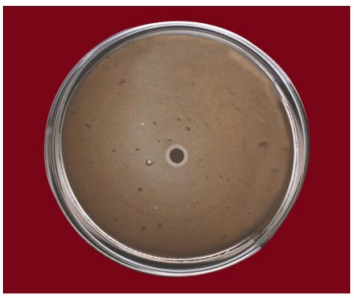
***In vitro* effect of chemicals on PGPR**



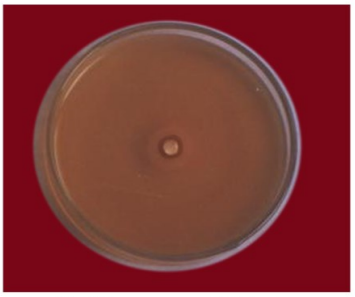
1a. *Azospirillum* sp. + Copper oxychloride



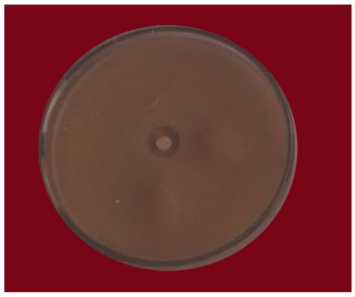
1b. *Azospirillum* sp. + Paraquat



1c. *P. fluorescens* + Carbaryl



1d. *P. fluorescens* + Metalaxyl



1e. *B. subtilis* + Tridemorph

Table 13: Effect of herbicides on PGPR.

(Diameter of inhibition zone (cm)

Herbicides	*Azospirillum* sp.	*P. fluorescens*	*B. subtilis*
H_0 -Control	0	0	0
H_1-Glyphosate -800g ai/ha	0	0.7	0.7
H_2-2,4-D -1 kg ai/ha	0	0	0
H_3-Butachlor -1.25 kg ai/ha	0	0.7	0.7
H_4-Pretilachlor -750 g ai/ha	0	0.7	0.7
H_5- Paraquat -600g ai/ha	3.6	1.2	0

Mean of three replications.

Azospirillum sp. CD= 0.035. *P. fluorescens* CD= 0.03. *B. subtilis* CD= 0.044

Table 14: Interactive effect of fungicides and herbicides on *Azospirillum* sp.

(Diameter of inhibition zone (cm)

Herbicides / Fungicides	H_0- Control	H_1- Glyphosate	H_2- 2,4-D	H_3- Butachlor	H_4- Pretilachlor	H_5- Paraquat
F_0-Control	0 (0.7)	0.37 (0.9)	0 (0.7)	0.27 (0.85)	0.42 (0.94)	3.7 (2.04)
F_1- Mancozeb	1.21 (1.3)	1.81 (1.5)	1.71 (1.47)	1.72 (1.48)	1.7 (1.48)	3.9 (2.1)
F_2-Carbendazim	0.13 (0.78)	0.29 (0.86)	0 (0.7)	0.46 (0.95)	0.32 (0.87)	1.49 (1.28)
F_3-Metalaxyl	1.05 (1.24)	1.5 (1.38)	0.59 (0.99)	0.5 (0.97)	0.45 (0.92)	1.31 (1.17)
F_4-Copper oxychloride	3.27 (1.92)	2.02 (1.49)	1.66 (1.33)	1.48 (1.28)	1.5 (1.28)	2.77 (1.67)
F_5-Tridemorph	1.12 (1.25)	2.28 (1.67)	1.7 (1.53)	1.4 (1.36)	1.48 (1.4)	3.34 (1.89)

CD = 0.014 . Values in the parentheses are $\sqrt{x} + 0.05$ transformed. F x H Significant

Table 15: Interactive effect of fungicides and herbicides on *P. fluorescens*

(Diameter of inhibition zone (cm)

Herbicides / Fungicides	H_0- Control	H_1- Glyphosate	H_2- 2,4-D	H_3- Butachlor	H_4- Pretilachlor	H_5- Paraquat
F_0-Control	0.11 (0.77)	0.29 (0.86)	0 (0.707)	0.32 (0.87)	0.28 (0.86)	0.58 (1.01)
F_1- Mancozeb	0.1 (0.76)	0.12 (0.77)	0.1 (0.76)	0.1 (0.76)	0 (0.7)	0 (0.7)
F_2-Carbendazim	0.11 (0.77)	0 (0.7)	0 (0.7)	0 (0.707)	0.11 (0.77)	0 (0.7)
F_3-Metalaxyl	0.17 (0.79)	0.12 (0.77)	0.22 (0.83)	0 (0.7)	0.23 (0.83)	0.49 (0.96)
F_4-Copper oxychloride	0.7 (1.09)	0.1 (0.76)	0.1 (0.76)	0.21 (0.82)	0.1 (0.76)	0 (0.7)
F_5-Tridemorph	0.1 (0.76)	0.128 (0.78)	0.1 (0.76)	0.1 (0.76)	0 (0.7)	0 (0.7)

CD = 0.0129 .Values in the parentheses are √x + 0.05 transformed. F x H Significant

Table 16: Interactive effect of fungicides and herbicides on *B. subtilis.*

***(*Diameter of inhibition zone (cm)**

Herbicides / Fungicides	H_0- Control	H_1- Glyphosate	H_2- 2,4-D	H_3- Butachlor	H_4- Pretilachlor	H_5- Paraquat
F_0-Control	0.13 (0.77)	0.51 (0.98)	0.14 (0.78)	0.65 (1.1)	0.36 (0.90)	0.58 (1.01)
F_1- Mancozeb	1.3 (1.33)	1.79 (1.50)	1.56 (1.43)	1.65 (1.46)	1.63 (1.45)	1.68 (1.47)
F_2-Carbendazim	0.42 (0.93)	0.1 (0.76)	0 (0.7)	0.56 (1.01)	0.48 (0.97)	1.97 (1.57)
F_3-Metalaxyl	0.89 (1.16)	1.58 (1.43)	1.16 (1.28)	1.05 91.24)	1.02 (1.23)	1.41 (1.36)
F_4-Copper oxychloride	0.83 (1.15)	0.24 (0.84)	0.83 (1.15)	1.02 91.22)	0.71 (1.1)	1.59 (1.41)
F_5-Tridemorph	0.71 (1.1)	0.44 (0.94)	0.5 (0.98)	0.76 (1.12)	0.78 (1.13)	1.42 (1.3)

CD = 0.018. Values in the parentheses are √x + 0.05 transformed. F x H Significant

Table 17: Interactive effect of fungicides and insecticides on *Azospirillum* sp.

(Diameter of inhibition zone (cm)

Insecticides / Fungicides	I_0-Control	I_1-Lindane	I_2-Chlorpyriphos	I_3-Carbaryl	I_4-Lamda cyhalothirn	I_5-Imidacloprid
F_0-Control	0.5 (0.9)	1.0 (1.10	0.9 (1.1)	0.8 (1.1)	0.6 (0.9)	1.0 (1.1)
F_1- Mancozeb	2.4 (1.7)	2.1 (1.6)	2.2 (1.6)	2.1 (1.6)	1.5 (1.4)	1.9 (1.5)
F_2-Carbendazim	0 (0.7)	0.6 (0.9)	0.7 (1.0)	0.2 (0.8)	0.6 (1)	0.6 (1)
F_3-Metalaxyl	1.9 (1.5)	1.6 (1.4)	0.7 (1.1)	0.2 (0.8)	0.6 (1)	0.5 (0.9)
F_4-Copper oxychloride	2.4 (1.7)	0.6 (0.9)	0.6 (0.9)	1.9 (1.4)	3.5 (2.0)	3.7 (2.03)
F_5-Tridemorph	2.2 (1.6)	2.1 (1.6)	1.9 (1.5)	1.8 (1.5)	1.5 (1.4)	1.9 (1.5)

CD 0.014 values in the parentheses are √x + 0.05 transformed. F x I Significant

Table 18: Interactive effect of fungicides and insecticides on *P. fluorescens.*

(Diameter of inhibition zone (cm)

Insecticides / Fungicides	I_0-Control	I_1-Lindane	I_2-Chlorpyriphos	I_3-Carbaryl	I_4-Lamda cyhalothirn	I_5-Imidacloprid
F_0-Control	0.55 (1.0)	0 (0.7)	0 (0.7)	0.46 (0.9)	0.45 (0.9)	0.12 (0.77)
F_1- Mancozeb	0.22 (0.81)	0.11 (0.8)	0 (0.7)	0 (0.7)	0 (0.7)	0.3 (0.89)
F_2-Carbendazim	0.11 (0.8)	0 (0.7)	0 (0.7)	0 (0.7)	0.11 (0.8)	0 (0.7)
F_3-Metalaxyl	0.17 (0.79)	0.17 (0.79)	0.12 (0.77)	0.21 (0.83)	0.22 (0.83)	0.33 (0.88)
F_4-Copper oxychloride	0.21 (0.87)	0.3 (0.88)	0.41 (0.93)	0.1 (0.76)	0.1 (0.76)	0.1 (0.76)
F_5-Tridemorph	0.1 (0.76)	0 (0.7)	0.1 (0.76)	0.13 (0.78)	0 (0.7)	0.1 (0.76)

CD 0.0129 Values in the parentheses are √x + 0.05 transformed. F x I Significant

Compatible combinations for *P. fluorescens* were carbendazim with lindane, chlorpyriphos, carbaryl and imidacloprid. The combination of tredimorph with lindane and lamda cyhalothrin was also compatible to *P. fluorescens*. Similarly, combination of mancozeb with chlorpyriphos, carbaryl and lamda cyhalothrin did not show any inhibition to *P. fluorescens*. In the case of *B. subtilis,* (Table 19) the compatible combination was carbendazim with all insecticides. The highest inhibiting combination for *B. subtilis* was the combination of mancozeb with all insecticides.

4.7.6. **Interactive effect of insecticides and Herbicides PGPR**

The data on the interactive effect of insecticides and herbicides on PGPR are furnished in the Tables 20, 21and 22. From the Table 20, it is found that lindane with paraquat was an inhibitory combination to the growth *Azospirillum* sp. producing an inhibition zone of 3.3 cm. The combinations of 2, 4-D with carbaryl and with lamda cyhalothirn were least inhibitory to the bacterial growth. The inhibition zone ranged from 0.67 to 0.69 cm in this case. Table 21 shows that the insecticide and herbicide combinations were least inhibitory and produced a zone of 0.1 to 0.2 cm to *P. fluorescens.* Growth inhibition was not observed in the combinations of chlorpyriphos with glyphosate and with 2, 4 - D. The combination of carbaryl with paraquat was found most inhibitory to *B. subtilis* where as the combination of lindane with 2, 4-D was least inhibitory to *B. subtilis* (Table 22).

4.7.7. **Interactive effect of fungicides and insecticides on PGPR in presence herbicide glyphosate**

The results presented in tables 23, 24 and 25 show the interactive effect of fungicides and insecticide in the presence herbicide glyphosate. From Table 23, it was found that the combinations of the fungicide copper oxychloride with the insecticides carbaryl and lamda cyhalothrin in presence of glyphosate are the most inhibitory combinations to *Azospirillum* sp (3.2 cm). However, copper oxychloride with lindane, chlorpyriphos and carbaryl in presence of herbicide glyphosate did not show any inhibition of the growth of *Azospirillum* sp. In the case of *P. fluorescens*, (Table 24) the combination of tredimorph with carbaryl in presence of glyphosate

Table 19: Interactive effect of fungicides and insecticides on *B. subtilis*

(Diameter of inhibition zone (cm)

Insecticides / Fungicides	I_0-Control	I_1-Lindane	I_2-Chlorpyriphos	I_3-Carbaryl	I_4-Lamda cyhalothirn	I_5-Imidacloprid
F_0-Control	0.35 (0.9)	0.35 (0.89)	0.48 (0.97)	1.01 (1.23)	0.13 (0.78)	0.1 (0.76)
F_1- Mancozeb	1.49 (1.4)	1.12 (1.27)	1.82 91.52)	1.69 (1.48)	1.84 (1.53)	1.67 (1.47)
F_2-Carbendazim	0.47 (0.92)	0.66 (1.04)	0.52 (0.97)	0.77 (1.09)	0.49 (0.93)	0.63 (1.01)
F_3-Metalaxyl	0.97 (1.18)	1.08 (1.25)	0.97 (1.21)	1.24 (1.3)	1.64 (1.46)	1.21 (1.3)
F_4-Copper oxychloride	0.56 (1.01)	1.13 (1.23)	0.98 (1.18)	0.99 (1.21)	0.76 (1.12)	0.8 (1.12)
F_5-Tridemorph	0.87 (1.15)	0.81 (1.08)	1.03 (1.2)	0.79 (1.09)	0.66 (1.08)	0.45 (0.96)

CD = 0.018. Values in the parentheses are $\sqrt{x} + 0.05$ transformed. F x I Significant

Table 20: Interactive effect of insecticides and herbicides on *Azospirillum* sp.

(Diameter of inhibition zone (cm)

Herbicides / Insecticides	H_0-Control	H_1-Glyphosate	H_2-2,4-D	H_3-Butachlor	H_4-Pretilachlor	H_5-Paraquat
I_0-Control	0.47 (0.95)	1.6 (1.4)	1.56 (1.35)	1.36 (1.29)	1.39 (1.31)	2.96 (1.79)
I_1-Lindane	1.22 (1.23)	1.03 (1.18)	0.89 (1.1)	0.73 (1.08)	0.7 (1.06)	3.33 (1.88)
I_2-Chlorpyriphos	1.26 (1.24)	1.03 (1.18)	0.73 (1.05)	0.81 (1.1)	0.66 (1.04)	2.5 (1.61)
I_3-Carbaryl	1.21 (1.23)	1.18 (1.19)	0.41 (0.9)	0.65 (0.0)	0.61 (1.0)	2.7 (1.67)
I_4-Lamda cyhalothrin	1.37 (1.31)	1.58 (1.38)	0.98 (1.12)	1.16 (1.2)	1.27 (1.25)	1.98 (1.41)
I_5-Imidacloprid	1.24 (1.24)	1.8 (1.5)	1.1 (1.2)	1.13 (1.2)	1.3 (1.2)	2.96 (1.78)

CD 0.014. Values in the parentheses are $\sqrt{x} + 0.05$ transformed. I x H Significant

Table 21: Interactive effect of selected insecticides and herbicides on *P. fluorescens.*

(Diameter of inhibition zone (cm)

Herbicides / Insecticides	H_0- Control	H_1- Glyphosate	H_2- 2,4-D	H_3- Butachlor	H_4- Pretilachlor	H_5- Paraquat
I_0-Control	0.68 (1.07)	0.12 (0.77)	0 (0.7)	0.12 (0.77)	0.12 (0.77)	0.32 (0.87)
I_1-Lindane	0.1 (0.76)	0.1 (0.76)	0.1 (0.76)	0.1 (0.76)	0 (0.7)	0.28 (0.86)
I_2-Chlorpyriphos	0.1 (0.76)	0 (0.7)	0 (0.7)	0.11 (0.77)	0.22 (0.83)	0 (0.7)
I_3-Carbaryl	0.21 (0.82)	0.13 (0.77)	0.22 (0.83)	0.21 (0.82)	0.11 (0.77)	0.15 (0.79)
I_4-Lamda cyhalothrin	0.1 (0.76)	0.17 (0.8)	0.1 (0.76)	0 (0.7)	0.27 (0.85)	0.22 (0.83)
I_5-Imidacloprid	0.1 (0.76)	0.23 (0.84)	0.1 (0.76)	0.2 (0.82)	0 (0.707)	0.33 (0.88)

CD = 0.0129. Values in the parentheses are $\sqrt{x} + 0.05$ transformed. I x H Significant

Table 22: Interactive effect of insecticides and herbicides on *B. subtilis*

(Diameter of inhibition zone (cm)

Herbicides / Insecticides	H_0- Control	H_1- Glyphosate	H_2- 2,4-D	H_3- Butachlor	H_4- Pretilachlor	H_5- Paraquat
I_0-Control	0.37 (0.9)	0.96 (1.15)	0.77 (1.09)	0.8 (1.12)	0.93 (1.19)	0.88 (1.12)
I_1-Lindane	0.71 (1.09)	0.59 (1.0)	0.5 (0.97)	0.9 (1.18)	0.64 (1.05)	1.79 (1.49)
I_2- Chlorpyriphos	0.65 (1.04)	0.79 (1.11)	0.76 (1.08)	0.9 (1.18)	0.9 (1.18)	1.74 (1.47)
I_3-Carbaryl	1.02 (1.23)	0.77 (1.1)	0.67 (1.05)	1.19 (1.29)	1.01 (1.22)	1.84 (1.52)
I_4-Lamda cyhalothrin	0.67 (1.05)	0.99 (1.15)	0.8 (1.1)	0.99 (1.18)	0.71 (1.07)	1.35 (1.34)
I_5- Imidacloprid	0.86 (1.14)	0.57 (0.97)	0.69 (1.06)	0.89 (1.17)	0.78 (1.09)	1.06 (1.19)

CD = 0.018. Values in the parentheses are $\sqrt{x} + 0.05$ transformed. I x H Significant

Table 23: Interactive effect of fungicides and insecticide in the presence herbicide glyphosate on *Azospirillum* sp.

(Diameter of inhibition zone (cm)

Insecticides / Fungicides	I_0-Control	I_1- Lindane	I_2- Chlorpyriphos	I_3- Carbaryl	I_4-Lamda cyhalothrin	I_5- Imidacloprid	Mean
F_0-Control	0 (0.7)	0.73 (1.11)	0.76 (1.12)	0 (0.7)	0 (0.7)	0.7 (1.09)	0.36 (0.90)
F_1- Mancozeb	3.03 (1.88)	1.5 (1.41)	2.13 (1.62)	1..5 (1.41)	0.86 (1.16)	1.8 (1.51)	1.80 (1.50)
F_2-Carbendazim	0 (0.7)	0. (0.7)	0 (0.7)	0. (0.7)	0.76 (1.12)	0.96 (1.21)	0.28 (0.86)
F_3-Metalaxyl	1.9 (1.54)	1.6 (1.47)	1.46 (1.40)	0 (0.7)	2..3 (1.67)	1.66 (1.47)	1..5 (1.37)
F_4-Copper oxychloride	2.56 (1.75)	0 (0.7)	0 (0.7)	3..2 (1..92)	3.2 (1.92)	3.16 (1.91)	2..02 (1.48)
F_5-Tridemorph	2.16 (1.63)	2.3 (1.67)	1.83 (1.52)	2.36 (1.69)	2..36 (1..69)	2.63 (!.77)	2..27 (1.66)
Mean	1.61 (1.37)	1.03 (1.18)	1.03 (1.18)	1.17 (1.19)	1..58 (1.38)	1.82 (1.49)	1..37 (1.301)

CD= 0.035. Values in the parentheses are √x + 0.05 transformed . F x I x H Significant

Table 24: Interactive effect of fungicides and insecticide in the presence of herbicide glyphosate on *P. fluorescens.*

(Diameter of inhibition zone (cm)

Insecticides / Fungicides	I_0- Control	I_1- Lindane	I_2- Chlorpyriphos	I_3- Carbaryl	I_4-Lamda cyhalothrin	I_5- Imidacloprid	Mean
F_0- Control	0.7 (1.09)	0 (0.7)	0 (0.7)	0 (0.7)	1.03 (1.24)	0 (0.7)	0.29 (0.86)
F_1- Mancozeb	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0.12 (0.77)
F_2- Carbendazim	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)
F_3- Metalaxyl	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0.7 (1.1)	0.12 (0.77)
F_4-Copper oxychloride	0 (0.7)	0.6 (1)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0.1 (0.76)
F_5-Tridemorph	0 (0.7)	0 (0.7)	0 (0.7)	0.77 (0.13)	0 (0.7)	0 (0.7)	0.13 (0.78)
Mean	0.12 (0.77)	0.1 (0.76)	0 (0.7)	0.13 (0.78)	0.17 (0.8)	0.23 (0.84)	0.13 (0.78)

Values in the parentheses are √x +0.05 transformed CD= 0.03. F x I x H Significant

was found to record an inhibition zone of 0.77 cm. Excepting this combination, all other combinations of fungicide and insecticide with glyphosate were compatible to the growth of *P. fluorescens*. As far as *B. subtilis* was concerned, the combination of mancozeb with imidacloprid was the most inhibiting (2.5cm, Plate II e) insecticide where as, carbendazim combined with all insecticide in the presence of glyphosate were compatible to the growth of *B. subtilis* (Table 25).

4.7.8 **Interactive effect of the insecticides and fungicides on PGPR in presence of herbicide 2, 4-D**

Tables 26, 27 and 28 furnishes the data on the interactive effect of the insecticides and fungicides in the presence of herbicide 2, 4-D. Table 26 revealed that, in the presence of 2,4-D, the combination of copper oxychloride with lamda cyhalothrin was the most inhibitory to the growth of *Azospirillum* sp. (3.63 cm,Plate II b). The growth was not affected in the interactions where carbendazim was combined with all insecticide. As far as *P. fluorescens* was concerned, all the combinations were found compatible with no or minimal inhibitory effect (Table 27). In the case of *B. subtilis*, the highest inhibition was observed when mancozeb was applied with cholrpyriphos (2.1 cm). The interaction of all insecticides with carbendazim along with 2, 4-D was compatible to the growth of *B. subtilis*.

4.7.9 **Interactive effect of the insecticides and fungicides on PGPR in presence of herbicide Butachlor**

The interactive effects of the insecticides and fungicides in presence of herbicide Butachlor are presented in Tables 29, 30 and 31. The results presented in the Table 29 showed that the combination of copper oxychloride with the insecticide lamda cyhalothrin and imidacloprid in presence of butachlor was highly inhibitory to *Azospirillum* sp. and recorded an inhibition zone of 3.4 cm and 3.5 cm respectively. The compatible combination which did not show any inhibition on the growth of *Azospirillum* sp. was carbendazim with lindane and chlorpyriphos.Similarly,the fungicide metalaxyl with carbaryl, lamda cyhalothrin and imidacloprid did not

Table 25: Interactive effect of selected fungicides and insecticide in the presence herbicide glyphosate on *B. subtilis.*

(Diameter of inhibition zone(cm)

Insecticides / Fungicides	I_0- Control	I_1- Lindane	I_2- Chlorpyriphos	I_3- Carbaryl	I_4-Lamda cyhalothrin	I_5- Imidacloprid	Mean
F_0- Control	0.7 (1.09)	0.6 (1.04)	0.7 (1.09)	1.06 (1.25)	0. (0.70)	0 (0.70)	0.51 (0.98)
F_1- Mancozeb	1.86 (1.53)	1.26 (1.32)	1.8 (1.51)	1.33 ((1.35)	2.0 (1.58)	2.5 (1.73)	1.79 (1.50)
F_2- Carbendazim	0 (0.7)	0. (0.7)	0 (0.7)	0.6 (0.04)	0 (0.70)	0. (0.70)	0.1 (0.76)
F_3- Metalaxyl	1..9 (1.54)	1.66 (1.47)	1.5 (1.41)	0.76 (1.12)	2..23 (1.65)	1.4 (1.37)	1.57 (1.43)
F_4-Copper oxychloride	0 (0.7)	0 (0.7)	0 (0.7)	0.83 (1.15)	0.6 (1.04)	0 (0.7)	0.23 (0.83)
F_5-Tridemorph	1.26 (1.32)	0 (0.7)	0.7 (1.12)	0 (0.70)	0.6 (1.04)	0 (0.70)	0.43 (0.93)
Mean	0.95 (1.15)	0.58 (0.99)	0.79 (1.09)	0.76 (1.10)	0.98 (1.14)	0.56 (0.96)	0.77 (1.07)

Values in the parentheses are √x +0.05 transformed CD= 0.044. Fx Ix H Significant

Table 26: Interactive effect of the insecticides and fungicides in the presence of herbicide 2,4-D on *Azospirillum* sp.

(Diameter of inhibition zone (cm)

Insecticides / Fungicides	I_0- Control	I_1- Lindane	I_2- Chlorpyriphos	I_3- Carbaryl	I_4-Lamda cyhalothrin	I_5- Imidaclopri d	Mean
F_0- Control	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)
F_1- Mancozeb	2.56 (1.75)	1.73 (1.49)	1.8 (1.51)	1.7 (1.48)	0.73 (1.10)	1.7 (1.48)	1.70 (1.47)
F_2- Carbendazim	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)
F_3- Metalaxyl	1.67 (1.47)	1.06 (1.25)	0.8 (1.14)	0 (0.7)	0 (0.7)	0 (0.7)	0.58 (0.99)
F_4-Copper oxychloride	3.26 (1.94)	0 (0.7)	0 (0.7)	0 (0.7)	3.63 (2.03)	3.06 (1.88)	1.66 (1.33)
F_5-Tridemorph	1.83 (1.52)	2.53 (1.74)	1.8 (1.51)	0.73 (1.11)	2.06 (1.60)	1.23 (1.31)	1.7 (1.53)
Mean	1.55 (1.35)	0.88 (1.10)	0.73 (1.04)	0.40 (0.90)	0.97 (1.12)	1.09 (1.15)	0.94 (1.11)

Values in the parentheses are √x + 0.05 transformed CD= 0.035. F x I x H Significant

Table 27: Interactive effect of the insecticides and fungicides in the presence of herbicide 2,4-D on *P. fluorescens.*

(Diameter of inhibition zone (cm)

Insecticides / Fungicides	I_0- Control	I_1- Lindane	I_2- Chlorpyriphos	I_3- Carbaryl	I_4-Lamda cyhalothrin	I_5- Imidacloprid	Mean
F_0- Control	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)
F_1- Mancozeb	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0.6 (1)	0.1 (0.77)
F_2- Carbendazim	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)
F_3- Metalaxyl	0 (0.7)	0 (0.7)	0 (0.7)	0.6 (1)	0.7 (1.1)	0 (0.7)	0.22 (0.83)
F_4-Copper oxychloride	0 (0.7)	0 (0.7)	0.6 (1)	0 (0.7)	0 (0.7)	0 (0.7)	0.1 (0.77)
F_5-Tridemorph	0 (0.7)	0 (0.7)	0.6 (1)	0 (0.7)	0 (0.7)	0 (0.7)	0.1 (0.77)
Mean	0 (0.7)	0 (0.7)	0.2 (0.82)	0.1 (0.77)	0.12 (0.77)	0.1 (0.77)	0.09 (0.76)

Values in the parentheses are √x +0.05 transformed CD= 0.03, F X I X H Significant

Table 28: Interactive effect of the insecticides and fungicides in the presence of herbicide 2,4-D on *B. subtilis.*

(Diameter of inhibition zone (cm)

Insecticides / Fungicides	I_0-Control	I_1- Lindane	I_2- Chlorpyriphos	I_3- Carbaryl	I_4-Lamda cyhalothrin	I_5- Imidacloprid	Mean
F_0- Control	0 (0.7)	0 (0.7)	0 (0.7)	0.9 (1.2)	0 (0.7)	0 (0.7)	0.14 (0.78)
F_1- Mancozeb	1.6 (1.4)	0.9 (1.2)	2.1 (1.61)	1.6 (1.4)	1.8 (1.6)	1.5 (1.4)	1.6 (1.4)
F_2- Carbendazim	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)
F_3- Metalaxyl	1.2 (1.3)	1.03 (1.23)	1.0 (1.20)	0.8 (1.12)	1.7 (1.5)	1.3 (1.34)	1.2 (1.3)
F_4-Copper oxychloride	0.6 (1.0)	1 (1.2)	0.9 (1.2)	0.8 (1.1)	0.83 (1.2)	0.8 (1.1)	0.83 (1.2)
F_5-Tridemorph	1.2 (1.3)	0 (0.7)	0.6 (1)	0 (0.7)	0.6 (1)	0.6 (1)	0.5 (0.9)
Mean	0.8 (1.1)	0.5 (0.9)	0.8 (1.1)	0.7 (1.04)	0.8 (1.1)	0.7 (1.1)	0.7 (1.1)

Values in the parentheses are √x +0.05 transformed CD= 0.044. F x I x H Significant

Table 29: Interactive effect of the insecticides and fungicides in the presence of herbicide butachlor on *Azospirillum.*

(Diameter of inhibition zone (cm)

Insecticides / Fungicides	I_0-Control	I_1-Lindane	I_2-Chlorpyriphos	I_3-Carbaryl	I_4-Lamda cyhalothrin	I_5-Imidacloprid	Mean
F_0- Control	0 (0.7)	0.86 (1.16)	0.76 (1.12)	0 (0.7)	0 (0.7)	0 (0.7)	0.27 (0.85)
F_1- Mancozeb	2.66 (1.77)	1.6 (1.448)	2.1 (1.61)	1.83 (1.52)	0.86 (1.16)	1.26 (1.32)	1.72 (1.47)
F_2- Carbendazim	0 (0.7)	0 (0.7)	0 (0.7)	1.16 (1.29)	0.73 (1.11)	0.83 (1.15)	0.45 (0.94)
F_3- Metalaxyl	1.1 (1.26)	0.90 (1.18)	1.0 (1.22)	0 (0.7)	0 (0.7)	0 (0.7)	0.50 (0.96)
F_4-Copper oxychloride	1.9 (1.54)	0 (0.7)	0 (0.7)	0 (0.7)	3.53 (2.00)	3.46 (1.99)	1.48 (1.27)
F_5-Tridemorph	2.46 (1.72)	1.03 (1.23)	0.96 (1.2)	0.86 (1.16)	1.8 (1.51)	1.23 (1.31)	1.39 (1.36)
Mean	1.35 (1.28)	0.73 (1.07)	0.80 (1.09)	0.64 (1.01)	1.15 (1.20)	1.33 (1.20)	0.97 (1.14)

Values in the parentheses are √x + 0.05 transformed CD= 0.035. F x I x H Significant

Table 30: Interactive effect of the insecticides and fungicides in the presence of herbicide butachlor on *P. fluorescens.* (Diameter of Inhibition zone (cm)

Insecticides / Fungicides	I_0-Control	I_1-Lindane	I_2-Chlorpyriphos	I_3-Carbaryl	I_4-Lamda cyhalothrin	I_5-Imidacloprid	Mean
F_0- Control	0.7 (1.1)	0 (0.7)	0 (0.7)	1.23 (1.32)	0 (0.7)	0 (0.7)	0.32 (0.87)
F_1- Mancozeb	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0.6 (1.05)	0.1 (0.76)
F_2- Carbendazim	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)
F_3- Metalaxyl	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)
F_4-Copper oxychloride	0 (0.7)	0.6 (1)	0.63 (1.06)	0 (0.7)	0 (0.7)	0 (0.7)	0.21 (0.82)
F_5-Tridemorph	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0.6 (1)	0.1 (0.76)
Mean	0.12 (0.77)	0.1 (0.76)	0.11 (0.76)	0.21 (0.82)	0 (0.7)	0.2 (0.82)	0.12 (0.77)

Values in the parentheses are √x +0.05 transformed CD= 0.03, F x I x H Significant

cause any inhibition to *Azospirillum* sp. The combination of copper oxychloride with the insecticides lindane, chlorpyriphos and carbaryl were found compatible in the case of *P. fluorescens*. The interactive effect of insecticides and fungicides in the presence of butachlor were found compatible to *P. fluorescens* in other combinations (Table 30). The combinations of mancozeb with chlorpyriphos and copper oxychloride with carbaryl were found most inhibitory to the growth of *B. subtilis* and the zones ranged from 0.9 to 2.0 cm. The interaction of carbendazim with insecticides, lamda cyhalothrin and imidacloprid in presence of butachlor were found least inhibitory to the growth of *B. subtilis* (0.6cm).

4.7.10. **Interactive effect of the fungicides and insecticides on PGPR in presence of herbicide Pretilachlor**

The results of the study on the interaction of fungicides and insecticides on PGPR in presence of pretilachlor are described in Tables 32, 33 and 34. From the Table 32, it was revealed that the combinations of copper oxychloride with lamda cyhalothrin and imidacloprid were found highly inhibitory to *Azospirillum* sp. and the inhibition zone was 3.6 cm. The safe combinations for the growth of *Azospirillum* sp. were carbendazim with lindane, carbaryl and imidacloprid. These combinations did not show any inhibition. In the case of *P. fluorescens* the interactive effect of fungicides and insecticides in the presence of pretilachlor were found least inhibitory in the most of the combinations. As far as *B. subtilis* was concerned, the highest inhibiting combination was that of mancozeb with chlorpyriphos (1.9 cm). The combinations of carbendazim with lamda cyhalothrin and imidacloprid were found compatible to *B. subtilis* (Table 34).

4.7.11. **Interactive effect of the fungicides and insecticides on PGPR in presence of herbicide Paraquat**

Tables 35, 36 and 37 show the results of the study on the interactive effect of fungicides and insecticides on PGPR in presence of paraquat. As revealed in Table 35, for *Azospirillum* sp., paraquat alone and in combination with all fungicides and

Table 31: Interactive effect of the insecticides and fungicides in the presence of herbicide butachlor on *B. subtilis*.

(Diameter of inhibition zone (cm)

Insecticides / Fungicides	I_0-Control	I_1-Lindane	I_2-Chlorpyriphos	I_3-Carbaryl	I_4-Lamda cyhalothrin	I_5-Imidacloprid	Mean
F_0- Control	0.7 (1.1)	0.7 (1.1)	0.8 (1.12)	1.2 (1.3)	0 (0.7)	0.6 (1)	0.7 (1.1)
F_1- Mancozeb	1.8 (1.5)	1 (1.2)	2 (1.6)	1.8 (1.5)	1.97 (1.45)	1.3 (1.3)	1.65 (1.46)
F_2- Carbendazim	0 (0.7)	0.9 (1.2)	0.6 (1)	0.7 (1.1)	0.6 (1)	0.6 (1)	0.6 (1)
F_3- Metalaxyl	0.7 (1.1)	0.9 (1.2)	0.7 (1.1)	0.8 (1.12)	1.86 (1.5)	1.3 (1.34)	1.05 (1.23)
F_4-Copper oxychloride	0.7 (1.1)	1.2 (1.3)	0.8 (1.13)	1.97 (1.57)	0.8 (1.14)	0.7 (1.1)	1.02 (1.22)
F_5-Tridemorph	0.9 (1.2)	0.7 (1.1)	0.7 (1.1)	0.8 (1.14)	0.7 (1.1)	0.8 (1.14)	0.76 (1.12)
Mean	0.8 (1.12)	0.9 (1.18)	0.93 (1.18)	1.19 (1.29)	0.99 (1.18)	0.89 (1.17)	0.95 (1.19)

Values in the parentheses are √x + 0.05transformed CD= 0.044. F x I x H Significant

Table 32: Interactive effect of the fungicides and insecticides in the presence of herbicide pretilachlor on *Azospirillum.*

(Diameter of inhibition zone (cm)

Insecticides / Fungicides	I_0-Control	I_1-Lindane	I_2-Chlorpyriphos	I_3-Carbaryl	I_4-Lamda cyhalothrin	I_5-Imidacloprid	Mean
F_0- Control	0 (0.7)	0.6 (1.04)	0.60 (1.04)	0.60 (1.04)	0 (0.7)	0.70 (1.09)	0.41 (0.94)
F_1- Mancozeb	2.36 (1.69)	1.73 (1.49)	1.5 (1.41)	1.96 (1.57)	1.23 (1.31)	0 (0..7)	0.32 (0.87)
F_2- Carbendazim	0 (0.7)	0 (0.7)	0.70 (1.09)	0 (0.7)	1.23 (1.31)	0 (0.7)	0.32 (0.87)
F_3- Metalaxyl	1.83 (1.52)	0.86 (1.16)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0.45 (0.92)
F_4-Copper oxychloride	1.80 (1.51)	0 (0.7)	0 (0.7)	0 (0.7)	3.60 (2.02)	3.66 (2.20)	1.51 (1.28)
F_5-Tridemorph	2.33 (1.68)	1.0 (1.12)	1.16 (1.29)	1.06 (1.25)	1.63 (1.46)	1.70 (1.48)	1.48 (1.39)
Mean	1.38 (1.30)	0.70 (1.06)	0.66 (1.044	0.60 (0.99)	1.26 (1.24)	1.26 (1.24)	0.98 (1.14)

Values in the parentheses are √x +0.05 transformed CD= 0.035. F x I x H Significant

Table 33: Interactive effect of the fungicides and insecticides in the presence of herbicide pretilachlor on *P. fluorescens.*

(Diameter of inhibition zone (cm)

Insecticides / Fungicides	I_0- Control	I_1- Lindane	I_2- Chlorpyriphos	I_3- Carbaryl	I_4-Lamda cyhalothrin	I_5- Imidacloprid	Mean
F_0- Control	0.7 (1.1)	0 (0.7)	0 (0.7)	0 (0.7)	0.97 (1.2)	0 (0.7)	0.28 (0.86)
F_1- Mancozeb	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)
F_2- Carbendazim	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0.63 (1.06)	0 (0.7)	0.11 (0.77)
F_3- Metalaxyl	0 (0.7)	0 (0.7)	0.7 (1.1)	0.67 (1.08)	0 (0.7)	0 (0.7)	0.23 (0.83)
F_4-Copper oxychloride	0 (0.7)	0 (0.7)	0.6 (10	0 (0.7)	0 (0.7)	0 (0.7)	0.1 (0.76)
F_5-Tridemorph	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)
Mean	0.12 (0.77)	0 (0.7)	0.22 (0.83)	0.11 (0.76)	0.27 (0.85)	0 (0.7)	0.12 (0.77)

Values in the parentheses are√x +0.05 transformed CD= 0.03. F x I x H Significant

Table 34 Interactive effect of the fungicides and insecticides in the presence of herbicide pretilachlor on *B. subtilis.*

(Diameter of inhibition zone (cm)

Insecticides / Fungicides	I_0-Control	I_1- Lindane	I_2- Chlorpyriphos	I_3- Carbaryl	I_4-Lamda cyhalothrin	I_5- Imidacloprid	Mean
F_0- Control	0.7 (1.10	0 (0.7)	0.6 (10	0.9 (1. 2)	0 (0.7)	0 (0.7)	0.4 (0.9)
F_1- Mancozeb	1.7 (1.5)	1 (1.2)	1.9 (1.6)	1.7 (1.5)	1.6 (1.4)	1.8 (1.5)	1.6 (1.5)
F_2- Carbendazim	0.6 (1)	0.7 (1.10	1 (1.21)	0.6 (1)	0 (0.7)	0 (0.7)	1.6 (1.5)
F_3- Metalaxyl	0.9 (1.2)	0.9 (1.2)	0.7 (1.1)	1.1 (1.3)	1.2 (1.3)	1.4 (1.4)	1.02 (1.23)
F_4-Copper oxychloride	0.7 (1.10	0.6 (1)	0.7 (1.1)	0.7 (1.1)	0.83 (1.2)	0.8 (1.14)	0.7 (1.1)
F_5-Tridemorph	0.9 (1.2)	0.6 (1)	0.9 (1.2)	1 (1.21)	0.7 (1.1)	0.7 (1.1)	0.8 (1.14)
Mean	0.9 (1.2)	0.6 (1)	0.9 (1.2)	1 (1.2)	0.7 (1.1)	0.8 (1.14)	0.8 (1.14)

Values in the parentheses are √x +0.05 transformed CD= 0.044

F x I x H Significant

Table 35: The interactive effect of the fungicides and insecticides in the presence of herbicide paraquat on *Azospirillum.*

(Diameter of inhibition zone (cm)

Insecticides / Fungicides	I_0- Control	I_1- Lindane	I_2- Chlorpyriphos	I_3- Carbaryl	I_4-Lamda cyhalothrin	I_5- Imidacloprid	Mean
F_0- Control	2.9 (1.84)	3.53 (2.01)	3.43 (1.98)	3.93 (2.10)	3.83 (2.08)	4.36 (2.20)	3.66 (2.03)
F_1- Mancozeb	3.10 (1.89)	4.56 (2.25)	4.06 (2.13)	4.2 (2.16)	3.8 (2.07)	3.7 (2.04)	3.90 (2.09)
F_2- Carbendazim	0 (0.7)	3.60 (2.02)	3.46 (1.99)	0 (0.7)	0 (0.7)	1.86 (1.53)	1.48 (1.27)
F_3- Metalaxyl	3.83 (2.08)	4.0 (2.12)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	1.30 (1.17)
F_4-Copper oxychloride	3.63 (2.03)	0 (0.7)	0 (0.7)	4.46 (2.22)	3.60 (2.17)	4.26 (2.18)	2.76 (1.67)
F_5-Tridemorph	4.3 (2.19)	4.26 (2.18)	4.03 (2.12)	3.86 (2.09)	0 (0.7)	3.56 (2.01)	3.33 (1.88)
Mean	2.96 (1.72)	3.32 (1.88)	2.5 (1.60)	2.74 (1.66)	1.97 (1.40)	2.96 (1.78)	2.74 (1.69)

Values in the parentheses are √x + 0.05 transformed CD= 0.035. F x I x H Significant

Table 36: The interactive effect of the fungicides and insecticides in the presence of herbicide paraquat on *P. fluorescens.*

(Diameter of inhibition zone (cm)

Insecticides / Fungicides	I_0- Control	I_1- Lindane	I_2- Chlorpyriphos	I_3- Carbaryl	I_4-Lamda cyhalothrin	I_5- Imidacloprid	Mean
F_0- Control	1.2 (1.29)	0 (0.7)	0 (0.7)	0.9 (1.18)	0.7 (1.09)	0.7 (1.09)	0.58 (1.01)
F_1- Mancozeb	0.7 (1.09)	0.67 (1.08)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.70)	0.22 (0.83)
F_2- Carbendazim	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)
F_3- Metalaxyl	0 (0.7)	1.0 (1.29)	0 (0.7)	0 (0.7)	0.63 (1.06)	1.3 (1.34)	0.4 (0.95)
F_4-Copper oxychloride	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)
F_5-Tridemorph	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)
Mean	0.31 (0.87)	0.27 (0.85)	0 (0.7)	0.13 (0.78)	0.22 (0.83)	0.33 (0.87)	0.21 (0.82)

Values in the parentheses are √x + 0.05 transformed CD= 0.03. F x Ix H Significant

Table 37: The interactive effect of the fungicides and insecticides in the presence of herbicide paraquat on *B. subtilis.*

(Diameter of inhibition zone (cm)

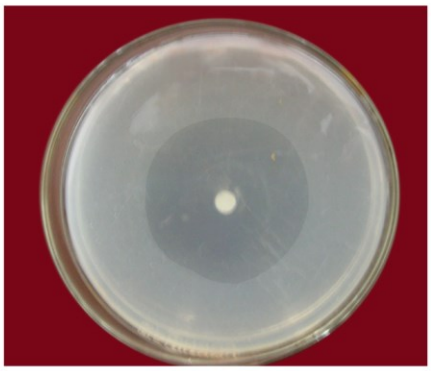
Insecticides / Fungicides	I_0-Control	I_1-Lindane	I_2-Chlorpyriphos	I_3-Carbaryl	I_4-Lamda cyhalothrin	I_5-Imidacloprid	Mean
F_0- Control	0 (0.7)	0.7 (1.1)	0.8 (1.14)	1.3 (1.32)	0.8 (1.14)	0 (0.7)	0.6 (1)
F_1- Mancozeb	1.4 (1.38)	1.33 (1.35)	1.67 (1.47)	2.03 (1.59)	1.8 (1.52)	2.8 (1.82)	1.69 (1.48)
F_2- Carbendazim	2.2 (1.64)	1.77 (1.5)	1.53 (1.43)	2 (1.58)	2.33 (1.68)	1.97 (1.57)	1.97 (1.57)
F_3- Metalaxyl	1.07 (1.25)	1.1 (1.27)	1 (1.2)	1.9 (1.55)	1.77 (1.51)	0.7 (1.1)	1.41 (1.36)
F_4-Copper oxychloride	0.6 (1.05)	3 (1.87)	2.6 (1.76)	0.87 (1.17)	0.67 (1.08)	1.8 (1.52)	1.59 (1.41)
F_5-Tridemorph	0 (0.7)	2.8 (1.83)	2.8 (1.83)	2.1 (1.6)	0.8 (1.14)	0 (0.7)	1.42 (1.3)
Mean	0.88 (1.12)	1.79 (1.49)	1.74 (1.47)	1.84 (1.52)	1.35 (1.34)	1.06 (1.19)	1.44 (1.36)

Values in the parentheses are $\sqrt{x} + 0.05$ transformed CD= 0.044

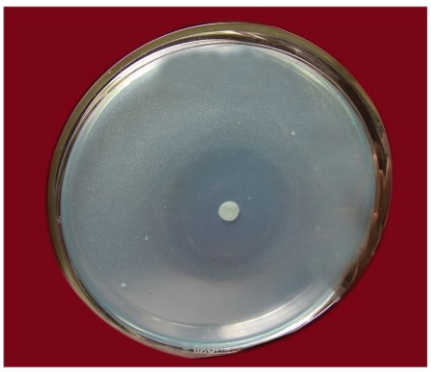
F x I x H Significant

PLATE - 2

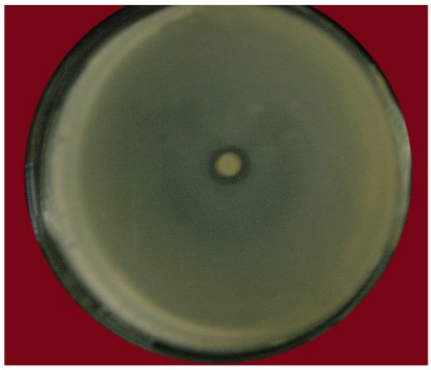
In vitro effect of combinations of chemicals on PGPR



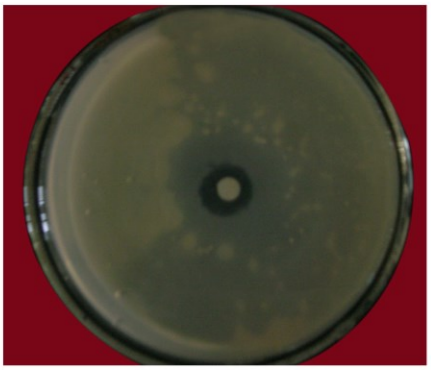
2a. *Azospirillum* sp. + CoCl + Carbaryl + Paraquat



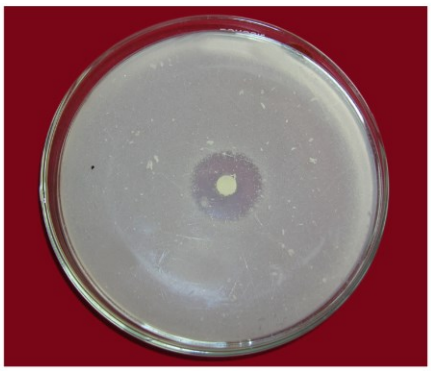
2b. *Azospiriuillum* sp. + CoCl + L. cyhalothrin + 2,4-D



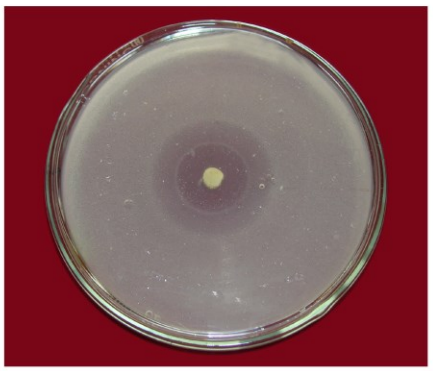
2c. *P. fluorescens* + Metalaxyl + L. cyhalothrin + Paraquat



2d. *P. fluorescens* + Metalaxyl + Imidacloprid + Paraquat



2e. *B. subtilis* + Mancozeb + Imidacloprid + Glyphosate



2f. *B. subtilis* + Mancozeb + Imidacloprid + Paraquat

insecticides was highly inhibitory. The combination of copper oxychloride with carbaryl in presence of paraquat was the most inhibitory combination against the growth of *Azospirillum* sp. (4.46 cm Plate II a). The compatible combinations in the presence of paraquat were carbendazim with carbaryl and lamda cyhalothrin. Along with this, the combinations of metalaxyl with the insecticides chlorpyriphos, carbaryl, lamda cyhalothrin and imidacloprid were also found non inhibitory to *Azospirillum* sp. As far as *P. fluorescens* is concerned, all combinations in presence of paraquat were least inhibitory (Table 36, Plate II c and d). The combinations of tredimorph with the insecticide lindane and chlorpyriphos and also the combination of mancozeb with imidacloprid were found highly inhibitory to the growth of *Bacillus subtilis* (2.8 cm Plate II f). The non inhibiting combination in the presence of paraquat was tredimorph with imidacloprid.

4.8 *In vitro* EVALUATION OF PGPR TO DIFFERENT DOSES OF PLANT PROTECTION CHEMICALS.

4.8.1 ***In vitro*** **evaluation of *Azospirillum* sp. to different doses of fungicides, insecticides and herbicides**

The results of the study are presented in Table 38. From the results furnished in Table 33, it was found that out of five fungicides tested, *Azospirillum* sp. was not sensitive to carbendazim and tredimorph. Inhibition zone of 0.9 to 1.06 cm diameter was recorded in the lower doses of metalaxyl (0.05 per cent), copper oxychloride (0.2 per cent). At the recommended doses of the fungicides, the diameter of inhibition ranged from 1.06 to 1.26 cm. At the higher doses also the same size of inhibition zone was recorded. Carbendazim and tredimorph did not show any response to any doses tested. Mancozeb at a lower dose of 0.2 per cent produced an inhibition zone of 1.16 cm. At the recommended dose of 0.3 per cent and at higher dose of 0.4 per cent, the zone was 1.26cm. Metalaxyl at its lower dose of 0.05 per cent produced a zone of 0.9 cm diameter. At the middle dose of 0.1 per cent and at higher dose of 0.2 per cent, the diameter of inhibition zone was 1.06 cm. Copper

Table 38: *In vitro* evaluation of *Azospirillum* sp. to different doses of chemicals

(Zone of Inhibition in cm)

Chemicals	Lower dose	Middle dose	Higher Dose
T_1-Mancozeb	1.16	1.26	1.26
T_2-Carbendazim	0	0	0
T_3-Metalaxyl	0.9	1.06	1.06
T_4-Copperoxychloride	1.06	1.16	1.16
T_5-Tridemorph	0	0	0
T_6-Lindane	0	0	0
T_7-Chlorpyriphos	0	0	0.7
T_8-Carbaryl	0.6	0.6	0.6
T_9-Lamda cyhalothrin	0	0	0
T_{10}-Imidacloprid	0	0	0
T_{11}-Glyphosate	0	0	0.6
T_{12}-2,4-D	0	0	0
T_{13}-Butachlor	0	0	0.7
T_{14}-Pretilachlor	0	0	0.97
T_{15}-Paraquat	3.5	3.6	4.1

Mean of three replications

oxychloride showed inhibition to *Azospirillum* sp. to all the three doses tested. The lowest dose of 0.2 per cent recorded the minimum inhibition of 1.06 cm. Maximum zone of inhibition was noticed at recommended and higher doses *ie*, at 0.3 and 0.4 per cent (1.16cm).

The three insecticides lindane, lamda cyhalothrin and imidacloprid did not cause any inhibition to *Azospirillum* sp. at any doses tested. Chlorpyriphos inhibited *Azospirillum* sp. at the higher dose of 0.1 ml (0.7 cm). At the lower (0.025 ml) and recommended doses (0.05 ml) of this insecticide, there was no growth inhibition. At all the doses of carbaryl, it inhibited the growth of *Azospirillum* sp. The lower dose of 0.1 per cent showed an inhibition of 0.6 cm. The same inhibition zone was obtained with the recommended dose (0.2 per cent) and higher dose (0.3 per cent).

Among the herbicides, only 2, 4-D did not inhibit the growth of *Azospirillum* sp. at any of the doses tested. The herbicides glyphosate and butachlor inhibited the growth only at the higher dose of 0.5ml and 0.6 ml respectively. At the lower dose of 0.3 ml, the inhibition zone recorded was 3.5 cm for paraquat, but at the dose of 0.4 ml it was 3.6 cm. When the dose was increased to 0.5 ml, the diameter of inhibition zone was also increased to 4.1 cm.

4.8.2. ***In vitro*** **evaluation of** ***P. fluorescens*** **to different doses of fungicides, insecticides and herbicides**

The data on this study are presented in the Table 39. The table shows that the two fungicides, carbendazim and tredimorph did not inhibit *P. fluorescens* at any of the doses tested. All the three doses of mancozeb tested, caused inhibition of *P. fluorescens*. The lower dose (0.2 per cent) and recommended dose (0.3 per cent) recorded an inhibition zone of 1.1 cm. This was increased with increase in concentration to 0.4 per cent. Metalaxyl at lower dose of 0.05 per cent and at recommended dose of 0.1 per cent, recorded an inhibition zone of 0.8 cm. An increase of the zone of inhibition (0.9 cm) was found at the higher dose of 0.2 per cent.

Table 39: *In vitro* evaluation of *P. fluorescens* to different doses of chemicals

(Zone of Inhibition in cm)

Chemicals	Lower dose	Middle Dose	Higher Dose
T_1-Mancozeb	1.1	1.1	1.13
T_2-Carbendazim	0	0	0
T_3-Metalaxyl	0.8	0.8	0.9
T_4-Copperoxychloride	1.5	1.53	1.6
T_5-Tridemorph	0	0	0
T_6-Lindane	0	0	0
T_7-Chlorpyriphos	0	0	0
T_8-Carbaryl	0	0	0
T_9-Lamda cyhalothrin	0	0	0
T_{10}-Imidacloprid	0	0	0
T_{11}-Glyphosate	0	0	0
T_{12}-2,4-D	0	0	0
T_{13}-Butachlor	0	0.7	0.7
T_{14}-Pretilachlor	0	0.7	0.7
T_{15}-Paraquat	0.	0.7	0.7

Mean of three replications

The results showed that copper oxychloride recorded an inhibition zone of 1.5 cm at its lower dose (0.2per cent) and the inhibition was increased with increasing concentration. At the dose of 0.3 per cent, the zone was 1.53 cm and at higher dose of 0.4 per cent, the zone was increased to 1.6 cm.

All the five insecticides tested namely lindane, chlorpyriphos, carbaryl, lamdacyhalothrin and imidacloprid did not show any inhibition to *P. fluorescens* at any of the doses tested.

The herbicides glyphosate and 2, 4-D did not inhibit the growth of *P. fluorescens* at any of the doses tested. Butachlor, pretilachlor and paraquat did not inhibit the bacterial growth at the lower dose. But these herbicides produced an inhibition zone of 0.7 cm diameter at their recommended and higher doses.

4.8.3. ***In vitro*** **evaluation of *B. subtilis* to different doses of fungicides, insecticides and herbicides.**

The results of the experiment are furnished in the Table 40. It was found that the fungicide carbendazim did not inhibit *B. subtilis* growth at any of the three doses tested. The fungicide mancozeb, Metalaxyl+M and copper oxychloride inhibited the growth of *B. subtilis* at all the three concentrations.

All the five insecticides lindane, chlorpyriphos, carbaryl, lamda cyhalothrin and imidacloprid did not inhibit the growth of *B. subtilis* at any of the three doses tested.

Among the herbicides, glyphosate and 2, 4-D did not inhibit *B. subtilis* at any of the three doses tested. Butachlor did not inhibit the bacterial growth at lower and middle doses. It showed an inhibition of 0.8 cm at the higher dose. The herbicide pretilachlor recorded an inhibition of 0.7 cm at three concentrations tested. Paraquat inhibited the growth at lower and middle dose and gave an inhibition of 0.6 cm. At the higher dose paraquat produced an inhibition zone of 0.87 cm.

Table 40: *In vitro* evaluation of *B. subtilis* to different doses of chemicals

(Zone of Inhibition in cm)

Chemicals	Lower dose	Middle Dose	Higher Dose
T_1-Mancozeb	1.03	1.07	1.4
T_2-Carbendazim	0	0	0
T_3-Metalaxyl	0.7	0.87	1.07
T_4-Copperoxychloride	0.6	0.6	0.63
T_5-Tridemorph	1.07	1.17	1.27
T_6-Lindane	0	0	0
T_7-Chlorpyriphos	0	0	0
T_8-Carbaryl	0	0	0
T_9-Lamda cyhalothrin	0	0	0
T_{10}-Imidacloprid	0	0	0
T_{11}-Glyphosate	0	0	0
T_{12}-2,4-D	0	0	0
T_{13}-Butachlor	0	0	0.8
T_{14}-Pretilachlor	0.7	0.7	0.7
T_{15}-Paraquat	0.6	0.63	0.87

Mean of three replications

4.9 TOXIC EFFECT OF CARBENDAZIM, CHLORPYRIPHOS AND 2, 4-D AT HIGHER DOSES

Among fungicides, carbendazim was found least inhibitory to PGPR. This chemical is being used extensively in rice fields**.** Among insecticides, all except carbaryl was found compatible to PGPR. But among the safe insecticides, chlorpyriphos is the one being used often in rice to control rice gall midge. In the case of herbicides use of 2, 4-D is very common in rice fields to kill sedges and dicot weeds. Thus, these three commonly used chemicals were selected for further studies on possible inhibition at higher doses.

4.9.1 **Toxic effect of chemicals at higher doses**

The results of the experiment to find out the safety limit of relatively safe chemicals selected, on the growth of three organisms are presented in the Table 41.

It is clear from the table that the growth of *P. fluorescens* was not at all affected by any of the higher dose of the chemicals tested. All the three chemicals were safe to *P. fluorescens* even at the highest dose tested. But this was not seen in the case of *Azospirillum* sp. and *B. subtilis*. It was found that *Azospirillum* sp. was inhibited when it was exposed to 0.3 g 100 ml^{-1} and above dose of carbendazim. The table revealed that application of chlorpyriphos was deleterious to *Azospirillum* sp. at 0.2 ml. However, 2, 4-D turned lethal to *Azospirillum* sp. from 0.4 g concentration onwards. The results revealed that *B. subtilis* was compatible with carbendazim and 2, 4-D even at the higher doses tested. But it was contradictory when it was exposed to chlorpyriphos. All the doses tested starting from 0.2 ml was found inhibitory to *B subtilis*. Whenever, there was inhibition of growth when exposed to a lethal dose of the chemical, there were not much variation in the size of inhibition zone. It was either 0.6 or 0.7cm.

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Table 41: Toxic effect of selected chemicals at higher doses.

(Zone of inhibition in cm)

Chemicals	Concentration (g $100ml^{-1}$)	*Azospirillum* sp.	*P. fluorescens*	*B. subtilis*
Carbendazim	0.2	0	0	0
	0.3	0.6	0	0
	0.4	0.6	0	0
Chlorpyriphos	0.1	0	0	0
	0.2	0.6	0	0.6
	0.3	0.7	0	0.7
2,4-D	0.3	0	0	0
	0.4	0	0	0
	0.5	0.6	0	0

Mean of three replications

4.10 SURVIVAL OF PGPR IN SOIL INOCULATED WITH PLANT PROTECTION CHEMICALS

4.10.1. **Population of *Azospirillum* sp. in soil**

Results of this experiment are given in the Table 42. After inoculation of *Azospirillum* sp. in to the soil, the population recorded was 35 X 10^8 cfu g^{-1} of soil.

Next day after chemical application, the maximum bacterial population was noticed in the treatment with lamdacyhalothrin (27.67 X 10^8 cfu g^{-1} of soil) and minimum population was in the treatment with glyphosate and the fungicide mancozeb (11.67 X 10^8 cfu g^{-1} of soil).

Among the fungicides, the maximum population of *Azospirillum* sp. was accounted in tredimorph (24.33 X 10^8 cfu g^{-1}) but the count was decreased as time passed. However, at the sixth week of chemical application, the count was increased. In the case of carbendazim treated soil, count noticed on next day after application was 22.0 X 10^8 cfu g^{-1} and showed an increasing trend for the next fortnight, then again decreased at sixth week. The population was reached up to 25.33 X 10^8 cfu g^{-1}. This was the maximum among the fungicide treatments.

Among the insecticides, on the next day of chemical application the maximum *Azospirillum* sp. population was recorded in lamda cyhalothrin (27.67 X 10^8 cfu g^{-1}) and the maximum count was for carbaryl (17.67 X 10^8 cfu g^{-1}). After six weeks of chemical application, the insecticide treatments, which showed the maximum population, were lamda cyhalothrin (20.3 X 10^8 cfu g^{-1}) and Chlorpyriphos (20.67 X 10^8 cfu g^{-1}). The bacterial population on the fourth and sixth weeks after chemical application were maximum in Chlorpyriphos (22.3 X 10^8 cfu g^{-1}) followed by lamda cyhalothrin (21.67 X 10^8 cfu g^{-1}).The results revealed that 2, 4-D application increased *Azospirillum* sp. population to the maximum on the next day of chemical application (17.33 X 10^8 cfu g^{-1}). There after it showed a decreasing trend as the period of application increased. After six weeks of application, the

Table 42: Population changes of *Azospirillum* in the sterilized soil as influenced by fungicides, insecticides and herbicides at their recommended dose.

(Population of *Azospirillum* sp.in soil(X 10^{8} cfu $g^{-1}$of soil)

Sl. no	Treatments	Days after chemical application			
		One day	Two weeks	Four weeks	Six weeks
1	F1-Mancozeb	11.67^{h}	9^{f}	15.67^{bcd}	12^{ef}
2	F2-Carbendazim	22^{bcd}	22.3^{ab}	20^{abc}	25.33^{a}
3	F3-Metalaxyl	12.3^{fgh}	9.67^{ef}	8.67^{e}	9^{f}
4	F4-Copperoxychloride	20^{cde}	16.3^{cd}	15^{cd}	13.67^{cdef}
5	F5-Tridemorph	24.33^{abc}	22.3^{ab}	20^{abc}	21.67^{ab}
6	I1-Lindane	25.67^{ab}	25^{a}	22.3^{a}	18.33^{bcd}
7	I2-Chlorpyriphos	24.33^{abc}	23.67^{ab}	22.3^{a}	20.67^{ab}
8	I3-Carbaryl	17.67^{def}	19^{bc}	20.67^{ab}	17.33^{bcde}
9	I4-Lamda cyhalothrin	27.67^{a}	26^{a}	21.67^{a}	20.3^{ab}
10	I5-Imidacloprid	23.67^{abc}	21.67^{ab}	21.67^{a}	19^{bc}
11	H1-Glyphosate	11.67^{h}	10.67^{ef}	10.33^{de}	10.33^{f}
12	H2-2, 4-D	17.33^{defg}	14.3^{de}	15^{cd}	13^{def}
13	H3-Butachlor	14.3^{fgh}	12.3^{def}	14.33^{d}	11.67^{ef}
14	H4-Pretilachlor	15^{efgh}	12.3^{def}	15^{cd}	11.67^{ef}
15	H5-Paraquat	12^{gh}	11.67^{def}	10.33^{de}	9^{f}

Figures followed by same letter do not differ significantly according to DMRT

Mean of three replications

maximum bacterial population was observed in the case of 2, 4-D (13.0 X 10^8 cfu g^{-1}) among the herbicides.

4.10.2 **Population of *P. fluorescens* in soil**

After the inoculation of *P. fluorescens,* the population was 28 X 10^8 cfu g^{-1} of soil. Results on this are furnished in the Table 43.

Next day after chemical application, maximum *P. fluorescence* population was noticed in the treatment with carbendazim. Population was then decreased as the time passed but the same treatment recorded the maximum population always. After two weeks, the treatment with lamdacyhalothrin also was on par with carbendazim. On next day of chemical application, the minimum population was recorded for paraquat. Butachlor was also statistically on par with this treatment. During the sixth week, metalaxyl was found detrimental to *P. fluorescens* and recorded the minimum population.

In the case of *P. fluorescens,* the maximum bacterial count was recorded in soil treated with carbendazim (25.67 X 10^8 cfu g^{-1}). The population was decreased after two weeks and thereafter showed a steady trend. After six weeks of chemical application, the count was maximum again in the treatment with carbendazim. The minimum population was recorded for metalaxyl in all cases.

Among the insecticides, maximum population of *P. fluorescens* was observed in the treatment lamda cyhalothrin (24.3X 10^8 cfu g^{-1}). The bacterial population showed a decreasing trend there after. In the case of chlorpyriphos, the population on the next day of chemical application was 21.33 X 10^8 cfu g^{-1}, which was decreased to 17.67 X 10^8 cfu g^{-1} after two weeks onwards, the population was increased and showed a steady growth up to six weeks (19.67 X 10^8 cfu g^{-1}).

Among the herbicides, maximum bacterial population was observed in pretilachlor (17.67 X 10^8 cfu g^{-1}) After six weeks, the maximum count was observed in butachlor (14.67 X 10^8 cfu g^{-1} of soil) and 2,4 -D(14.33 X 10^8 cfu g^{-1}).

Table 43: Population changes of *P. fluorescens* in the sterilized soil as influenced by fungicides, insecticides and herbicides at their recommended dose.

(Population of *P. fluorescens* in soil(X 10^{8} cfu $g^{-1}$of soil)

Sl. no	Treatments	Days after chemical application			
		One day	Two weeks	Four weeks	Six weeks
1	F1-Mancozeb	15^{def}	14.67^{abcd}	12^{bc}	11.33^{ef}
2	F2-Carbendazim	25.67^{a}	21^{a}	21.67^{a}	21^{a}
3	F3-Metalaxyl	13^{ef}	11.67^{cd}	10.67^{c}	9^{f}
4	F4-Copperoxychloride	17.67^{cde}	16.67^{abcd}	15.67^{abc}	12.67^{cdef}
5	F5-Tridemorph	19.67^{bcd}	18^{abc}	19.67^{a}	11.67^{def}
6	I1-Lindane	21.67^{abc}	21^{a}	17.67^{ab}	16.33^{abcde}
7	I2-Chlorpyriphos	21.33^{abc}	17.67^{abcd}	19.67^{a}	19.67^{abc}
8	I3-Carbaryl	18^{cde}	20.67^{a}	19.67^{a}	19.67^{abc}
9	I4-Lamda cyhalothrin	24.3^{ab}	20.33^{a}	20^{a}	20^{a}
10	I5-Imidacloprid	21.67^{abc}	20^{ab}	20.3^{a}	18.67^{abcd}
11	H1-Glyphosate	13^{ef}	14.67^{abcd}	16^{abc}	12.33^{def}
12	H2-2, 4-D	15.3^{def}	14.67^{abcd}	12.67^{bc}	14.33^{abcdef}
13	H3-Butachlor	11.33^{f}	12^{cd}	11.33^{c}	14.67^{abcdef}
14	H4-Pretilachlor	17.67^{cde}	13.67^{bcd}	10^{c}	13^{bcdef}
15	H5-Paraquat	10.67^{f}	11.33^{d}	11^{c}	11.33^{ef}

Figures followed by same letter do not differ significantly according to DMRT

Mean of three replications

4.10.3. **Population of *B. subtilis* in soil**

After inoculation of *B. subtilis,* the soil population recorded was 25 X 10^8 cfu g^{-1} of soil. Observations on this study are given in the Table 44.

Among the treatments, maximum soil population of *B subtilis* after the chemical application was recorded in lindane (23 X 10^8 cfu g^{-1}). The lowest soil population was recorded in the treatments where soil was applied with paraquat and Butachlor (9.3 X 10^8 cfu g^{-1}, 10.33 X 10^8 cfu g^{-1}). After four weeks of chemical application maximum bacterial population was in Imidacloprid applied soil and the minimum population was in soil applied with mancozeb, metalaxyl+M, glyphosate, 2, 4-D, butachlor and paraquat. After six weeks, the treatment which recorded maximum population, was again lindane which was followed by other insecticides. The deleterious chemicals after six weeks included paraquat and metalaxyl+M. 2, 4-D and mancozeb were at par with these chemicals statistically.

Among fungicides, maximum bacterial population was observed in the treatment with tredimorph (21.67 X 10^8 cfu g^{-1}), but the population was decreased as the period increased. The same trend was observed in the case of other fungicides also, carbendazim came in third position and it had a population of 19.33 X 10^8 cfu g^{-1} of soil on the next day of application.

Among insecticides, lindane was found less inhibitory to *B. subtilis* and the next safe chemical was lamda cyhalothrin. Here also, the population showed a decreasing trend as time passed. In the case of other insecticides, the population first increased and thereafter followed a decreasing trend.

In the case of *B. subtilis,* the safe herbicide found was pretilachlor and the highly inhibiting one was paraquat.

Table 44: Population changes of *B. subtilis* in the sterilized soil as influenced by fungicides, insecticides and herbicides at their recommended dose.

(Population of *B. subtilis* in soil (X 10^{8} cfu $g^{-1}$of soil)

Sl. no	Treatments	Days after chemical application			
		One day	Two weeks	Four weeks	Six weeks
1	F1-Mancozeb	13^{ef}	12^{de}	10^{c}	10.67^{c}
2	F2-Carbendazim	19.33^{abcd}	19^{abc}	15.33^{bc}	12.67^{bc}
3	F3-Metalaxyl	10^{f}	11.33^{de}	11.33^{c}	10.33^{c}
4	F4-Copperoxychloride	19.33^{abcd}	16.33^{abcd}	20.33^{ab}	17.67^{ab}
5	F5-Tridemorph	21.67^{ab}	20^{ab}	20^{ab}	18^{ab}
6	I1-Lindane	23^{a}	22.67^{a}	20.33^{ab}	19.67^{a}
7	I2-Chlorpyriphos	19.33^{abcd}	21.33^{ab}	19.67^{ab}	18^{ab}
8	I3-Carbaryl	18.33^{abcde}	20.33^{ab}	21.33^{ab}	17.33^{ab}
9	I4-Lamda cyhalothrin	22.33^{ab}	21.33^{ab}	20^{ab}	19.33^{ab}
10	I5-Imidacloprid	18^{abcde}	18.67^{abc}	21.67^{a}	17.67^{ab}
11	H1-Glyphosate	14.67^{cdef}	14.67^{bcd}	11^{c}	12.67^{bc}
12	H2-2,4-D	14.33^{def}	13^{cde}	12.67^{c}	10.67^{c}
13	H3-Butachlor	10.33^{f}	13.33^{cde}	12^{c}	15.33^{abc}
14	H4-Pretilachlor	17^{bcde}	19^{abc}	16^{abc}	13.67^{abc}
15	H5-Paraquat	9.3^{f}	8^{e}	12^{c}	10.33^{c}

Figures followed by same letter do not differ significantly according to DMRT

Mean of three replications

4.11 EFFECT OF SELECTED PESTICIDES ON THE POPULATION OF PGPR IN THE RHIZOSPHERE OF RICE.

The results of the pot culture experiments conducted to study the effect of selected plant protection chemicals at the recommended doses on the population of *Azospirillum* sp., *P. fluorescens* and *B. subtilis* in rice rhizosphere are presented below.

4.11.1. **Population of *Azospirillum* sp.in soil**

The natural population of *Azospirillum* sp. in the rice field soil was 35 X 10^8 cfu g^{-1} of the soil. Before chemical application, the population of *Azospirillum* sp. showed a general increase and it was 55 X 10^8 cfu g^{-1} of the soil. The data on the population are presented in the Table 45.

In the treatment where no plant protection chemical was applied, the maximum population was 59 X 10^8 cfu g^{-1} of the soil. But subsequently, the population showed a drastic decrease after one month and the trend was continued in the next month also. But a gradual increase in the population was observed after three months.

In carbendazim (0.1 per cent) treatment, the bacterial population was decreased from 55 X 10^8 cfu g^{-1} to 47 X 10^8 cfu g^{-1} on the next day of chemical application. The population also showed a drastic decrease to 6.3 X 10^8 cfu g^{-1} of soil after one month. After two months, the population was increased to 15.7 X 10^8 cfu g^{-1} and again at third month it was decreased to 12 X 10^8 cfu g^{-1}.

The bacterial population in the rhizosphere of rice plants treated with chlorpyriphos (500g ai/hectare) also recorded a decrease of population from 55 X 10^8 cfu g^{-1} to 40.7 X 10^8 cfu g^{-1} next day after the chemical application. One month after, there was a slow decrease in the population to 38.7 X 10^8 cfu g^{-1}. On the second

Table 45: Changes in population of *Azospirillum* sp.in rice soils.

(Population of *Azospirillum* sp. in pot soil(X 10^8 cfu g^{-1}soil)

Treatments	Day/ month after chemical application			
	One Day	One month	Two month	Three month
T_1-*Azospirillum* + Carbendazim	47	6.3	15.7	12
T_2-*Azospirillum* + Chlorpyriphos	40.7	38.7	9	9.7
T_3-*Azospirillum* + 2,4-D	23.7	8.3	8.3	14.7
T_{10}-*Azospirillum* sp.	59	15.3	8.3	11.7

Mean of three replications

month, the population drastically decreased to 9 X 10^8 cfu g^{-1} and a steady population was observed on the third month.

In the rhizosphere of plants treated with the herbicide 2, 4-D (1 kg ai/ha), the population was decreased from 55 X 10^8 cfu g^{-1} to 23.7 X 10^8 cfu g^{-1} of soil. After one month of chemical application the population was again decreased to 8.3 X 10^8 cfu g^{-1} and the same count was obtained in the second month also. But on the third month the population was increased to 14.7 X 10^8 cfu g^{-1}.

After three months of chemical application, the maximum bacterial population was observed in the treatments with 2, 4-D and carbendazim compared to control.

4.11.2 Population of *P. fluorescens* in soil

The natural population of *P. fluorescens* was 18 X 10^8 cfu g^{-1} soil. After the inoculation of *P. fluorescens* along with rice seedlings the population was increased to 35 X 10^8 cfu g^{-1} of soil. After fifteen days of bacterial inoculation the chemicals were applied at their recommended dose. The data on these experiments are given in the Table 46.

In the treatment where *P. fluorescens* was applied alone, the population was increased after 16 days of bacterial inoculation (47 X 10^8 cfu g^{-1} soil). But after one month, the population was suddenly decreased to 19.3 X 10^8 cfu g^{-1} soil and there after continued the decreasing trend.

In carbendazim treatment the population was found to reduce from 35 X 10^8 cfu /g soil to 28.3 X 10^8 cfu g^{-1} soil on the next day after chemical application. The population showed a decrease to 18.7 X 10^8 cfu g^{-1} soil after one month. On second month, the population again decreased but after that it showed an increasing trend (16.7 X 10^8 cfu g^{-1} soil).

Table 46: Changes in Soil Population of *P. fluorescens* in rice soil.

(Population of *P. fluorescens* in pot soil *(*X 10^8 cfu g^{-1}soil)

Treatments	Day/ month after chemical application			
	One day	One month	Two month	Three month
T_4-*P. fluorescens* + Carbendazim	28.3	18.7	11.7	16.7
T_5-*P. fluorescens* + Chlorpyriphos	31.7	24	24.3	21
T_6-*P. fluorescens* + 2,4-D	20.6	9.3	5.7	6
T_{11}-*P. fluorescens*	47	19.3	8.7	5.7

.

Mean of three replications

Decreasing trend of *P. fluorescens* population was also seen in rice plants treated with chlorpyriphos (31.7 X 10^{8} cfu g^{-1} soil). The population gradually decreased on first month and then showed a steady growth in second month. On third month, it again decreased to 21 X 10^{8} cfu g^{-1} soil.

Similar result was recorded in the treatments with 2,4-D also. The population came down to 20.6 X 10^{8} cfu g^{-1} soil which was again decreased to 9.3 X 10^{8} cfu g^{-1} soil after one month. After three months, the population was again decreased to 6 X 10^{8} cfu g^{-1} soil.

An overall review revealed that the maximum bacterial count maintained after three months was in the treatment with chlorpyriphos followed by those with carbendazim.

4.11.3 **Population of *B. subtilis* in soil**

The natural soil population of *B. subtilis* was very low (28 X 10^{8} cfu g^{-1} soil) compared to other beneficial microorganisms. After two weeks of inoculation, the count was increased to 36 X 10^{8} cfu g^{-1} soil. The results of the study are presented in the Table 47.

In the treatment where *B. subtilis* was applied alone, an increase in the population (41 X 10^{8} cfu g^{-1} soil) was observed. The population showed a drastic decrease after one month and thereafter a steady decreasing trend was noticed. After three months, the population recorded was 10 X 10^{8} cfu g^{-1} soil which was very low compared to initial count.

It was found that in the soils treated with carbendazim, the population was decreased to 20.7 X 10^{8} cfu g^{-1} soil from 36 X 10^{8} cfu g^{-1} soil which again drastically decreased to 6 X 10^{8} cfu g^{-1}. After two months of chemical application, the count showed a slow increase and after three months it reached to 10.3 X 10^{8} cfu g^{-1}.

Table 47: Changes in the soil population of *B. subtilis* in rice soils

(Population of *B. subtilis* in pot soil ($X10^8$ cfu g^{-1}soil)

Treatments	Day/ month after chemical application			
	One day	One month	Two month	Three month
T_7- *B. subtilis* + Carbendazim	20.7	6	7.7	10.3
T_8- *B. subtilis*+ Chlorpyriphos	21	17.7	8.7	10.7
T_9- *B. subtilis* + 2,4-D	18.7	10	7.3	6
T_{12}- *B. subtilis*	41	11.7	9.7	10

Mean of three replications

The soils treated with chlorpyriphos also showed a decreased population of 21 X 10^{8} cfu g^{-1} soil. It showed a steady decrease in first and second month. On third month, the count was increased to 10.7 X 10^{8} cfu g^{-1}. The treatment with 2, 4-D also showed the same trend as in the case of carbendazim.

4.11.4 **Effect of combined application of PGPR and plant protection chemicals on biometric characters of rice**

4.11.4.1 ***Effect on plant height***

The results are presented in the Table 48. At the time of tillering, the treatment T_5 recorded the maximum height (72.9 cm). The treatments T_{12}, T_{11},T_{10}, T_8, T_7, T_4, T_2 and T_1 were statistically on par with T_5. At the time of panicle emergence, the treatment T_{10} gave the maximum height of 110 cm. The treatments T_8, T_7, T_5 were on par with T_{10} (*Azospirillum* sp. alone). At the time of grain maturity again T_{10} recorded the maximum height and T_{12}, T_7 and T_5 were on par.

4.11.4.2 ***Effect on number of leaves***.

The data on leaf number are given in the Table 49. Significant difference in the number of leaves was observed at the time of tillering. Maximum number were recorded (5.7) in the plants where PGPR were applied alone (T_{10}, T_{11}, T_{12}) and the minimum number of 2.8 was in T_3 (*Azospirillum* sp. with 2, 4-D). At panicle emergence, the maximum leaf number was noticed in T_4 (*P. fluorescens* and carbendazim) followed by T_5 and the minimum number was in T_9 (*B. subtilis* with 2, 4-D). At grain maturity, maximum leaves were in T_2 (*Azospirillum* sp. with chlorpyriphos) and minimum were in T_6 and T_9 (*P. fluorescens*+2,4-D and *B. subtilis*+2,4-D).

Table 48: Effect of combined application of PGPR and plant protection chemicals on plant height

Treatments	Time of observations			
	One day after chemical application	Tillering	Panicle emergence	Grain maturity
T_1	27.8[a]	66.6[a]	96.8[ab]	96.8[ab]
T_2	28.34[a]	67.6[a]	95.8[ab]	98[ab]
T_3	28.1[a]	28.4[b]	58.4[ab]	58.4[b]
T_4	30.7[a]	65.2[a]	96.4[ab]	99[ab]
T_5	30.1[a]	72.9[a]	103[a]	104.2[a]
T_6	28.8[a]	44.2[ab]	63.2[ab]	63.2[ab]
T_7	30.1[a]	69.9[a]	104.4[a]	105.6[a]
T_8	31.2[a]	66.8[a]	100.4[a]	101.6[ab]
T_9	29.4[a]	28.2[b]	51.8[b]	65.8[ab]
T_{10}	29.8[a]	65.5[a]	110[a]	110[a]
T_{11}	26.5[a]	75[a]	101[ab]	102[ab]
T_{12}	29[a]	70.5[a]	101[ab]	103[a]
	NS			

Mean of five replications

Figures followed by same letter do not differ significantly according to DMRT

NS: Non significant

T_1 - Soil application of *Azospirillum* sp + Carbendazim

T_2 - Soil application of *Azospirillum* sp + Chlorpyriphos

T_3 - Soil application of *Azospirillum* sp + 2,4-D

T_4 - Soil application of *Pseudomonas fluorescens* + Carbendazim

T_5 - Soil application of *Pseudomonas fluorescens* + Chlorpyriphos

T_6 - Soil application of *Pseudomonas fluorescens* + 2, 4-D

T_7 - Soil application of *Bacillus subtilis* + Carbendazim

T_8 - Soil application of *Bacillus subtilis* + Chlorpyriphos

T_9 - Soil application of *Bacillus subtilis* +2,4-D

T_{10} - Soil application of *Azospirillum* sp.

T_{11} - Soil application of *Pseudomonas fluorescens*

T_{12} - Soil application of *Bacillus subtilis*

Table 49: Effect of combined application of PGPR and plant protection chemicals on number of leaves, tillers and productive tillers

Treatments	One day after chemical application	Tillering		Panicle Emergence			Grain maturity		
	No. of leaves	No. of leaves	No.of tillers	No.of leaves	No.of tillers	No.of productive tillers	No.of leaves	No.of Tillers	No.of productive Tillers
T_1	4^{ab} (2.16)^{ab}	5.2^{abc} (2.4)^{abc}	1.6^{b} (1.4)^{ab}	5.6^{ab} (2.5)^{ab}	1.8^{ab} (1.5)^{ab}	1.8^{abc} (1.5)^{ab}	7.4^{ab} (2.8)^{abc}	4.4^{bc} (2.2)^{bc}	4.2^{ab} (2.2)^{ab}
T_2	3.8^{ab} (2.07)^{ab}	4.2^{abc} (2.2)^{abc}	2.4^{a} (1.7)^{a}	5.2^{abc} (2.4)^{ab}	2.4^{a} (1.7)^{a}	2.4^{a} (1.7)^{a}	9^{a} (3.1)^{a}	8.4^{a} (3)^{a}	5.4^{a} (2.4)^{a}
T_3	4.2^{ab} (2.2)^{ab}	2.8^{cd} (1.6)^{cd}	0.6^{c} (1)^{c}	3.2^{bc} (1.7)^{b}	1^{cd} (1.2)^{bcd}	1^{cd} (1.2)^{bcd}	5^{ab} (2.1)^{abc}	2.6^{bc} (1.6)bc	2^{bc} (1.5)^{bc}
T_4	4.2^{ab} (2.2)^{ab}	5.4^{ab} (2.4)^{ab}	1.4^{b} 91.4)^{b}	6^{a} (2.5)^{a}	1.6^{abc} (1.4)^{abc}	1.6^{abc} (1.4)^{ab}	7.8^{ab} (2.9)^{abc}	3.8^{bc} (2)^{bc}	2.8^{bc} (1.8)^{abc}
T_5	4ab (2.1)ab	5^{abc} (2.3)^{abc}	1.6^{b} (1.4)^{ab}	5.8^{a} (2.5)^{ab}	2.4^{a} (1.7)^{a}	2^{ab} (1.6)^{a}	7.2^{ab} (2.7)^{abc}	5^{b} (2.3)^{b}	3.6^{abc} (2)^{abc}
T_6	4.2^{ab} (2.2)^{ab}	3^{bcd} (1.7)^{bcd}	0.6^{c} (1)^{c}	3.2^{b} (1.7)^{b}	1^{bc} (1.2)^{bc}	0.6^{d} (1)^{cd}	4^{b} (1.9)^{c}	2.4^{c} (1.6)^{c}	1.6^{c} (1.3)^{c}
T_7	3.6^{ab} (2.02)^{ab}	5.6^{a} (2.5)^{a}	1.8^{ab} (1.5)^{ab}	5.2^{abc} (2.4)^{ab}	2^{a} (1.6)^{a}	1.6^{abc} (1.4)^{ab}	8.2^{ab} (2.9)^{ab}	3.8^{bc} (2.1)^{bc}	3.4^{abc} (2)^{abc}
T_8	4.6^{a} (2.3)^{a}	5.2^{abc} (2.4)^{abc}	1.6^{b} (1.4)^{ab}	5.6^{ab} (2.5)^{ab}	$1.^{6abc}$ (1.4)^{abc}	1.4^{bcd} (1.4)^{abc}	7.6ab (2.8)abc	4^{bc} (2.1)^{bc}	3.2^{abc} (1.9)^{abc}
T_9	3.2^{b} (1.8)^{b}	1.8^{d} (1.3)^{d}	0.4c (0.9)^{c}	3^{c} (1.7)^{b}	0.8^{c} (1.1)^{c}	0.6^{d} (1)^{d}	4^{b} (1.98)^{bc}	2.2^{c} (1.6)^{bc}	2.25^{bc} (1.4)^{bc}
T_{10}	4.3^{a} (2.2)^{a}	5.7^{a} (2.5)^{a}	2^{ab} (1.6)^{ab}	5.3^{abc} (2.4)^{ab}	2^{a} (1.6)^{a}	2^{ab} (1.6)^{a}	7^{ab} (2.7)^{abc}	4.3^{bc} (2.2)^{bc}	3.7^{abc} (2)^{abc}
T_{11}	4.3^{a} (2.2)^{a}	5.7^{a} (2.5)^{a}	2^{ab} (1.6)^{ab}	5.3^{abc} (2.4)^{ab}	2^{a} (1.6)^{a}	2^{ab} (1.6)^{a}	7^{ab} (2.7)^{abc}	4.3^{bc} (2.2)^{bc}	3.7^{abc} (2)^{abc}
T_{12}	4.3^{a} (2.2)^{a}	5.7^{a} (2.5)^{a}	2^{ab} (1.6)^{ab}	5.3^{abc} (2.4)^{ab}	2^{a} (1.6)^{a}	2^{ab} (1.6)^{a}	7^{ab} (2.7)^{abc}	4.3^{bc} (2.2)^{bc}	3.7^{abc} (2)^{abc}

Mean of five replications NS: Non significant

Values in the parentheses are $\sqrt{x} + 0.05$ transformed; Figures followed by same letter do not differ significantly according to DMRT

T_1 - Soil application of *Azospirillum* sp + Carbendazim

T_2 - Soil application of *Azospirillum* sp + Chlorpyriphos

T_3 - Soil application of *Azospirillum* sp + 2,4-D

T_4 - Soil application of *Pseudomonas fluorescens* + Carbendazim

T_5 - Soil application of *Pseudomonas fluorescens* + Chlorpyriphos

T_6 - Soil application of *Pseudomonas fluorescens* + 2, 4-D

T_7 - Soil application of *Bacillus subtilis* + Carbendazim

T_8 - Soil application of *Bacillus subtilis* + Chlorpyriphos

T_9 - Soil application of *Bacillus subtilis* +2,4-D

T_{10} - Soil application of *Azospirillum* sp.

T_{11} - Soil application of *Pseudomonas fluorescens*

T_{12} - Soil application of *Bacillus subtilis*

4.11.4.3 ***Effect on number of tillers and productive tillers***.

The mean number of tillers and productive tillers at various growth stages are given in the above table (Table 49). Maximum tiller count was observed consistently in all stages with the treatment T_2 (*Azospirillum* sp. with chlorpyriphos). Similarly the numbers of productive tillers were maximum in the same treatment. Minimum tillers were observed in T_9 (*B. subtilis* with 2, 4-D). At panicle emergence stage lowest productive tillers were noticed in T_5 and T_9, however at grain maturity, the minimum number was recorded in T_6 *(P. fluorescens* with 2, 4-D).

4.11.5 **Post harvest observations**

The results on post harvest observations are presented in the Table 50.

4.11.5.1 ***Effect on shoot fresh weight***

Among the treatments the maximum fresh weight of 13.5g was recorded for the treatment T_2 (*Azospirillum* sp. with chlorpyriphos). The treatments T_{10},T_{11},T_{12} and T_5 came next. The minimum fresh weight of 4.7 g was recorded in T_3 (*Azospirillum* sp. with 2, 4-D).

4.11.5.2 ***Effect on shoot Dry weight***

The treatments showed the same results as in the case of fresh weight. The maximum shoot dry weight of 6.9g was recorded in T_2 (*Azospirillum* sp. with chlorpyriphos). The treatments T_1,T_5,T_{10},T_{11} and T_{12} recorded the next. Minimum dry weight of 2.2g was recorded in T_3.

4.11.5.3 ***Effect on root fresh weight***

The treatment T_5 *(P. fluorescens* with chlorpyriphos) recorded maximum root fresh weight of 17.3 g and the control treatments T_{10},T_{11},T_{12} and T_4 were at par with T_5. Minimum root dry weight was recorded in T_3 and T_6.

Table 50: Effect of combined application of PGPR and plant protection chemicals on fresh and dry weight of shoot, root and panicle and root length

Treatments	Shoot fresh weight(g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Root length (cm)	Panicle fresh weight (g)	Panicle dry weight (g)
T_1	10.6^{abc}	4.9^{ab}	10.1^{abc}	1.7^{bcd}	15.6^{abc}	4.1^{ab}	1.2^{abc}
T_2	13.5^{a}	6.9^{a}	14.5^{ab}	2.5^{ab}	15.2^{abc}	4.6^{ab}	1.3^{abc}
T_3	4.7^{c}	2.2^{c}	6.3^{c}	0.7^{c}	9.1^{c}	2.7^{b}	0.7^{c}
T_4	9.4^{abc}	4^{bc}	15.4^{a}	2.3^{a}	19.4^{ab}	4.6^{ab}	1.3^{abc}
T_5	11.5^{ab}	5.1^{ab}	17.3^{a}	3.3^{a}	21.8^{a}	4.8^{ab}	1.5^{ab}
T_6	6^{bc}	3.3^{bc}	6.8^{c}	0.7^{c}	8.9^{c}	2.9^{b}	1^{bc}
T_7	8.7^{abc}	4.4^{abc}	11.7^{abc}	1.8^{abc}	15.6^{abc}	4.8^{ab}	1.6^{ab}
T_8	8.1^{abc}	4^{bc}	10.4^{abc}	1.2^{abc}	16.2^{abc}	4.8^{ab}	1.5^{ab}
T_9	6.4^{bc}	3^{bc}	7.6^{bc}	1.0^{bc}	10.4^{bc}	2.8^{b}	0.8^{c}
T_{10}	11.2^{ab}	4.9^{ab}	15.5^{a}	2.1^{a}	17.4^{abc}	5.03^{a}	1.8^{a}
T_{11}	11.2^{ab}	4.9^{ab}	15.5^{a}	2.1^{a}	17.4^{abc}	5.03^{a}	1.8^{a}
T_{12}	11.2^{ab}	4.9^{ab}	15.5^{a}	2.1^{a}	17.4^{abc}	5.03^{a}	1.8^{a}

Mean of five replications

NS: Non significant

Figures followed by same letter do not differ significantly according to DMRT

T_1 - Soil application of *Azospirillum* sp + Carbendazim

T_2 - Soil application of *Azospirillum* sp + Chlorpyriphos

T_3 - Soil application of *Azospirillum* sp + 2,4-D

T_4 - Soil application of *Pseudomonas fluorescens* + Carbendazim

T_5 - Soil application of *Pseudomonas fluorescens* + Chlorpyriphos

T_6 - Soil application of *Pseudomonas fluorescens* + 2, 4-D

T_7 - Soil application of *Bacillus subtilis* + Carbendazim

T_8 - Soil application of *Bacillus subtilis* + Chlorpyriphos

T_9 - Soil application of *Bacillus subtilis* +2,4-D

T_{10} - Soil application of *Azospirillum* sp.

T_{11} - Soil application of *Pseudomonas fluorescens*

T_{12} - Soil application of *Bacillus subtilis*

4.11.5.4 ***Effect on root dry weight***

Maximum dry weight of 3.3g was observed in the treatment T_5 and the minimum value was recorded in the treatments T_3 and T_6.

4.11.5.5 ***Effect on root length***

The treatment T_5 recorded the maximum root length of 21.8 cm and the treatments T_3 and T_6 were recorded the minimum root length of 9.1cm and 8.9 cm respectively.

4.11.5.6 ***Effect on panicle fresh weight***

Among the treatments, maximum panicle fresh weight of 5.03 g was recorded in the treatments T_{10}, T11 and T_{12}. The minimum value was recorded in T_3 (2.7g). The treatments T_6 and T_9 were at par with T_3 statistically.

4.11.5.7 ***Effect on panicle dry weight***

Here also the same trend was noticed. The maximum panicle dry weight of 1.8 g was recorded in the treatments T_{10},T_{11} and T_{12}. The minimum dry weight of 0.7g was recorded in T_3 and T_9 was statistically at par with T_3.

4.11.6 **Nutrient content**

Effect of different treatments on plant nutrient content was analyzed and the results are given in the table 51.

Table 51: Effect of combined application of PGPR and plant protection chemicals on nitrogen, phosphorus and potassium content in plant sample

Treatments	N(per cent)	P (per cent)	K (per cent)
T_1	4.3^{a}	0.23^{d}	1.26^{def}
T_2	3.6^{b}	0.23^{d}	1.34^{bcd}
T_3	1.9^{c}	0.24^{d}	1.28^{cde}
T_4	4.4^{a}	0.23^{d}	1.28^{cde}
T_5	4.3^{a}	0.27^{ab}	1.37^{bc}
T_6	4.5^{a}	0.23^{d}	1.26^{def}
T_7	4.4^{a}	0.21^{e}	1.13^{g}
T_8	4.3^{a}	0.26^{abc}	1.17^{fg}
T_9	4.2^{a}	0.25^{bcd}	1.21^{efg}
T_{10}	4.51^{a}	0.28^{a}	1.39^{b}
T_{11}	4.3^{a}	0.25^{bcd}	1.36^{bc}
T_{12}	4.3^{a}	0.283^{a}	1.57^{a}

Mean of three replications

NS: Non significant

Figures followed by same letter do not differ significantly according to DMRT

T_1 - Soil application of *Azospirillum* sp + Carbendazim

T_2 - Soil application of *Azospirillum* sp + Chlorpyriphos

T_3 - Soil application of *Azospirillum* sp + 2,4-D

T_4 - Soil application of *Pseudomonas fluorescens* + Carbendazim

T_5 - Soil application of *Pseudomonas fluorescens* + Chlorpyriphos

T_6 - Soil application of *Pseudomonas fluorescens* + 2, 4-D

T_7 - Soil application of *Bacillus subtilis* + Carbendazim

T_8 - Soil application of *Bacillus subtilis* + Chlorpyriphos

T_9 - Soil application of *Bacillus subtilis* +2,4-D

T_{10} - Soil application of *Azospirillum* sp.

T_{11} - Soil application of *Pseudomonas fluorescens*

T_{12} - Soil application of *Bacillus subtilis*

4.11.6.1 ***Nitrogen***

Among the treatments where *Azospirillum* sp. was applied, the maximum N content was recorded in T_{10} (4.51 per cent). The lowest N content of 1.95 per cent was observed in T_3. The treatments where *P. fluorescens* was inoculated, the maximum N content of 4.5 per cent were noticed in T_6. The minimum N content was recorded for T_5 and T_{11}. In the *B. subtilis* applied treatments, maximum N content was observed in T_7 (4.4 per cent). The lowest value was recorded for T_9 (4.2 per cent).

4.11.6.2 ***Phophorus***

In *Azospirillum* sp. inoculated treatments, the maximum P content was recorded in T_{10} (0.28 per cent). The minimum P content of 0.23 per cent was recorded in T_1 and T_2. Among the treatments applied with *P. fluorescens,* the maximum P content of 0.27 per cent was recorded with T_5 and the minimum content were observed for T_4 and T_6. The treatments which were inoculated with *B. subtilis,* the maximum P content was recorded in T_{12} (0.283 per cent) and the minimum content was noticed in the treatment T_7 (0.21per cent)

4.11.6.4 ***Potassium***

Among the treatments where *Azospirillum* sp. was applied, the maximum K content was recorded in T_{10} (1.39 per cent). The lowest potassium content of 1.26 per cent was observed in T_1. The treatments where *P. fluorescens* was inoculated the maximum K content of 1.37 per cent was noticed in T_5. The minimum K value was recorded for T_6 (1.26 per cent). The treatments that were inoculated with *B. subtilis* the maximum potassium content were recorded in T_{12} (1.57 per cent). The minimum K value was for T_7 (1.13 per cent).

4.12 COMPATIBILITY CHART OF PGPR IN COMBINATION WITH AGRICULTURAL CHEMICALS

Combatibility chart describing the effect of chemicals on PGPR are presented in Table 52.

Table 52: **Compatibility chart of PGPR in combination with agricultural chemicals**

Chemicals		PGPR		
		Azospirillum sp.	*P. fluorescens*	*B.subtilis*
Fungicides	Mancozeb	N	Q	Q
	Carbendazim	C	Q	C
	Metalaxyl	Q	N	C
	Copper oxychloride	N	N	Q
	Tridemorph	C	Q	Q
Insecticides	Lindane	C	C	C
	Chlorpyriphos	C	C	C
	Carbaryl	C	Q	C
	Lamda cyhalothrin	C	C	C
	Imidacloprid	C	C	C
Herbicides	Glyphosate	C	Q	Q
	2, 4-D	C	C	C
	Butachlor	C	Q	Q
	Pretilachlor	C	Q	Q
	Paraquat	N	N	C

C – Compatible, **Q** – Questionable (Inhibition zone < 1 cm), **N** – Not compatible

Among fungicides, carbendazim was found more or less compatible with *Azospirillum* sp. and *B. subtilis* where as compatibility with *P. fluorescens* was questionable. The fungicide copper oxychloride was found not compatible with *Azospirillum* sp. and *P. fluorescens*. All insecticides except carbaryl were found compatible with *P. fluorescens* but all the insecticides were compatible to *Azospirillum* sp. and *B. subtilis*. Among the herbicides paraquat was confirmed inhibitory to *Azospirillum* sp. and *P. fluorescens*.

Compatibility charts showing the effect of combinations of fungicides , insecticides and herbicides are furnished in Tables 53- 61.

Table 53: Compatibility of *Azospirillum* sp. in combination with fungicides and insecticides

Chemicals	Lindane	Chlorpyriphos	Carbaryl	Lamda cyhalothrin	Imidacloprid
Mancozeb	N	N	N	N	N
Carbendazim	Q	Q	Q	Q	Q
Metalaxyl	N	Q	Q	Q	Q
Copper oxychloride	Q	Q	N	N	N
Tridemorph	N	N	N	N	N

Q – Questionable (Inhibition zone < 1 cm), N – Not compatible

Table 54: Compatibility of *Azospirillum* sp. in combination with fungicides and herbicides

Chemicals	Glyphosate	2, 4 -D	Butachlor	Pretilachlor	Paraquat
Mancozeb	N	N	N	N	N
Carbendazim	Q	C	Q	Q	N
Metalaxyl	N	Q	Q	Q	N
Copper oxychloride	N	N	N	N	N
Tridemorph	N	N	N	N	N

C – Compatible, Q – Questionable (Inhibition zone < 1 cm), N – Not compatible.

Table 55: Compatibility of *Azospirillum* sp. in combination with insecticides and herbicides

Chemicals	Glyphosate	2, 4 -D	Butachlor	Pretilachlor	Paraquat
Lindane	N	Q	Q	Q	N
Chlorpyriphos	N	Q	Q	Q	N
Carbaryl	N	Q	Q	Q	N
Lamda cyhalothrin	N	Q	N	N	N
Imidacloprid	N	N	N	N	N

Q – Questionable (Inhibition zone < 1 cm), N – Not compatible.

The combinations of mancozeb with all insecticides and tridemorph with all insecticides were not compatible with *Azospirillum* sp. Mancozeb, copper oxychloride and tridemorph when combined with herbicides were found inhibitory to the growth of *Azospirillum* sp. Combination of imidacloprid with all herbicide was found inhibiting the growth of *Azospirillum* sp. The combination of carbendazim with 2, 4-D was noticed as the compatible one among these combinations.

Table 56: Compatibility of *P. fluorescens* in combinations with fungicides and insecticides

Chemicals	Lindane	Chlorpyriphos	Carbaryl	Lamda cyhalothrin	Imidacloprid
Mancozeb	Q	C	C	C	Q
Carbendazim	C	C	C	Q	C
Metalaxyl	Q	Q	Q	Q	Q
Copper oxychloride	Q	Q	Q	Q	Q
Tridemorph	C	Q	Q	C	Q

C – Compatible, Q – Questionable (Inhibition zone < 1 cm).

Table 57: Compatibility of *P. fluorescens* in combinations with fungicides and herbicides

Chemicals	Glyphosate	2, 4 -D	Butachlor	Pretilachlor	Paraquat
Lindane	Q	Q	Q	C	C
Chlorpyriphos	C	C	C	Q	C
Carbaryl	Q	Q	C	Q	Q
Lamda cyhalothrin	Q	Q	Q	Q	C
Imidacloprid	Q	Q	Q	C	C

C – Compatible, Q – Questionable (Inhibition zone < 1 cm)

Table 58: Compatibility of *P. fluorescens* in combinations with insecticides and herbicides

Chemicals	Glyphosate	2, 4 -D	Butachlor	Pretilachlor	Paraquat
Lindane	Q	Q	Q	C	Q
Chlorpyriphos	C	C	Q	Q	C
Carbaryl	Q	Q	Q	Q	Q
Lamda cyhalothrin	Q	Q	C	Q	Q
Imidacloprid	Q	Q	Q	C	Q

C – Compatible, Q – Questionable (Inhibition zone < 1 cm)

The combinations were least inhibitory to *P. fluorescens* compared to *Azospirillum* sp.

Table 59: Compatibility of *B. subtilis* in combination with fungicides and insecticides

Chemicals	Lindane	Chlorpyriphos	Carbaryl	Lamda cyhalothrin	Imidacloprid
Mancozeb	N	N	N	N	N
Carbendazim	Q	Q	Q	Q	Q
Metalaxyl	N	Q	N	N	N
Copper oxychloride	N	Q	Q	Q	Q
Tridemorph	Q	N	Q	Q	Q

Q – Questionable (Inhibition zone < 1 cm), N – Not compatible.

Table 60: Compatibility of *B. subtilis* in the combination with fungicides and herbicides

Chemicals	Glyphosate	2, 4 -D	Butachlor	Pretilachlor	Paraquat
Lindane	N	N	N	N	N
Chlorpyriphos	Q	C	Q	Q	N
Carbaryl	N	N	N	N	N
Lamda cyhalothrin	Q	Q	N	Q	N
Imidacloprid	Q	Q	Q	Q	N

C – Compatible, Q – Questionable (Inhibition zone < 1 cm), N – Not compatible

Table 61: Compatibility of *B. subtilis* in combination with insecticides and herbicides

Chemicals	Glyphosate	2, 4 -D	Butachlor	Pretilachlor	Paraquat
Lindane	Q	Q	Q	Q	N
Chlorpyriphos	Q	Q	Q	Q	N
Carbaryl	Q	Q	N	N	N
Lamda cyhalothrin	Q	Q	Q	Q	N
Imidacloprid	Q	Q	Q	Q	N

Q – Questionable (Inhibition zone < 1 cm), N – Not compatible

As like for *Azospirillum* sp. the combination of mancozeb with all insecticides inhibited the growth of *B. subtilis*. Lindane and carbaryl with all herbicides were also found detrimental to *B. subtilis.* Paraquat when combined with all insecticides was found not compatible to this bacterium.

5. DISCUSSION

Agriculture depends mainly on fertilizers and plant protection chemicals in enhancing production to meet the present needs. Recently, the increasing concern over health hazards and environmental pollutions, made the scientists and farmers to think of alternate ways to improve production in an ecofriendly way. In this context, the world agriculture is now changing to non chemical methods. Using Plant Growth Promoting Rhizobacteria (PGPR) to replace many chemical pesticides and fertilizers are gaining importance now. Bacteria belonging to the genera *Azospirillum, Pseudomonas* and *Bacillus* have been found to have enormous potential as plant growth promoting agents and are now being used in agriculture as bioinoculants. Minakshi *et al.* (2005) have reported that an increase in crop yield as high as 160 per cent using PGPR were found in their experiments.

PGPR are free living, root colonizing bacteria that have beneficial effects on plants. They reduce disease severity and enhance yield of many crops. Many PGPR do have great role in integrated disease management (IDM) systems. However, only a little work has been published on integration of PGPR with other integrated disease management tools including chemicals. Only the integration of several tools including the use of PGPR and chemicals bring stability to production and disease management.

It is a fact that many farmers are using fungicides and insecticides together for the management of pests and diseases. As part of this, farmers are even advised to use biological agents and biofertilizers indiscriminately along with plant protection chemicals and fertilizers without the backing of sufficient scientific data. Hence, information on the effect of chemicals on PGPR is of much scientific and practical importance. Now, scientific data on the compatibility aspects of PGPR and agricultural chemicals is very scanty. It was with this background, the present study on ‘Population dynamics of plant growth promoting rhizobacteria under the influence of agricultural chemicals’ was undertaken.

The plant growth promoting rhizobacteria used for this study was comprised of *Azospirillum* sp*., Pseudomonas fluorescens* and *Bacillus subtilis.* The *Azospirillum* sp. and *Pseudomonas fluorescens* cultures available in the Department of Plant Pathology and *Bacillus subtilis* culture from TNAU were used for the study. These cultures were subjected to cultural and biochemical tests. The results of cultural and biochemical tests confirmed the identity of each organisms.

An experiment was conducted to test the effect of selected agricultural chemicals and their combinations on the PGPR at the recommended dose. The results of the experiment revealed that among the fungicides, copper oxychloride was extremely inhibitory to *Azospirillum* sp. and *P. fluorescens.* Tridemorph was found as the highly inhibiting fungicide against *B. subtilis*. Inhibition by copper oxychloride can well be attributed to the well known toxic effect of copper on microorganisms. The inhibitory effect of fungicides on the growth of beneficial microorganisms was studied earlier also by many scientists. In an experiment, Govindarajan and Purushothaman (1988) reported that captan inhibited the growth of *Azospirillum brasilense*. In a similar work, Gallori *et al.* (1992) found that captan and thiram reduced the growth rate of *A. brazilense.* In the present study also, the dithiocarbamate fungicide mancozeb was found inhibitory to the growth of *Azospirillum* sp. The inhibitory effect of mancozeb on the growth of *P. fluorescens* was reported by Elkins and Lindow (1999). But in the current study, it was found mancozeb was least inhibitory to *P. fluorescens*. Population *B. subtilis* was found to be inhibited by hexaconazole. Such a result was reported by Kalam and Mukherjee (2001) also. In another report, Guven *et al.* (2003) described the inhibitory effect of maneb and mancozeb on *B. subtilis* even at 0.1 ppm concentration. Present studies under *in vitro* condition revealed that carbendazim, mancozeb and tridemorph were least inhibitory to *P. fluorescens* whereas carbendazim was compatible with *Azospirillum* sp. and *B. subtilis*. These results are found to be in line with the works done by many workers. Guang *et al.* (1999) in an experiment, found positive influence on *P. fluorescens* by carbendazim. Similarly, compatibility of carbendazim with *P. fluorescens* was reported by Laha and Venkataraman (2001). In another study, Mathew (2003) reported that *P. fluorescens* strain P_{11} was compatible with

mancozeb and carbendazim. Joseph *et al.* (2003) found that the interaction of *P. fluorescens* with mancozeb did not inhibit the antagonist even at the highest concentration of the fungicide.

In the case of *B. subtilis,* carbendazim and metalaxyl were found compatible. This was substantiated by the report of Laha and Venkataraman (2001) who found that *Bacillus* strain B44 was compatible with carbendazim at 500 ppm and 1000 ppm concentrations. In another experiment, Van Eden and Korsten (2004) reported that carbendazim had no detrimental effect on *B. subtilis* population. But they found that the combination of *B. subtilis* and copper oxychloride had a negative effect on the survival of the organism.

The study on the effect of insecticides on PGPR revealed that none of them were inhibitory to the growth of *Azospirillum* sp. and *B. subtilis* at the recommended doses. Similarly, all insecticides except carbaryl were highly compatible with *P. fluorescens*. The effect of insecticides was studied by many scientists. Contradictory to the findings of the present study, Vlassak and Livens (1975) found that the pesticide oxamyl had a harmful effect on the nitrogenase activity in soil. However, studies conducted by Alvarez and Sleiman (1983), supported the present study and found that none of the insecticides tested affected the growth rate of *A. brasilense* and *A. lipoferum.* The inhibitory effect of the insecticide dicofol on *A. lipoferum* population was found out by Mano *et al.* (1988). But in another study, Rangaswami *et al.* (1989) reported that monocrotophos and quinalphos up to 5 kg/ha level, were stimulatory to *Azospirillum* sp. which was supportive to the findings of the present study. Kalam and Mukherjee (2001) in a study reported that, carbofuran inhibited the enzyme activity of *B. Subtilis.* In a study, Swarnali *et al.* (2004) found that monocrotophos and carbaryl were compatible with *B. subtilis* and this was in line with the findings of the current study where it was found that carbaryl was compatible with *B. subtilis.*

Among the herbicides tested, paraquat was the only one found highly inhibitory to *Azospirillum* sp. and *P. fluorescens.* In the case of *P. fluorescens,* 2, 4-D

was found compatible where as for *B. subtilis,* 2, 4-D and paraquat were highly compatible. The inhibitory effect of herbicides like atrazine and linuron on *A. lipoferum* was reported earlier by Alvarez and Sleiman (1983). Contradictory to the above result, Gadkari (1988) found that the herbicides metamitron and ethiozin did not affect the nitrogenase activity of *A. lipoferum* and *A. brasilens.* In an experiment, Adeleye *et al.* (2004) studied the effect of agioxone, alramex and 2, 4-D on *B. subtilis* and the results revealed 2, 4-D was most toxic to *B. subtilis.* However, results of the current study showed that 2, 4 –D was compatible with *B. subtilis*.

Among the different combinations of fungicides and herbicides, the combination of mancozeb with paraquat was highly inhibitory to *Azospirillum* sp. However, the herbicide 2, 4-D when combined with carbendazim was found compatible with *Azospirillum* sp. The combination of carbendazim with all herbicides except pretilachlor did not affect the growth of *P. fluorescens*. The combination of carbendazim with 2, 4-D was found compatible to *B. subtilis* where as paraquat with all fungicides was found highly inhibitory to *B. subtilis.* In a similar study, Nicwiadomska and Sawicka (2002) found that the fungicide carbendazim and thiram and the herbicide imazetapir affected the nitorgenase activity of microorganisms and these chemicals inhibited their multiplication.

In the experiment to study the combined effect of fungicides and insecticides, it was revealed that mancozeb with the combination of all insecticides were highly inhibitory to *Azospirillum* sp. In the case of *P. fluorescens,* the combination of copper oxychloride with carbaryl inhibited the bacterial growth. The highest inhibiting combination for *B. subtilis* was mancozeb with all insecticides. Inhibition by thiram in combination with the insecticides carbofuran and phorate on *Azospirillum* sp. growth was reported earlier by Raji and Pillai (2000).

The fungicide carbendazim, when combined with all insecticides tested, was compatible with *Azospirillum* sp. Similarly, the compatible combinations for *P. fluorescens* were carbendazim with lindane, chlorpyriphos, carbaryl and imidacloprid. In the case of *B. subtilis,* the compatible combination was carbendazim

with all insecticides. The systemic fungicide carbendazim when combined with insecticides and herbicides were found non inhibitory to the three micro organisms. Deleterious effects of the herbicides or insecticides might have been neutralized when these were mixed with a safe chemical. However, the mechanism of such action needs further investigations.

The interactive effect of insecticides and herbicides on PGPR revealed that the combination of lindane and paraquat was highly inhibitory to *Azospirillum* sp. In the case of *B. subtilis,* the combination of carbaryl with paraquat was most inhibitory to this bacterium. The combination of 2, 4-D with carbaryl and with lamda cyhalothrin was least inhibitory to *Azospirillum* sp. All insecticide and herbicide combinations were also least inhibitory to *P. fluorescens.* The combination of lindane with 2, 4-D was found least inhibitory to *B. subtilis.* In a similar study, Prast (2006) suggested that all herbicides and insecticides *viz.* glyphosate, nonanomic acid and dichlorprop-p and insecticides potassium oil, malathion and pyrethrin influenced nitrification process by *Azospirillum* sp. In an experiment, Mathew (2003) found that *P. fluorescens* strain P_{11} was compatible with mancozeb, carbendazim, imidacloprid, etofenprox, chlorpyriphos and triazophos at the recommended dosages.

Studies on the population of *Azospirillum* sp. in soil presented in the figures 1, 2 and 3 revealed that the next day after chemical application, the bacterial population was decreased from the initial population of 35 x 10^8 cfu g^{-1} to 22 X 10^8 cfu g^{-1} in all treatments with chemicals. Minimum decrease in population was recorded in the soil treated with lamda cyhalothrin (27.67 X 10^8 cfu g^{-1}) and the maximum decrease in population was recorded in the treatment with the herbicide glyphosate (11.67 X 10^8 cfu g^{-1}). As time progressed, varying changes in the population was observed. After two weeks, maximum *Azospirillum* sp. count was recorded in lindane treated soil (Figure2) and the minimum was in mancozeb treated soil (Figure 1). The maximum bacterial population of 22.3 x 10^8 cfu g^{-1} was recorded for lindane and chlorpyriphos treated soils after four weeks of chemical application (Figure 2). The minimum population was observed for butachlor treated soil during this period (Figure 3).

Fig : 2 Population of *Azospirillum* sp.in soil after application of insecticides

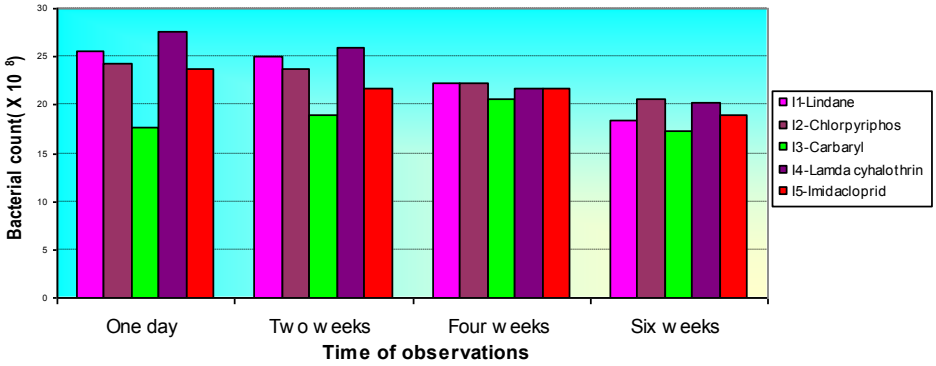


Fig: 1 Population of *Azospirillum* sp. in soil after application of fungicides

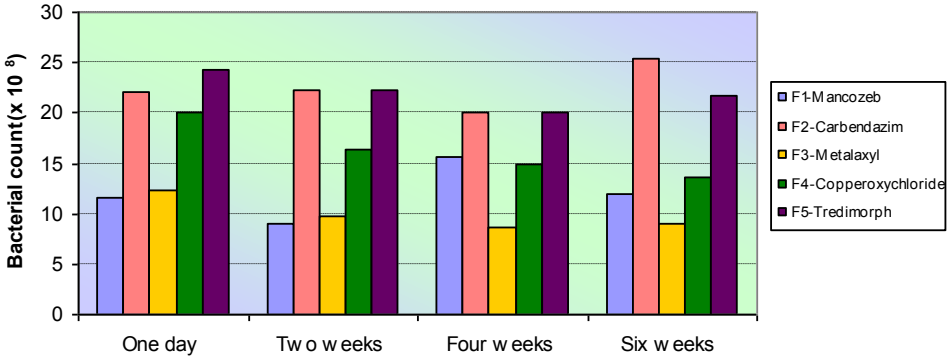
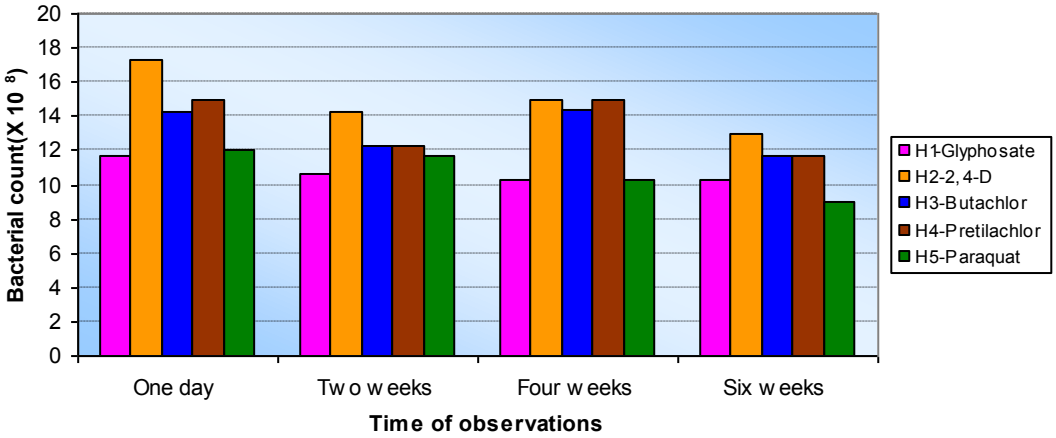


Fig : 3 Population of *Azospirillum* sp.in soil after application of herbicides



The stimulating effect of hexachlorocyclohexane on the population of *Azospirillum* sp. was reported earlier by Mahapatra and Rao (1981). In an experiment, Jena *et al.* (1987) reported that the insecticide carbofuran increased the Indole Acetic Acid production and nitrogen fixation by *Azospirillum* sp. Contrary to these findings, Govindarajan and Purushothaman (1988) reported that the seed dressing fungicide captan decreased the *Azospirillum brasilense* population. Ciocco *et al.* (1997) studied in detail about the effect of thiram and mancozeb on *A. brasilense* and found that these fungicides inhibited the growth of this bacterium even at lower concentrations.

The initial population of *P. fluorescens* in soil was found to decrease in all treatments with the test chemicals after the chemical application. On next day of chemical application, the cell count of 10.67 x 10^8 cfu g^{-1} was found in the treatment with paraquat. Here, the population was decreased from an initial population of 28x10^8 cfu g^{-1}. The maximum *P. fluorescens* population of 25.67 x 10^8 cfu g^{-1} was observed for Carbendazim treated soil. Experiments conducted by Laha and Venkataraman (2001) also support these results. They reported that *P. fluorescens* was compatible with Carbendazim at 500 ppm and 1000 ppm concentrations.

Population data of *P. fluorescens* in soil after chemical application presented in Figures 4, 5 and 6. It is clear from the figures that, the treatment with carbendazim maintained the population steady till sixth week (Figure 4). After two weeks, other treatments were also found on par with carbendazim. Another inference from the experiment was that, generally the insecticides maintained a higher population (Figure 5) compared to herbicides and fungicides. These points out that, insecticides are relatively safe to be used with *P. fluorescens,* compared to fungicides and herbicides. Mathew (2003) also reported that *P. fluorescens* strain P_{11} was compatible with imidacloprid, etofenprox and chlorpyriphos at the recommended dose for field use.

Fig : 4 Population of *P. fluorescens* in soil after application of fungicides

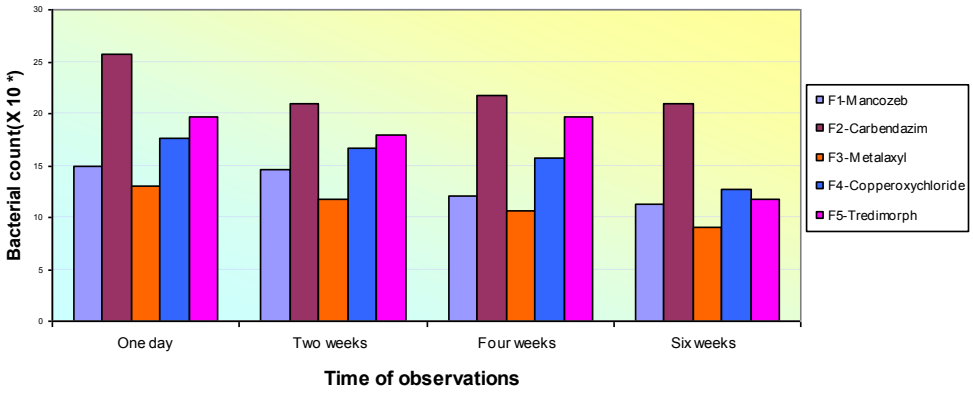


Fig : 5 Population of *P . fluorescens* in soil after application of insecticides

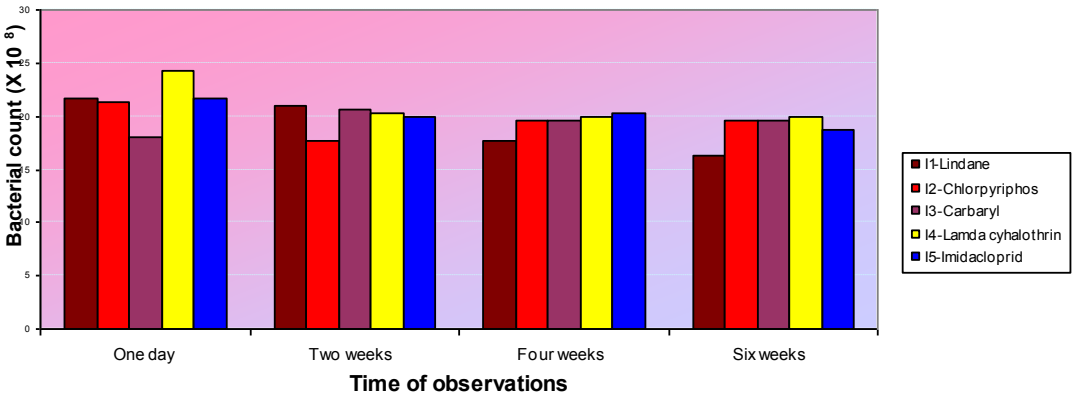
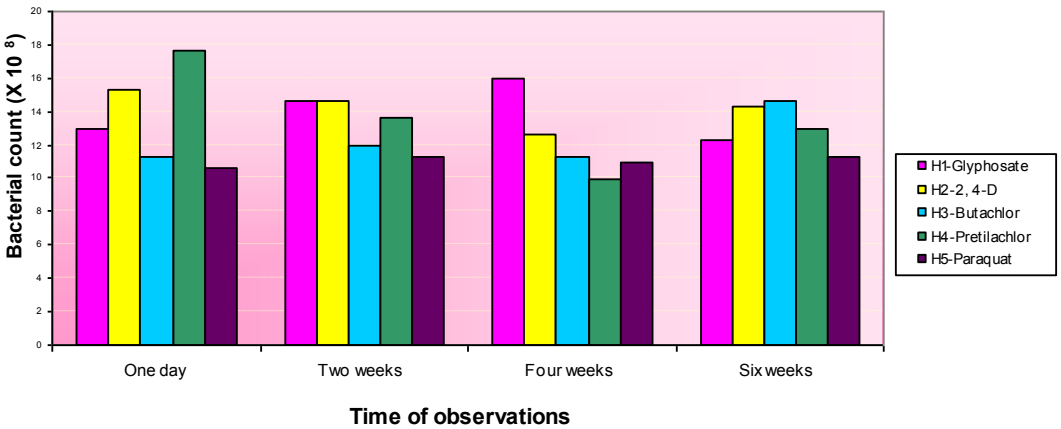


Fig : 6 Population of *P. fluorescens* in soil after application of herbicides



a low population of the test bacteria throughout this experiment. However, a contradictory report was published by Sabet *et al*. (2000). He reported that a low dose of metalaxyl and copper oxychloride in combination with *P. fluorescens* effectively reduced the damping off in tomato. In another experiment, Mathew (2003) and Joseph *et al.* (2003) reported that mancozeb was compatible with *P. fluorescens*

The figures 7, 8 and 9 presents the changes in the population of *B. subtilis* when exposed in chemicals applied soil. It is clear from the figures that the initial population of 25 x 10^8 cfu g^{-1} showed a decreasing trend after the chemical application. On the next day of chemical application, the population reached a minimum of 23 x 18^8 cfu g^{-1} when applied with the insecticide Lindane (Figure 8). The results revealed that among all the treatments, the insecticides were comparatively safe to *B. subtilis*. Population of PGPR was brought down to lower level when applied with all herbicides. The fungicides, mancozeb and metalaxyl were also similar in their effects. The population of *B. subtilis* was lower when applied with paraquat, metalaxyl and butachlor (Figure 7, 9). In a similar study, Kalam and Mukherjee (2001) reported that the fungicide hexaconazole strongly affected the count of *B. subtilis* in soil. Studies by Guven *et al.* (2003) also reported the inhibitory effect of mancozeb on *B. subtilis.* In another study, Adeleye *et al*. (2004) found out the toxic effect of herbicides and their results revealed a reduction in population initially.

Two weeks after application, the same trend mentioned in the above situation was noticed in the case of *B. subtilis* also. The trend continued up to the end. In general, detrimental chemicals found from the study include paraquat, metalaxyl, glyphosate and mancozeb. In a study, Van Eeden and Korsten (2004) found that the combination of *B. subtilis* and copper oxychloride had a negative effect on the survival of this organism. The safe chemicals for *B. subtilis* according to present study included all insecticides, carbendazim and butachlor.

Fig : 7 Population of *B. subtilis* in soil after application of fungicides

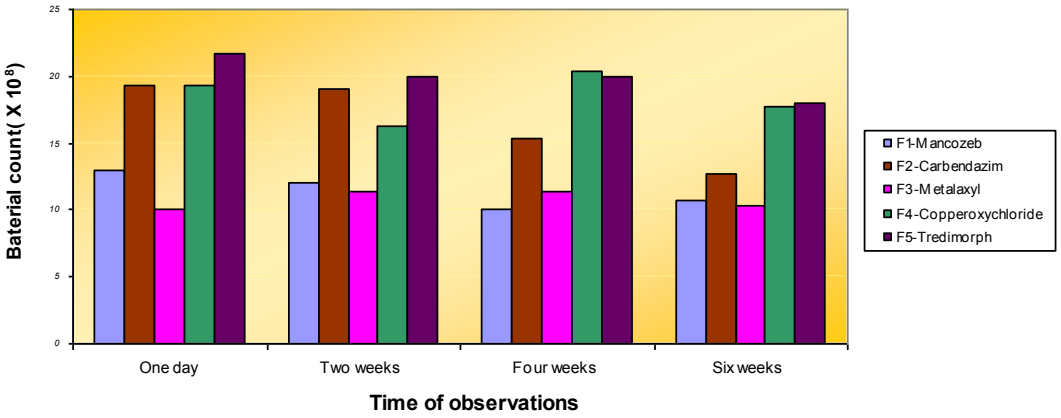


Fig : 8 Population of *B . subtilis* in soil after application of insecticides

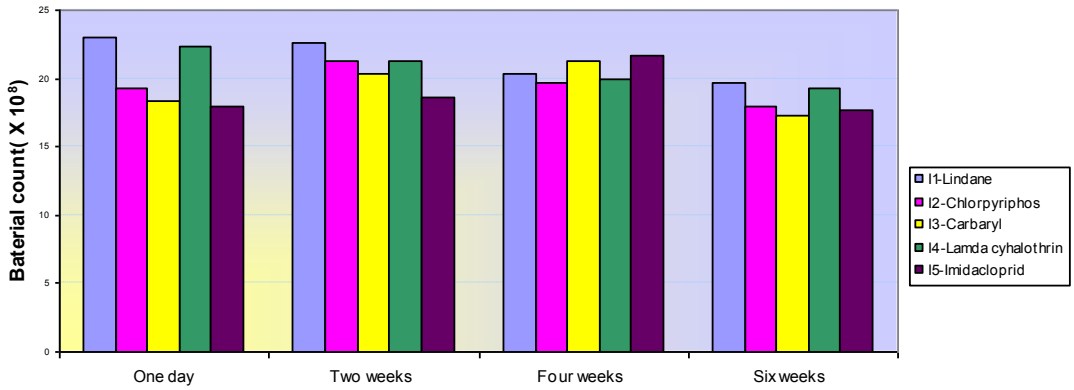
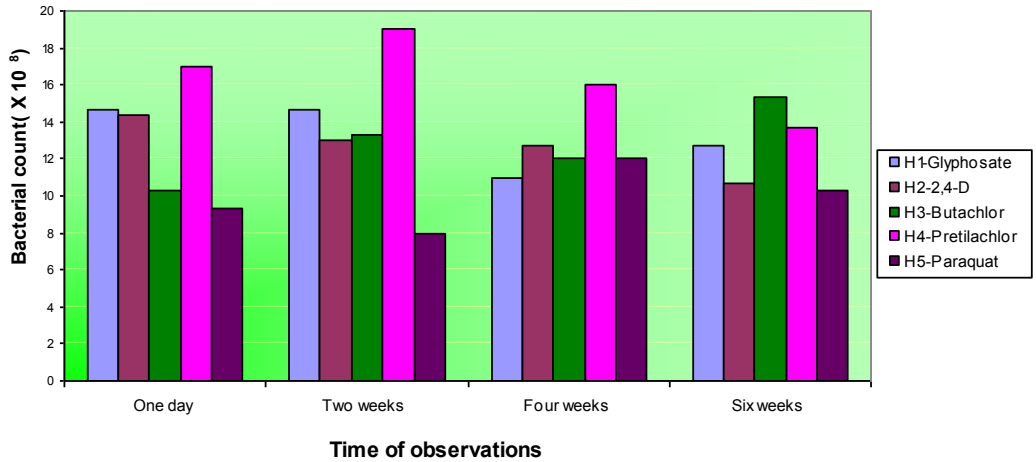


Fig : 9 Population of *B . subtilis* in soil after application of herbicides



There are contradictory reports also on the inhibition by agricultural chemical on the enzymatic activities of beneficial microorganisms under *in vitro* and *in vivo* conditions. Many workers (Mahapatra and Rao (1981), Jena *et al.* (1987), Rangaswami *et al*. (1989), Laha and Venkataraman (2001), Singh *et al.* (2003) and Thankamani *et al*. (2003)) reported the stimulatory effect of agricultural chemicals on the population of PGPR. On the other hand, the inhibitory effects of agricultural chemicals on the microbial activities of these beneficial microbes were also reported by many workers (Gadkari (1988), Mano *et al*. (1988), Elkins and Lindow (1999), Giraud *et al*. (2001), Bhavani (2004) and Van Eeden and Korsten (2004)).

Beneficial rhizobacteria and agricultural chemicals are also compared under field conditions to study the influence of chemicals on the microorganisms in the natural soil environment. This was conducted to confirm the results of the *in vitro* and *in vivo* study which were carried out under the controlled laboratory condition. Among the 15 chemicals, one chemical which was relatively safe to PGPR was selected. The selection was based on the *in vitro* study since under sterile controlled conditions the results may be more reliable. From the group of fungicides, carbendazim and tridemorph were found compatible with the three plant growth promoting rhizobacteria. Between these two fungicides, carbendazim was used in rice field for seed treatment, seedling treatment and foliar application. Also under the *in vitro* study, where the combination of fungicides, insecticides and herbicides were evaluated, carbendazim was found least inhibitory to the three rhizobacteria tested. Guang *et al. (*1999) and Laha and Venkatraman (2001) in their studies found that carbendazim was compatible with *P. fluorescens* and *B. subtilis* at its recommended dose. Among insecticides, all of them except carbaryl were found safe to these PGPR. The insecticide chlorpyriphos is being used to control the major rice pests stem borer and gall fly and also used for root dipping. Like carbendazim, chlorpyriphos was also found least inhibitory to the test microorganisms. Based on these criteria, it was selected as safe insecticide for the field experiment. In a study Mathew (2003) found that the recommended dose of chlorpyriphos was compatible with the beneficial microorganisms. The safe herbicides under *in vitro* study were 2, 4-D, butachlor, glyphosate and pretilachlor. In the combination studies 2, 4-D was

found less inhibitory to the PGPR and it is widely using in rice fields to control dicot weeds and sedges. In this experiment, the crop used was rice, a monocot in which 2, 4-D is being used widely.

In the next stage, a pot culture experiment was conducted to find the effect of selected chemicals on the population of PGPR in the rhizosphere of rice. After the application of carbendazim, chlorpyriphos and 2, 4-D, the population of *Azospirillum* sp. was decreased where as, a slight increase was noticed in the pot applied with *Azospirillum* sp. alone (59 x 10^8 cfu g^{-1}).From figure 10, it is evident that on the next day of chemical application, the lowest bacterial count was in the treatment where *Azospirillum* sp. was applied with 2, 4-D. Here a decrease to 23 X 10^8 cfu g^{-1} from 55 X 10 ^{8} cfu g^{-1} was noticed. Alvarez and Sleiman (1983) found that the herbicides atrazine and linuron increased the rate of acetylene reduction in *A. lipoferum.* Supported to this result, Gomez *et al.* (1999) reported that profenofos significantly reduced the dinitrogen fixation in *A. brasilense.* According to Sreenivasulu *et al.* (2001) maximum activity of all diazotroph isolate was observed after 14 days of its inoculation.

A drastic decrease was noticed in the population of *Azospirillum* sp. after one month in the treatments where carbendazim and 2, 4-D were applied. Treatment with chlorpyriphos showed a steady decrease in the population. But in second and third month, the population showed a decreasing trend. This decrease in the population was may be due to the stagnant water prevailing in the pots. The inability of the aerobic bacteria to compete effectively in a water logged situation may be a reason for the low population of these bacteria. The effect of chemicals also might have added to their poor competence. Another reason may be due to the lack of competitive ability against the native microflora. Bashan (1999) reported that inoculation with *Azospirillum* sp. often failed to increase the crop yield due to poor survival and lack of establishment and competitive ability against the native microflora. In another study Amalia *et al.* (1988) observed that the inoculation of *Panicum miliaceum* roots with a wild *Azospirillum* sp. strain and an IAA overproducing mutant resulted in an increase in root elongation by the wild strain

while the mutant was ineffective. Similarly, Pereira *et al.* (1998) also found failure of inoculated *Azospirillum* sp. in sorghum and rice.

In the case of *P. fluorescens,* the population immediately after the bacterial inoculation was $35x10^8$ cfu g^{-1}. On the next day of chemical application, the population was increased to 47 x 10^8 cfu g^{-1}(Figure 11). But in the treatments with chemical, the application recorded a decrease. After one month, the population in all treatments and control showed a decrease and this trend continued up to third month. Contradictory to such a result Guang *et al.* (1999) reported that when carbendazim was applied to soil, it enhanced the population of *P. fluorescens* strain P_{32}. Mathew (2003) in his study found that carbendazim and chlorpyriphos were compatible with *P. fluorescens* strain P_{11} at the recommended dose for field use.

It can be presumed that, decrease in the bacterial population after one month could be due to the anaerobic condition in the pots. The decrease in population may be due to poor root colonization by the bacteria. Srivastava *et al.* (1999) in their study on wheat found that the *P. fluorescens* population level increased during the early root expansion, and reaching a more or less constant level which was followed by a decline in population. In the present study also, at the initial period an increased population was noticed and as the plant grow, the population in the soil recorded a decreasing trend.

The data furnished in the Figure 12 indicate that population of *B. subtilis* was increased from 28 x 10^8 cfu g^{-1} to 36 x 10^8 cfu g^{-1} after inoculation. In the treatments inoculated with *B. subtilis* also, the population recorded a decrease after the application of the three chemicals. The decreasing trend continued up to the third month. The treatment applied with *B. subtilis* alone recorded an increase one day after chemical application but after one month, it also recorded a decreasing trend. Benizri *et al.* (2001) stated that colonization by beneficial bacteria was influenced by many biotic and abiotic parameters.

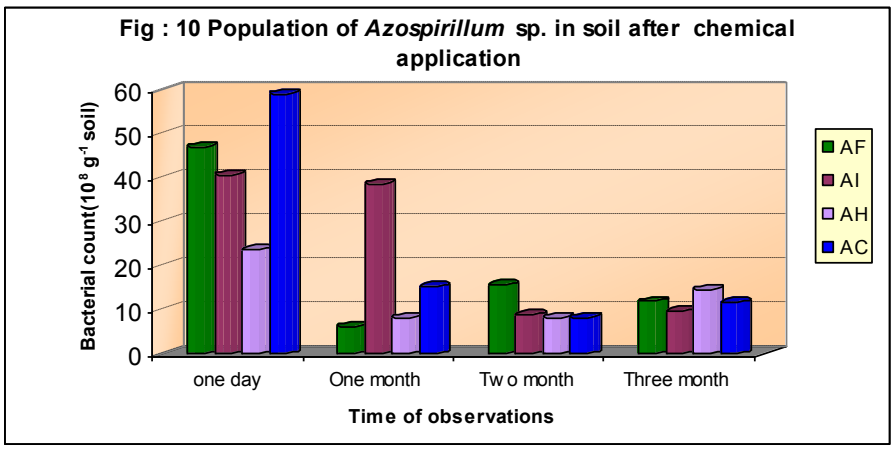


Fig : 11 Population of *P. fluorescens* in soil after chemical application

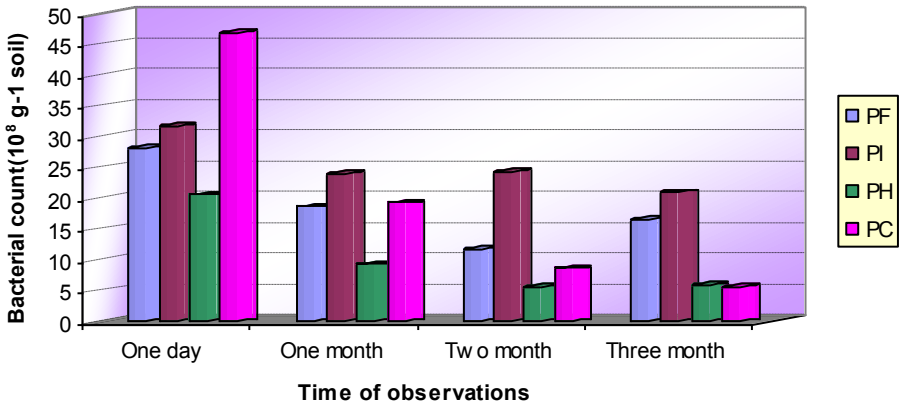
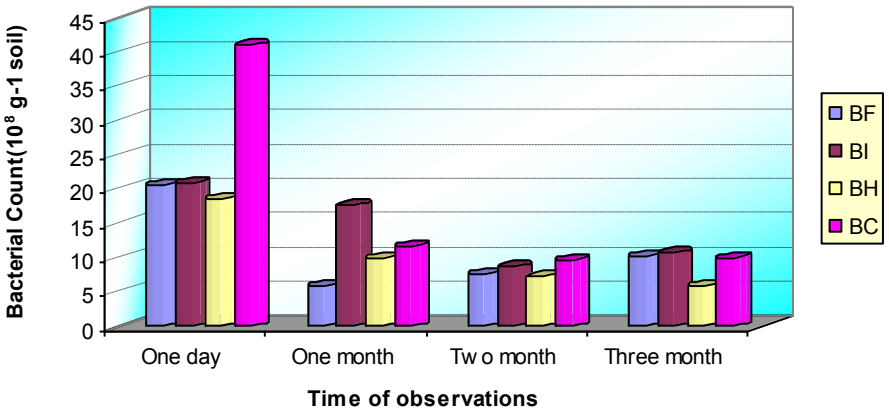


Fig : 12 Population of *B. subtilis* in soil after chemical application



The chemicals carbendazim, chlorpyriphos and 2, 4-D when applied to soil, resulted in decrease in the bacterial population. The anaerobic condition also might have enhanced the inhibitory effect of these chemicals. But the compatible nature of carbendazim and *Bacillus* spp. was reported by Laha and Venkataraman (2001). Guven *et al*. (2003) and Adeleye *et al.* (2004) found that fungicides, insecticides and herbicides adversely affected *B. subtilis* population. Under rice field condition, the application of carbendazim brought down the population of bacteria immediately after the chemical application (Saifunneesa, 2001). Literature on the fate of microbial population due to chemical treatments and PGPR application is scanty.

The present study had also attempted to correlate the growth parameters of rice with the influence of PGPR alone and in combination with chemicals. Studies on the growth parameter like plant height revealed that from the time of tillering onwards, there are significant differences among treatments. The results are presented in Figure 13. At the time of tillering, the maximum height recorded was in the treatment T_{11} (*P. fluorescens* alone). The treatment where *P. fluorescens* was applied with chlorpyriphos also was found on par. At the time of flowering and grain maturity, the superior treatment was T_{10} (*Azospirillum* sp. alone). This result was in line with the findings of Tien *et al.* (1979) where, he found that *Azospirillum* sp. produced phytohormones like Indole Acetic acid, Gibberllic acid and Kinetin which help the plants in enhancing biomass production. Chi *et al.* (1998) in his field trial found that after 30 - 40 days of *Azospirillum* sp. inoculation the seedlings appeared taller and more vigorous. In a similar study Kumar (1998) also reported that the application *P. fluorescens* with many crops increased the shoot height.

There were other treatments also found superior and on par with $T_{11.}$ These include the treatment T_5 (*P. fluorescens* with chlorpyriphos) and T_7 (*B. subtilis* with chlorpyriphos) along with these PGPR had a synergistic effect on the crop plants.

The results on number of leaves, number of tillers and productive tillers showed significant difference among the treatments. The maximum number of leaves was observed at the time of tillering where all the three PGPR were applied

separately. However, at the time of flowering the maximum number was in the treatment with *P. fluorescens* and carbendazim. At grain maturity, the maximum leaves were observed in *Azospirillum* sp. with chlorpyriphos treated pots. These results indicated that the chemicals along with these beneficial rhizobacteria had a positive influence on the vegetative growth of rice plants.

The study revealed that the maximum tillers and productive tillers were in the treatment with *Azospirillum* sp. applied with chlorpyriphos. This substantiates the synergistic action of chlorpyriphos and PGPR.

The study had also attempted to correlate the post harvest observations with the influence of treatments. The observations on fresh and dry weight of shoot, fresh and dry weight of root, length of root and fresh and dry weight of panicle were recorded and analyzed. The treatment chlorpyriphos applied with the PGPR *Azospirillum* sp. and *P. fluorescens* recorded maximum fresh and dry weight of shoot, fresh and dry weight of root and length of root. These results are supported by the findings of Hades and Okon (1987), Amalia *et al.* (1998), and Diby and Sarma (2006). The result points out that, such combinations of insecticides and PGPR are having some synergistic effect on plant growth.

In the case of panicle fresh and dry weight, the maximum value was recorded in the control treatments where the PGPR was applied alone (T_{10}, T_{11}, and T_{12}). Kumar (1998), Vivek *et al.* (2001), Sajindrenath *et al.* (2002) and Gopal *et al.* (2006) in their studies found the yield increasing capacity of plant growth promoting rhizobacteria.

Nutrient analysis of the plant samples were done to verify the effect of different treatments in the uptake of the three essential nutrients and the results are presented in Table 14. The maximum nitrogen content was recorded in the treatments where PGPR was applied alone and there are other treatments also which were found on par with these. The treatments *viz. Azospirillum* sp. with chlorpyriphos, *Azospirillum* sp. with 2, 4-D was found inferior. All other treatments contributed

Fig : 13 Effect of combinations of chemicals and PGPR on plant height of rice

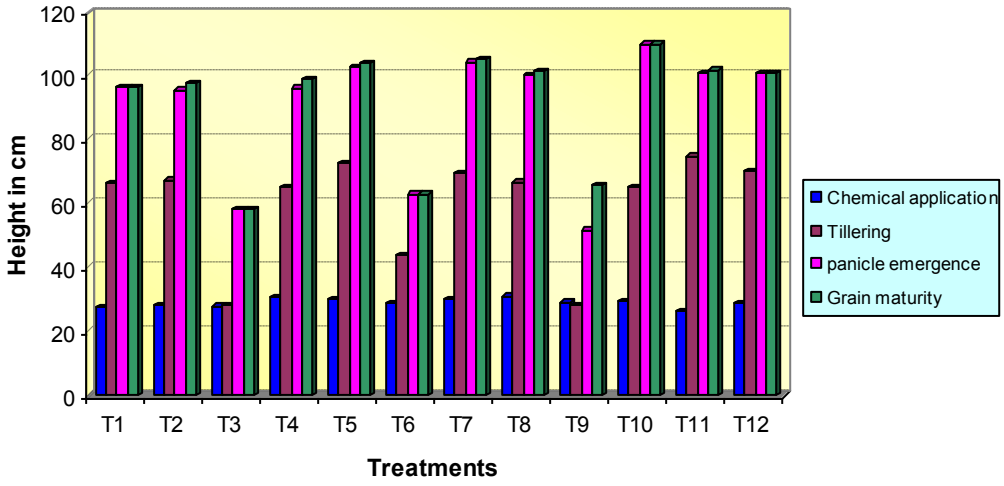
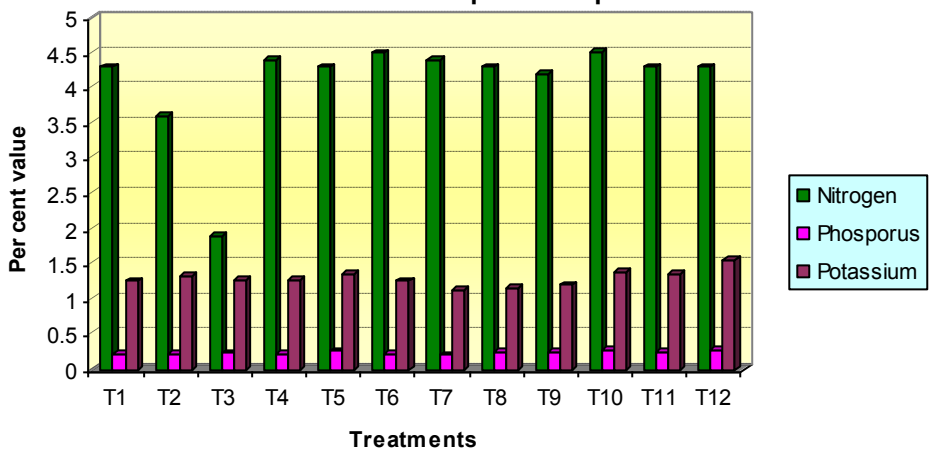


Fig :14 Effect of combinations of chemicals and PGPR on N, P and K content in plant sample



T_1-Azospirillum + Carbendazim

T_2-Azospirillum + Chlorpyriphos

T_3-Azospirillum +2,4-D

T_4-P.fluorescence+ Carbendazim

T_5-P.fluorescens+Chlorpyriphos

T_6-P.fluorescens+ 2,4-D

T_7-B.Subtilis + Carbendazim

T_8-B.Subtilis + Chloropyriphos

T_9-B.Subtilis+2,4-D

T_{10}-Azospirillum alone

T_{11}-P.fluorescens alone

T_{12}-B. Subtilis alone

positively to the uptake of nitrogen. As far as phosphorus uptake was concerned, the treatments where *Azospirillum* sp. and *B. subtilis* were applied alone (T_{10} & T_{12}) were found superior. The maximum potassium content was recorded in treatment with *B. subtilis.* Supportive to these results Kapulnik *et al.* (1982), Omar *et al.* (1989), Pietr *et al.* (1990), Berthelin *et al.* (1991) and Madhaiyan (1999) in their experiments found that application of *Azospirillum* sp. *, P. fluorescens* and *B. subtilis* increased N, P and K uptake by crop plants.

Based on the above studies compatibility charts were prepared on the compatibility of the three PGPR and the chemicals used in this study. The charts describe the compatible, not compatible and questionable combinations of PGPR and agricultural chemicals.

6. SUMMARY

The present experiments on 'Population dynamics of plant growth promoting rhizobacteria under the influence of agricultural chemicals' were carried out to explore the effects of selected agricultural chemicals on plant growth promoting rhizobacteria *viz. Azospirillum* sp*., P. fluorescens* and *B. subtilis*. The study was conducted at the Department of Plant Pathology, College of Horticulture, Kerala Agricultural University , Vellanikkara during the year 2003- 2007. The salient features of the experiment are summarized here.

The identity of *Azospirillum* sp*., P. fluorescens* and *B. subtilis* were established by laboratory tests. From the *in vitro* experiments conducted, it was found that the fungicide, carbendazim was compatible with *Azospirillum* sp. and *B. subtilis*. It was also found that carbendazim, mancozeb and tridemorph were lest inhibitory to *P. fluorescens.* Copper oxychloride was highly inhibitory to both *Azospirillum* sp. and *P. fluorescens* whereas, tridemorph was the highest inhibiting fungicide to *B. subtilis*. Effects of insecticides on the PGPR revealed that, none of the tested insecticides were inhibitory to *Azospirillum* sp. and *B. subtilis* at the recommended dose. Only carbaryl was found inhibitory to *P. fluorescens*. Among the herbicides, paraquat was the only one found inhibitory to *Azospirillum* sp. and *P. fluorescens*. 2, 4-D was found compatible with *P. fluorescens* whereas, 2, 4-D and paraquat were highly compatible to *B. subtilis.* Among the different combinations of fungicides and herbicides, the combination of mancozeb with paraquat was highly inhibitory to *Azospirillum* sp. The combination of carbendazim with all herbicides except pretilachlor did not affect the growth of *P. fluorescens.* The combination of carbendazim with 2, 4-D was found compatible to the growth of *B. subtilis* whereas, paraquat when combined with all fungicides was highly inhibitory to *B. subtilis*.

From the study to find out the combined effect of fungicides and insecticides it was revealed that, combinations of mancozeb with all insecticides were highly inhibitory to *Azospirillum* sp. In the case of *P. fluorescens,* copper oxychloride in

combination with carbaryl inhibited its growth. The highest inhibiting combination for *B. subtilis* was that of carbendazim with paraquat. The combination of carbendazim with all insecticides was supportive to the growth of *Azospirillum* sp. Similarly, the combinations of carbendazim with all insecticides except lamda cyhalothrin were compatible to *P. fluorescens.* In the case of *B. subtilis* the combinations of carbendazim with all insecticides were least inhibitory.

The interactive effects of insecticides and herbicides revealed that the combination of lindane with paraquat was highly inhibitory to *Azospirillum* sp. Combinations of all insecticides and herbicides were least inhibitory to the growth of *P. fluorescens*. The combinations of 2, 4-D with carbaryl and with lamda cyhalothrin were least inhibitory to *Azospirillum* sp. In the case of *B. subtilis*, lindane with 2, 4-D was the highest inhibiting combination.

Under *in vivo* conditions, after the application of chemicals, there was a decrease from the initial population in the case of all PGPR tested. The population showed a decreasing trend as time progressed. A drastic decrease in the population of all the PGPR was observed on the next day of chemical application. In the case of *Azospirillum* sp., up to two weeks, the positive influence on this bacterial population was recorded by lamda cyhalothrin. After two weeks, the maximum population was recorded for lindane and after four weeks the positive influence was for lindane and chlorpyriphos treated soils.

On the next day of chemical application, the decrease in the population of *P. fluorescens* was smallest in carbendazim treated soils. As time progressed, the same treatment supported *P. fluorescens* to reach the highest population till sixth week. Another influence noticed from the experiment was that, the insecticides supported to maintain higher population of *P. fluorescens* compared to fungicides and herbicides. These points out that, insecticides are relatively safe to be used with *P. fluorescens*. In the case of *B. subtilis,* lindane exerted a positive influence on the bacterial population in the initial stages. Gradually, as time passed the insecticides were found to be safe to the growth of *B. subtilis*.

From the *in vitro* studies, the deleterious chemicals against the three PGPR were found to be metalaxyl, mancozeb, butachlor, pretilachlor and paraquat. These chemicals were instrumental to maintain a low population of these PGPR throughout the experiment. From the studies, carbendazim, chlorpyriphos and 2, 4-D were selected for evaluation of the effects on the selected bacteria in a pot culture experiment.

In the pot culture experiment, rhizosphere population of *Azospirillum* sp., showed a decreasing trend in the treatments applied with carbendazim, chlorpyriphos and 2, 4-D. *P. fluorescens* population also exhibited a decreasing trend as the plants grow. Carbendazim, chlorpyriphos and 2, 4-D application decreased the population of *P. fluorescens.* The decreasing trend continued up to third month. The results of the study on the population of *B. subtilis* showed that the population of these bacteria also decreased after chemical application and the trend continued till third month. Probable reason for the decrease in the bacterial population can be attributed to the anaerobic condition in water filled pots. Supportive to this assumption the same decreasing trend of bacterial population was noticed in the pots where PGPR was applied alone.

Studies on the growth parameters recorded significant results. At the time of tillering, the maximum height recorded was in *P. fluorescens* applied plants. At the time of flowering and grain maturity, the superior treatment was *Azospirillum* sp. inoculation. Maximum number of leaves was observed at the time of tillering in the treatments were PGPR alone was applied. Treatment where *P. fluorescens* was inoculated along with carbendazim, the maximum number of leaves at the time of flowering was recorded. At the time of grain maturity, maximum number of leaves was observed in *Azospirillum* sp. with chlorpyriphos treated plants. The study also revealed that maximum tillers and productive tillers were observed in the treatment where *Azospirillum* sp. was combined with chlorpyriphos.

Post harvest characters like shoot weight, root weight, panicle weight and root length were varied significantly among treatments. Treatments where chlorpyriphos inoculated with *Azospirillum* sp. and *P. fluorescens* recorded the maximum shoot, root weight and root length. Maximum panicle weight was recorded in the treatments where the PGPR was applied alone. Nutrient analysis of the plant sample revealed that maximum nitrogen, phosphorus and potassium content were found in the treatments where the PGPR was applied alone.

From the entire study, it was revealed that the systemic fungicide carbendazim, all insecticides tested and the post emergent herbicides, 2, 4-D, butachlor and pretilachlor can be applied in the field along with growth promoting rhizobacteria. The bacteria should be applied at the time of sowing or transplanting and the application of chemicals should be done after two weeks of bacterial inoculation. From the study, it was observed that after the application of the three PGPR in soil, the population was increased. By the time the bacterial population increased in the rhizosphere, the bacteria were able to colonize the roots of the plants. But after two weeks, when the chemicals were applied, the population showed a decreasing trend. It was observed that after one month of bacterial application, the population drastically decreased. To overcome this and to maintain the rhizosphere population, inoculation of bacterial suspension at regular intervals during the crop period is necessary. It will also help the bacteria to overcome the deleterious effects of the chemicals applied in the field.

Since the present study was conducted in a small period, the effects may be confirmed only after conducting elaborate studies at various fields and conditions. Similar studies can also be conducted in the rhizosphere of other economically important crops.

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APPENDIX I

MEDIA COMPOSITION

(Ingredients per litre)

NITROGEN FREE BROMOTHYMOL BLUE (SEMI SOLID MALATE MEDIUM)

Malic acid	: 5.0g
KOH	: 4.0g
K_2HPO_4	: 0.5 g
$FeSO_4$ $7H_2O$	: 0.5g
$MnSO_4$ H_2O	: 0.01g
$MgSO_4$ $7H_2O$	: 0.10g
NaCl	: 0.02g
$CaCl_2$	: 0.01g
Na_2MoO_4	: 0.002g
Agar	: 1.75g
Distilled water	: 1000 ml
Bromothymol blue (0.5 per cent alcoholic solution)	: 2ml
pH	6.6-7

ROJO CONGO MEDIUM

Malic acid	: 5.0g
KOH	: 4.8g
K_2HPO_4	: 0.5g
Mg SO_4 $7H_2O$	: 0.2g
NaCl	: 0.02g
$CaCl_2$	: 0.1g
Yeast extract	: 0.5g
$FeSO_4$ $6H_2O$	: 0.015g
Agar	: 20.0g
Distilled water	: 1000.0 ml
Congo red (1:400 aqueous solution)	: 15.0 ml
pH	– 7.0

ACID FROM GLUCOSE

Glucose	: 10.0 g
Peptone	: 2.0g
$(NH_4)_2$ SO_4	: 1.0g
Mg SO_4	:1.0g

$FeCl_3$	: 0.002g
$MnSO_4$	: 0.002g
Distilled water	: 1000.0 ml
Bromothymol blue	: 2.0 ml
(Five percent alcoholic solution)	
pH -7.0	

KING'S B MEDIUM

Peptone	: 20.0 g
Glycerol	: 10.0 ml
K_2HPO_4	: 10.0 g
$MgSO_4.7H_2O$	: 1.5 g
Agar	: 20.0 g
Distilled water	: 1000.0 ml
pH	: 7.2 – 7.4

Arginine di hydrolase

Peptone	: 1.0 g
K_2HPO_4	:0.3 g
NaCl	: 5.0 g
Agar	: 3.0 g
Phenol red	: 0.01 g
L- Arginine monochloride	:10.0 g
Distilled water	: 1000.0 ml
pH	: 7.2

Urease Test

Peptone	: 1.0g
NaCl	: 5.0g
KH_2PO_4	:2.0g
Glucose	:1.0g
Phenol red(0.2%)	:6ml
Agar	: 20.0g
Distilled water	: 1000.0 ml
pH	: 6.8

Methyl red broth

Proteose peptone	: 5.0g
Glucose	: 5.0g
K_2HPO_4	: 2.0g
Distilled Water	:1000.0 ml
pH	:7.0

Nitrate Reduction

KNO_3 (Nitrate free)	: 1.0g
Peptone	: 10.0g
Beef Extract	: 5.0g
Distilled water	: 1000.0 ml
Agar	: 15.0 g
pH	: 7.0

Nutrient Agar

Peptone	: 5.0 g
Beef extract	: 1.0 g
NaCl	: 5.0 g
Agar	: 15.0 g
Distilled water	: 1000.0 ml

Potato Dextrose Agar

Potato	: 200.0 g
Dextrose	: 20.0 g
Agar	: 20.0 g
Distilled Water	: 1000.0 ml

Luria Bertani Medium (LB)

Tryptone	: 10.0 g
Yeast Extract	: 5.0 g
NaCl	: 5.0 g
Glucose	: 1.0 g
Distilled Water	: 1000.0 ml
pH	: 7.0

Cassamino Acid Broth

Peptone	: 10.0 g
Casamino acid	:1.0g
Glucose	: 5.0 g
Distilled Water	: 1000.0 ml
pH	: 6.8

Appendix II

Stains used in microbiological studies

1. Crystal violet

One volume saturated alcohol solution of crystal violet in four volumes of 1 per cent aqueous ammonium oxalate.

2. Gram's iodine

Iodine crystals	-	1.0 g
Potassium iodide	-	2.0 g
Distilled water	–	300 ml

3. Safranin

Safranin O	-	0.25 g
Ethanol (95%)	-	10.0 ml
Distilled water	-	100 ml

Dissolve safranin in ethanol and then in water and filter.

4. Malachite green

Malachite green	-	5.0 g
Distilled water	–	100 ml

POPULATION DYNAMICS OF PLANT GROWTH PROMOTING RHIZOBACTERIA UNDER THE INFLUENCE OF AGRICULTURAL CHEMICALS

By

BEETHI BALACHANDRAN

ABSTRACT OF THE THESIS

submitted in partial fulfillment of the
requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

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2007

ABSTRACT

Rhizobacteria that are beneficial to the plant growth and development are often referred to as plant growth promoting rhizobacteria (PGPR). Application of PGPR is often found to increase plant growth, development and yield. Now a days, use of PGPR is on an increasing trend in sustainable ecofriendly agriculture. Along with the micro organisms, the farming community is still applying plant protection chemicals and fertilizers in modern agriculture. Even though chemical application will help a lot to protect the crops from pest and diseases, their application may influence the PGPR also. Scientific data on the compatibility aspects of agricultural chemicals with PGPR is scanty. With this in view, the present study on ‘ Population dynamics of plant growth promoting Rhizobacteria under the influence of agricultural chemicals’ was taken up at College of Horticulture, Vellanikkara, during 2003-2007. Five numbers of fungicides, insecticides and herbicides were tested along with their combinations *in vitro* and in the field. The ultimate idea was to evolve a database for compatibility of chemicals and PGPR.

The studies revealed that the fungicide copper oxychloride was most deleterious to the growth of *Azospirillum* sp. and *P. fluorescens*, whereas, tridemorph inhibited the growth of *B. subtilis* under *in vitro* conditions. In the combination studies, it was found that the fungicide mancozeb when combined with all herbicides and all insecticides inhibited the growth of *Azospirillum* sp. The combinations of all fungicides with paraquat and the combination of copper oxychloride with carbaryl were found highly inhibitory to the growth of *B. subtilis*. The combination of the insecticide lindane with the herbicide paraquat was inhibitory to the growth of *Azospirillum* sp. In the case of *B. subtilis,* the combination of carbaryl with paraquat was deleterious.

Another result was that, the fungicide carbendazim, all insecticides except carbaryl and all herbicides except paraquat were least inhibitory to all the test organisms at their recommended doses under *in vitro* conditions. In the combination

studies, carbendazim with all insecticides and herbicides were found least inhibitory to the growth of PGPR. Also the combination of lindane, lamda cyhalothrin and chlorpyriphos with the herbicide 2, 4 –D were found least inhibitory to the growth of these beneficial rhizobacteria.

In the field situation, the chemicals carbendazim, chlorpyriphos and 2, 4-D were evaluated against these beneficial rhizobacteria and it was found that these chemicals affected the bacterial population immediately after their application to soil. All the bacterial populations recorded a decreasing trend as the rice plants grow. This trend was also noticed in the treatments where these PGPR were applied alone. The treatments significantly influenced the growth parameters of the rice plants and the post harvest observations like shoot weight, root weight, root length and panicle weight. Increase in growth parameters like plant height, production of tillers and productive tillers were recorded in the treatments where these PGPR were applied in combination with chlorpyriphos. In the post harvest observations like shoot weight, root weight and root length, positive influence was maximum in the same treatments. But the panicle weight and nutrient content were maximum in the treatments where all the beneficial rhizobacteria applied alone.

Based on the overall results on the studies, compatibility charts of agricultural chemicals and PGPR were prepared which will be a base for future recommendations on the use of chemicals and PGPR together.