EFFECT OF SELECTED PLANT PROTECTION CHEMICALS ON THE BENEFICIAL MICROORGANISMS IN COWPEA RHIZOSPHERE

By

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THESIS

Submitted in partial fulfilment of the
requirement for the degree

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COLLEGE OF HORTICULTURE
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DECLARATION

I hereby declare that the thesis entitled **EFFECT OF SELECTED PLANT PROTECTION CHEMICALS ON THE BENEFICIAL MICROORGANISMS IN COWPEA RHIZOSPHERE** is a bonafide record of research work done by me during the course of research and that the 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.

Vellanikkara
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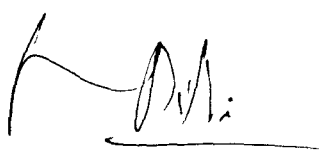
CERTIFICATE

Certified that the thesis entitled **EFFECT OF SELECTED PLANT PROTECTION CHEMICALS ON THE BENEFICIAL MICROORGANISMS IN COWPEA RHIZOSPHERE** is a record of research work done independently by Miss. Raji, P. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.



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CERTIFICATE

We, the undersigned members of the Advisory Committee of Miss. Raji, P., a candidate for the degree of Master of Science in Agriculture with major in Plant Pathology, agree that this thesis entitled **EFFECT OF SELECTED PLANT PROTECTION CHEMICALS ON THE BENEFICIAL MICROORGANISMS IN COWPEA RHIZOSPHERE** may be submitted by Miss. Raji, P. in partial fulfilment of the requirement for the degree.

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Raji. P

RAJI, P.

To my Parents

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Introduction

INTRODUCTION

India faces the twin challenges of producing sufficient food for her growing population from inelastic land area and to protect her environment from degradation. Productivity has to be augmented with the integrated use of such inputs which are not only cost effective but also are eco-friendly.

The nutritional security of the evergrowing population of the country can be assured only when the availability of sufficient nutrients to the plants is also assured. The fertility status of Indian soils with respect to available nitrogen is generally low, low to medium in phosphorus and medium to high in potash. Since soil is a non renewable resource, it should be managed properly otherwise fertile soil would deplete its essential plant nutrients and organic matter. In this situation, the concept of integrated plant nutrient supply system is of high importance. This implies the combined use of mineral fertilizers, organic manures and biofertilizers.

Global turnover of nitrogen per year is estimated to be around 200 million tonnes, out of which two third comes from biological sources. Nitrogen available from chemical fertilizers is estimated to be 80 million tonnes. Production of chemical fertilzers is energy intensive process and utilises

fast depleting non renewable sources of energy. Biofertilizers are environment friendly, low cost agricultural input playing a significant role in improving nutrient availability to the crop plants.

Biological nitrogen fixation contributes 69 per cent of the global nitrogen fixation. Among the nitrogen fixing organisms, Rhizobium and Azospirillum are two important bacterial genera. Legume - Rhizobium symbiosis is the most promising because it supplies approximately 80-90 per cent of the total nitrogen requirement of legumes. Legume inoculation is a long established and successful practice to ensure adequate nitrogen nutrition in place of fertilizer nitrogen in most agricultural soils throughout the world. Azospirillum is an associative microaerophilic nitrogen fixing bacteria found in association with several crop plants including legumes. Apart from nitrogen fixing ability, they have known to produce growth promoting substances (phytohormones) like indole compounds which enhance the growth of crop plants.

Cowpea is one of the most important legumes, which is cultivated for its pods as well as grains. Because of its short duration, adaptation to wide range of soil types and its drought tolerance, it forms an important crop in tropical regions especially Kerala. Cowpea - Rhizobium

symbiosis is well known for its high nitrogen fixing ability. Azospirillum, an important nitrogen fixing bacteria is also found in association with cowpea.

Like fertilizers, plant protection chemicals are also important inputs in modern agriculture. A large number of pesticides have been used to control pests and diseases. Even though this will help a lot to protect the crop from pests and diseases, these may also affect the non-target organisms including beneficial microorganisms. There is no clear cut estimate about the effect of plant protection chemicals on the beneficial microorganisms in cowpea rhizosphere. In this background, the present study was conducted with the following objectives:

1. To isolate the native strains of Azospirillum and Bradyrhizobium , their purification and maintenance.

2. To study the in vitro effect of selected plant protection chemicals at their recommended doses and their combinations on the growth of Azospirillum and Bradyrhizobium

3. To find out the effect of plant protection chemicals at recommended doses and their combinations on Azospirillum and Bradyrhizobium in cowpea rhizosphere in the field.

4. To determine the *in vitro* tolerance levels of *Azospirillum* and *Bradyrhizobium* to different doses of the selected plant protection chemicals.

Review of Literature

REVIEW OF LITERATURE

Beneficial effect of Azospirillum in many economically important plants including legumes as well as the beneficial legume - Rhizobium symbiosis have been studied for several years. The effect of plant protection chemicals on these beneficial microorganisms is an aspect of practical importance and scientific interest. Studies conducted by various workers in this field are reviewed here.

2.1 Effect of plant protection chemicals on Azospirillum

Alvarez and Sleiman (1983) studied the effect of some pesticides on Azospirillum lipoferum and A. brasilense in pure culture. They tested ten herbicides and three insecticides on four strains of both species of Azospirillum. None of the pesticides affected the growth rate of the bacteria. Atrazine and Linuron increased the rate of acetylene reduction in A. lipoferum during the initial days of incubation but had no effect on total nitrogenase activity.

The increase or decrease of the nitrogenase activity of A. lipoferum according to the cultural conditions by the herbicide Stomp (Pendimethalin) was reported by Gadkari (1987).

Jena et al. (1987) reported an increase in the accumulation of Indole Acetic Acid (IAA) and nitrogen fixation by application of Carbofuran to Azospirillum cultures isolated from rice roots and soils.

Influence of the herbicides Metamitron, Metribuzin, Ethiozin and Paraquat on growth and nitrogenase activity of A. lipoferum and A. brasilense was studied by Gadkari (1988). The nitrogenase activity of these strains was not affected by Metamitron (35 ppm and 70 ppm) and Ethiozin (20 ppm). On the other hand, Metribuzin (7 ppm and 14 ppm) and Ethiozin (50 ppm) caused marked decrease in nitrogenase activity.

Govindarajan and Purushothaman (1988) studied the effect of seed dressing fungicides and Azospirillum treatment on Pennisetum americanum. Seeds were treated with Captan or Thiram at 4g/kg seed, followed one hour later with an inoculum of A. brasilense. Other seeds were treated with bacterium followed by the fungicide or with the bacterium alone. Treated seeds were sown and the Azospirillum population was estimated after 7, 14, 21 and 28 days. Fungicide treatment before inoculation had little effect but after inoculation it caused a decrease in A. brasilense number on the seeds and in the rhizosphere of plants.

Mano et al. (1988) reported that under non nitrogen fixing conditions, an organochlorine insecticide Dicofol inhibited the growth of A. lipoferum totally by 250 and 500 ppm concentrations. But a latent growth was observed, suggesting selection of resistant mutants. Under nitrogen fixing conditions, nitrogenase activity was inhibited by increasing Dicofol concentrations upto 1000 ppm, but in some experiments, the growth of Azospirillum was stimulated by a concentration of one ppm.

The effect of Monocrotophos and Quinalphos on soil population and nitrogen fixing activity of Azospirillum sp. was reported by Rangaswami et al. (1989). Concentrations of the two insecticides upto 5 kg/ha level were either stimulatory or innocuous to population of Azospirillum in the soils. Four successive applications of the insecticides to the soil resulted in a significant increase in the population density. Also cultures of Azospirillum sp. isolated from insecticide treated soils exhibited greater nitrogen fixing activity.

Gallori et al. (1992) found out that fungicides Captan (0.5-2 mg/ml) and Thiram (3-7 mg/ml) caused a reduction in growth rate of A. brasilense, which was greater at higher concentrations and reduced nitrogenase activity.

2.2 Effect of plant protection chemicals on Bradyrhizobium

Different species of Bradyrhizobium respond differentially to the fungicides. This was reported by Mukewar and Bhide (1969). They tested Agrosan, Brassicol, Thiram, Captan and antibiotic Aureofungin on different species of Rhizobium. All Rhizobium spp. tested, grew well in presence of Agrosan, Brassicol and Aureofungin. However the growth of R. phaseoli, R. japonicum, R. trifolii and R. meliloti was inhibited by Thiram. Captan inhibited the growth of R. leguminosarum but not of other Rhizobium spp.

Increased nodulation, growth, yield and nitrogen uptake of Pisum sativum inoculated with R. leguminosarum by soil application of DDT upto 40 ppm was recorded by Pareek and Gaur (1969). But higher levels of DDT was found to be injurious.

In an in vitro study conducted by Oblisami et al. (1973) granular formulations of Endrin, Carbofuran and Disulfoton inhibited the growth of Rhizobium from redgram. The inhibitory effect of Endrin and Carbofuran increased with increasing concentration of active ingredients.

In vitro inhibitory effect of seed dressing fungicides Dithane M-45, Dithane Z-78, Flit 406, Ceresan wet and Aretan

at 1500, 2000 and 3000 ppm concentrations on Rhizobium sp. was reported by Lehri and Prasad (1976).

No deleterious effect was found on the nodulation of three groundnut varieties with the spraying of fungicides Benlate, Brestan, Ceresan, Dithane Z-78 and sulphur (Manickasundaram and Ramabadran, 1976). In another study conducted by Prasad and Ramani (1976) groundnut inoculated with Rhizobium showed no adverse effect upon seed treatment with wet Ceresan, on dry weight, total nitrogen content, nodulation and leg-haemoglobin content of nodules of plants. Soil application of Brassicol at recommended field rate (11 ppm) did not alter the groundnut - Rhizobium symbiosis. However, higher concentrations of 50 and 100 ppm were proved to be harmful. Foliar application of Dithane Z-78 and Copper oxychloride at the recommended field rate of 0.2 and 0.3 per cent respectively did not affect the symbiotic relationship. Soil application of Disyston and DDT at field rates increased significantly the dry weight, total nitrogen content, nodulation and leg-haemoglobin content. However at 200 ppm these insecticides exerted harmful effect on symbiosis.

The increased nodulation of soybean and berseem (Trifolium alexandrinum) by treatment of Bavistin was reported by Bandyopadhyay et al. (1983). Benomyl,

Radotiram and Preicur had no lethal effect on two soybean cultivars inoculated with Rhizobium japonicum in lab, greenhouse and field tests, but there were differences in the number of nodules formed. In most cases Benomyl was the least and Radotiram the most inhibitory (Heneberg et al., 1983).

The inhibitory effect of Captan and Thiram, on the growth of Rhizobium japonicum and its nodulation on the roots of soybean plants was reported by Abd el-Mo·nem and EL-Sawah (1984). Studies conducted by Jahuri and Agrawal (1984) showed that Dithane M-45 alone or in combination with Thiram gave the best yield and seed emergence of soybean in Macrophomina phaseolina infested soil. No adverse effect of these fungicides on seed inoculation of R. japonicum was observed.

In a study conducted by Chakraborty et al. (1985), the two protective fungicides namely Emisan-6 and Karathane were found to be inhibitory to all the test cultures of Rhizobium. Effect of eight fungicides viz., Ceresan (dry), Brassicol, Difolatan, Hexathir, Hexacap, Benlate, Bavistin, Calixin and an antibiotic Agrimycin at different concentrations were tested for their effect on R. leguminosarum. Out of the eight fungicides tested, Brassicol, Benlate, Bavistin and Calixin did not inhibit

the bacterial growth. Agrimycin was most inhibitory to growth followed by Ceresan, Difolatan, Hexacap and Hexathir. (Kakralia et al., 1985).

In a field experiment, Captan and Captafol markedly inhibited nodulation of clover (Trifolium alexandrinum) by Rhizobium trifolii (Ruiz sainz et al., 1985). Thomas and Vyas (1985) reported that Carbendazim, Triforine and Metalaxyl applied to plants grown from seeds treated with Rhizobium gave significant increase in both nodule number and nodule dry weight per pigeon pea plant. Triademefon decreased the nodule number and dry weight of pigeon pea plant. Thiram had no effect.

Anusuya (1986) obtained difference in nodulation in groundnut when different fungicides were used for seed tratment. Seed treatment with Bavistin and Triforine at one per cent concentration significantly reduced the number of nodules. However, 0.1 and 0.01 per cent concentrations of these two chemicals did not cause any reduction in number of nodules. Significant increase in nodulation was obtained by seed treatment with Agallol and Ceresan each at 0.01 per cent. These chemicals had adverse effect on nodulation at 0.1 and one per cent concentrations.

The effects of fungicide treatment of Rhizobium inoculated soybean seeds on subsequent nodulation and

nitrogen fixation were investigated by Diatloff (1986). Metalaxyl (25% WP), Benalaxyl (25% WP), Iprodione (50% WP) and Captafol (80% WP) were used for seed treatment. Captafol reduced the numbers of viable rhizobia. The other fungicides did not affect the bacterial growth. There were no significant differences in nodule number, wet weight of plants or nitrogen fixation between treated and control plants.

Experiments were conducted by Martinezviera and Casanova (1986) to study the interaction between ten strains of R. japonicum and the seed protectants Captan, Ceresan and TMTD applied to soybean seeds Cv. V-9 prior to inoculation. Under laboratory conditions, Captan and Ceresan inhibited development of all strains tested, with differing responses among strains according to their sensitivity. TMTD did not affect bacterial development. Field results showed that the number and size of the nodules were significantly reduced if Captan and Ceresan were applied. TMTD did not influence nodulation process.

Seed inoculation of groundnut with Rhizobium followed by fungicidal treatment with Carbendazim 1.5g/kg of seed considerably increased nodule numbers per plant, nodule dry weight and yield (Thomas et al., 1986).

An *in vitro* experiment was conducted by Joshi and Kulkarni (1987) to study the effect of pesticides on growth of *Rhizobium* spp. Three herbicides, one fungicide (Thiram) and four insecticides (Carbofuran, Phorate, Dursban and Temik) were tested. Two doses of each pesticide, one at the recommended field rate and other at 2-5 times higher were added to the broth, which was inoculated with 48 hour old *Rhizobium* culture. None of the pesticides inhibited the rhizobial growth. On the other hand Thiram, Carbofuran, Phorate, Dursban and Temik stimulated the rhizobial growth compared to control.

Gupta *et al*. (1988) studied the effect of pre and post inoculation seed treatment with fungicides on nodulation and grain yield of soybean. Rhizobial population per gram of seed was maximum with soybean seed treated with the inoculum alone followed by inoculation and post treatment of Thiram. Though Thiram was found to inhibit the growth of *R. japonicum*, it gave maximum number of nodules, dry weight of nodules, grain yield per plant and high crude protein. Kucey and Bonetti (1988) reported that the seed treatment with Captan reduced the number of nodules formed by field bean in which seed inoculation of *R. phaseoli* was done. Even though the seed applied Captan reduced the proportion of nodules formed by rhizobia, there was no reduction in the amount of ntrogen fixed.

Effect of Carbofuran on growth, nodulation, phytomass, chlorophyll and carotene content of Vigna radiata was studied by Singh and Saxena (1988). Out of different concentrations of Carbofuran viz., 0, 5, 10, 25, 50 and 100 ppm, five ppm Carbofuran showed no toxic effect, instead, the growth, number of nodules, phytomass and chlorophyll content were increased in comparison to control. However, 10, 25 and 50 and 100 ppm of Carbofuran were toxic and the plants showed inhibition of growth. El-Baharawy and Ghazal (1989) studied the effect of different pesticides on plant root nodules and rhizosphere microflora in cowpea. The insecticide Temik generally reduced the formation of efficient nodules although at certain concentrations tested, the dryweight of the plant and symbiotic nitrogen fixation were increased.

In an in vitro study, the growth of Rhizobium sp. was reduced by five antibiotics. Of the fungicides tested (Agrosan GN, Bavistin, Brassicol, Captan, Dithane M-45 and Thiram), Bavistin, Brassicol, and Dithane M-45 showed no inhibition of Rhizobium even at a concentration of 2000 ppm. (Patel et al., 1989).

Cowpea seed treatment with Carbendazim, Carbendazim + Carbosulfan and Carbosulfan was compatible with Rhizobium and had no inhibition on the nodulation. Seed treatment of

the pesticides along with Rhizobium inoculation exhibited better nodulation than uninoculated control (Ramadoss and Sivaprakasm, 1989). The influence of Phorate on growth and nodulation of Vigna radiata was reported by Singh and Mathur (1989). In sand culture trials, it was found that, 50, 100, 200, 400 or 600 mg Phorate increased root and shoot growth and number of nodules per plant.

The resistance of 50 Bradyrizobium japonicum strains to different fungicides (Captan, PCNB and TMTD) commonly used in soybean cultivation was investigated by Borges et al. (1990). Resistance to fungicides, Captan, PCNB and TMTD was found in 64, 44 and 54 per cent of strains for concentrations above 2.0, 0.05 and 0.11 mg/ml respectively, the normally recommended dosages for treatment of soybean. The herbicide Linuron adversely affected the nodulation and nitrogenase activity of Bradyrhizobium sp. in groundnut (Novo et al., 1990). Seed treatment with Carbendazim and Monocrotophos at the recommended levels not only reduced seed borne pathogens and harbouring pests but also significantly enhanced the plant growth and grain yield of green gram and black gram. But these seed dressing chemicals reduced the rhizobial population on the seed surface (Prabhakaran and Ramasami, 1990). Seed treatment with Carbendazim followed by Carbosulfan 25 STD or Acephate

75 SP did not affect the nodulation by Rhizobium in black gram (Logiswaran et al., 1991). Maheshwari and Gupta (1991) reported the diverse effects of two organocarbamate nematicides on nitrogen assimilation of R. japonicum 2002 in freeliving culture. They reported the inhibitory effects of Carbaryl and Carbofuran at concentrations between 50 and 300 mg/ml on growth of R. japonicum and also stimulation of nitrate uptake and nitrate reduction, by the two nematicides at concentrations below the LC 50 values.

Effect of agrochemicals and heavy metals on fast growing rhizobia and their symbiosis with small seeded legumes was investigated by Matrensson (1992). Symbiotic interactions were adversely affected by several agrochemicals. Bacterial induced root hair deformation necessary for nodulation decreased in the presence of Benomyl, Bentazone, Chlorosulfuron, Fenpropimorph, Mancozeb and Monopchlorophenoxy acetic acid. Fenpropimorph and Mancozeb did not cause root hair deformations at increasing concentrations indicating that they may inhibit nodulation under field conditions. Nodule development was inhibited at increased levels of Bentazone, Chlorsulfuron, Glyphosate and Mancozeb. Drymatter production of nodulated plants was adversely affected by Bentazone and Chlorsulfuron indicating disturbances in nodule formation.

Effect of a mixture of Chlorpyrifos and Lindane on symbiosis of Bradyrhizobium japonicum and soybean was studied by Revellin et al. (1992) and found that the insecticide mixture was innocous with respect to its effect on Bradyrhizobium soybean symbiosis in the field.

Materials and Methods

MATERIALS AND METHODS

A study on the **Effect of selected plant protection chemicals on the beneficial microorganisms in cowpea rhizosphere** was conducted during 1992-94 at the College of Horticulture, Vellanikkara, Trichur. The details of the materials used and techniques adopted during the course of investigation are presented hereafter.

3.1 Collection of root sample and isolation of Azospirillum

Roots of cowpea plants were collected for the isolation of Azospirillum from vegetable garden of College of Horticulture, Vellanikkara. Roots were collected in polyethylene bag along with a ball of earth adhering to it so as to prevent drying of roots. Azospirillum was isolated from the collected roots using Nitrogen Free bromothymol blue (NFb) medium (Semisolid malate medium) (Dobereiner et al., 1976) of the following composition.

Malic acid	5.0 g
KOH	4.0 g
K_2HPO_4	0.5 g

$FeSO_4$ $7H_2O$	0.5 g
$MnSO_4$ H_2O	0.01 g
$MgSO_4$ $7H_2O$	0.10 g
NaCl	0.02 g
$CaCl_2$	0.01 g
Na_2 MoO_4	0.002 g
Agar	1.75 g
Distilled water	1000.0 ml
Bromothymol blue (0.5 per cent alcholoic solution)	2.0 ml
pH 6.6 - 7.0	

The medium was transferred to test tubes, 10 ml each and sterilized in an autoclave at 121°C for 20 minutes.

Roots collected for isolation of Azospirillum were washed in tap water to remove the soil adhering to it. Roots were cut into bits of 0.5 - 1.0 cm length and surface sterilized with 0.1 per cent mercuric chloride solution for one minute. These surface sterilized roots bits were washed in four changes of sterile water and planted in semisolid malate medium in test tubes and incubated at 37°C for two days. They were observed for the presence of subsurface thin, pellicular growth of Azospirillum.

3.2 Purification of *Azospirillum*

Purification of *Azospirillum* was done by serial transfer of this pellicles into fresh semisolid malate medium. Loopful of the culture was stabbed into the fresh semisolid medium and incubated at 37°C for two days and observed for the development of white, pellicular growth.

3.3 Primary characterization of *Azospirillum*

Morphology, Gram reaction, cultural and physiological characters of isolated *Azospirillum* were studied for primary characterization.

3.3.1 Gram Staining

Hucker's modification of Gram staining was done. A smear of the bacterial isolate was prepared on a clean glass slide and heat fixed over a flame by gentle intermittent heating. It was stained with Hucker's ammonium crystal violet solution for one minute and then washed in a gentle stream of running tap water. After washing, it was flooded with Grams iodine solution for one minute and then decolourised with 95 per cent ethanol. After washing again in gentle stream of running tap water, the slide was stained with saffranin solution for one minute and the excess stain was washed off in tap water. After drying

between folds of filter paper, the slide was examined under light microscope for Gram reaction.

3.3.2 Cultural Characters

Cultural characters of the Azospirillum isolate were studied by growing them in different media and observing for the colony characters such as colour, shape etc.

3.3.2.1 Growth on Okon's medium (Okon et al., 1977) as modified by Kumari et al. (1980).

a.

K_2HPO_4	6.0 g
KH_2PO_4	4.0 g
Distilled water	500.ml

b.

$MgSO_4\ 7H_2O$	0.2 g
NaCl	0.1 g
$CaCl_2$	0.02 g
$NH_4\ Cl$	1.0 g
Malic acid	5.0 g
NaOH	3.0 g
Yeast extract	0.05 g
$Na_2\ MoO_4$	0.002 g
$MnSO_4\ H_2O$	0.001 g
H_3Bo_3	0.0014 g
$Cu\ (No_3)_2$	0.0004 g

$ZnSO_4$	0.0021 g
$FeCl_3$	0.002 g
Agar	15.0 g
Distilled water	500.ml

The agar was dissolved in 500 ml water by boiling. After cooling to about 45°C, the remaining ingredients were added to it.

c. Bromothymol blue 2.0 ml
(0.5 per cent alcoholic solution)

Solutions (a) and (b) were mixed, bromothymol blue indicator was added to this mixture and the pH was adjusted to 6.8 to 7.0 using 0.1 N potassium hydroxide solution. The medium was transferred into 250 ml conical flasks and sterilized by autoclaving at 121°C for 20 minutes.

A loopful of culture taken from the white pellicle of Azospirillum formed in semisolid malate medium was dispersed in five ml of sterile water. From this suspension, a loopful was taken and streaked on Okon's solid medium in sterlized petriplates. After incubation for five days at 37°C, they were observed for the development of thin, dry, slightly convex colonies and change in the colour of the medium to blue.

3.3.2.2 Growth on Rojo Congo (RC) medium (Caceres, 1982).

Malic acid	5.0 g
KOH	4.8 g
K_2HPO_4	0.5 g
$MgSO_4$ $7H_2O$	0.2 g
NaCl	0.02 g
$CaCl_2$	0.1 g
Yeast extract	0.5 g
$FeSO_4$ $6H_2O$	0.015 g
Agar	20.0 g
Distilled water	1000.0 ml
Congo red (1:400 aqueous solution)	15.0 ml
pH 7.0	

The medium was transferred to 250 ml conical flasks and sterilized in an autoclave at 121°C for 20 minutes.

A loopful of Azospirillum culture was taken from the, white pellicle formed in semisolid malate medium and dispersed in five ml sterile water. From this suspension a loopful was taken and streaked on RC medium in sterilized petriplates and incubated at 37°C for four days and observed for the development of scarlet bacterial colonies with rugose surface and undulating edges.

3.3.2.3 Growth on Potato Infusion Agar.

Potato	200.0 g
Malic acid	2.5 g
KOH	2.0 g
Sucrose	2.5 g
Agar	15.0 g
Vitamin solution	1.0 ml
Distilled water	1000.0 ml
Bromothymol blue	2.0 ml

(Vitamin solution prepared by dissolving 10 mg biotin and 20 mg pyridoxin in 100 ml sterile water).

Potatoes were washed, cut into small pieces and boiled in 500 ml distilled water for 30 minutes. The cooked potato solution was filtered through layers of cheese cloth. Malic acid was dissolved in 50 ml water, two drops of bromothymol blue (90.5 per cent solution in ethanol) was added and pH was adjusted to 7.0. Sucrose, agar and malic acid solution were added to the potato filtrate and made upto 1000 ml with distilled water. The medium was transferred to 250 ml conical flasks and sterilized by autoclaving at 121°C for 20 minutes. The vitamin solution was added aseptically before pouring the medium into petriplates.

A loopful of Azospirillum culture was dispersed in five ml sterile water. From this suspension, a loopful was taken and streaked on Potato Infusion Agar taken in sterile petriplates and incubated at 37°C for seven days and observed for the pink and wrinkled bacterial colonies.

3.3.3 Physiological characters

3.3.3.1 Utilisation of carbon sources

The ability of Azospirillum isolate to utilise different carbon sources was tested. The semisolid malate medium was modified by excluding bromothymol blue and replacing malic acid with one per cent carbon sources such as glucose lactose, sucrose and mannose. Ten ml quantities of the media were transferred to test tubes and sterilized in an autoclave at 115°C for 20 minutes.

The sterile media in test tubes were inoculated with a loopful of the culture grown in semisolid malate medium and incubated at 37°C for three days. They were then observed for the development of thin, white, subsurface, pellicular growth and compared with the growth in semisolid malate medium.

3.3.3.2 Acid from glucose

Acid production from glucose by the Azospirillum isolate was tested using the following medium.

Glucose	10.0 g
Peptone	2.0 g
$(NH_4)_2So_4$	1.0 g
$MgSo_4$	1.0 g
$FeCl_3$	0.002 g
$MnSo_4$	0.002 g
Distilled water	1000.0 ml
Bromothymol blue (Five per cent alcoholic solution).	2.0 ml
pH 7.0	

Five ml quantities of the medium were taken in test tubes and autoclaved at 121°C for 20 minutes. The sterilized medium was inoculated with 0.1 ml suspension of 48 hour old culture of Azospirillum and incubated at 37°C for four days. They were then observed for the change in colour of the medium from green to yellow which indicates acid production.

3.3.3.3 Test for dissimilation of nitrate

The Azospirillum isolate was tested for its ability to dissimilate nitrate. Semisolid malate medium was modified by adding five mM ammonium nitrate and 1.5 per cent agar. Five ml quantities of the medium were taken in test tubes and sterilized by autoclaving at 121°C for 20 minutes. The tubes were inoculated by stabbing with a

loopful of the Azospirillum culture and incubated at 37°C for five days. The dissimilation of nitrate was indicated by the shredding of agar blocks.

3.4 Maintenance of Azospirillum culture

After purification and primary characterization the Azospirillum culture was streaked on Okon's solid medium in petriplates. The single colonies typical of Azospirillum was transferred to test tube slants of the same medium. They were then stored in a refrigerator at 4°C. The culture was maintained with periodic purification and subculturing into fresh test tube slants.

3.5 Isolation of Bradyrhizobium, its purification and maintenance

Native strain of Bradyrhizobium was isolated from cowpea plants collected from vegetable garden of College of Horticulture, Vellanikkara. Plants were uprooted carefully with minimum disturbance to roots and nodules. Isolation of Bradyrhizobium was done in Yeast Extract Mannitol Agar medium (Allen, 1953) of the following composition.

Mannitol	10.0 g
K_2HPO_4	0.5 g
$Mg\ SO_4\ 7H_2O$	0.2 g
NaCl	0.1 g

$CaCo_3$	3.0 g
Yeast extract	1.0 g
Agar	15.0 g
Congo red (One per cent aqueous solution)	2.5 ml
Distilled water	1000.0 ml
pH 7.0	

Healthy pink coloured nodules along with little portion of the root attached to it were separated from the cowpea roots. The nodules were thoroughly washed in tap water and surface sterilized with 0.1 per cent mercuric chloride solution for one minute. The surface sterilized nodules after washing in five changes of sterile water were crushed in a small quantity of sterile water taken in a test tube. The suspension from the crushed nodules was streaked on YEMA medium in petriplates and incubated at room temperature for four days. Individual bacterial colonies showing the characteristics typical of Bradyrhizobium were purified by repeated streaking on YEMA medium.

3.6 Primary characterization of Bradyrhizobium

3.6.1 Growth on Glucose Peptone Agar

The composition of Glucose Peptone Agar medium is as follows.

Glucose	10.0 g
Peptone	20.0 g
NaCl	5.0 g
Agar	15.0 g
Distilled water	1000.0 ml
Bromocresol purple (1.6 per cent in ethanol)	10 ml
pH 7.2	

A loopful of the Bradyrhizobium isolate was streaked on this medium and then incubated at 28±2°C for seven days in a B.O.D. incubator. The extent of growth and change of colour of the medium, if any, were recorded.

Gram staining and microscopic examination of Bradyrhizobium were also done. After this primary characterization, Bradyrhizobium culture was transferred to YEMA slants in which congo red was excluded and they were stored in a refrigerator. Subculturing of the isolates was done at monthly intervals.

3.7 *In vitro* effect of selected plant protection chemicals at the recommended doses and their combinations on the growth of Azospirillum and Bradyrhizobium

The effect of selected plant protection chemicals at the recommended doses and their combinations on the growth of native strains of Azospirillum and Bradyrhizobium

isolated from the cowpea plant was tested by paper disc method. The experiment was conducted as a 5 X 4 factorial experiment in C.R.D. with three replications.

The treatments were as follows.

F - Fungicides	**I - Insecticides**
F_0 - Control	F_0 - Control
F_1 - Carbendazim 0.1%	I_1 - Carbofuran 12.5 ppm
F_2 - Thiram 0.2 %	I_2 - Phorate 5 ppm
F_3 - Bordeaux mixture 1%	I_3 - HCH 10 ppm
F_4 - Fytolan 0.3 %	

Treatment combinations were

F_0I_0	F_1I_0	F_2I_0	F_3I_0	F_4I_0
F_0I_1	F_1I_1	F_2I_1	F_3I_1	F_4I_1
F_0I_2	F_1I_2	F_2I_2	F_3I_2	F_4I_2
F_0I_3	F_1I_3	F_2I_3	F_3I_3	F_4I_3

The above concentrations of fungicides and insecticides and their combinations were prepared in sterile distilled water. Sterile filter paper discs of 10 mm diameter were dipped in the prepared solutions and were placed at the centre of the Okon's medium and YEMA medium seeded with 48 hour old cultures of Azospirillum and Bradyrhizobium respectively in sterile petriplates. Sterile filter paper discs dipped in sterile distilled water served as control.

The plates were incubated at room temperature and diameter of inhibition zones were recorded after three days.

3.8 Effect of selected plant protection chemicals at the recommended doses and their combinations on Azospirillum and Bradyrhizobium in cowpea rhizosphere in the field

The experiment was conducted as a 5 X 4 Factorial experiment in R.B.D. with three replications.

The land was ploughed two times and the weeds and stubbles were removed. After levelling the field the total experimental area was divided into six blocks. In each block, twenty beds of one m^2 size were taken. Irrigation channels were provided between the blocks. Bunds were made around the field.

Seeds of cowpea variety Pusa Komal procured from the Department of Olericulture, College of Horticulture, Vellanikkara was used. Bradyrhizobium was inoculated by mixing three days old pure culture of the bacteria uniformly with the seeds. Bradyrhizobium treatment was given in 60 plots. Sowing was done on the same day of inoculation. In the remaining 60 plots untreated seeds were sown for inoculating Azospirillum later. Sowing was done at a spacing of 45 cm X 15 cm, with two seeds per hole. Thinning was done after the germination of seeds retaining one healthy plant at each spot.

Soil inoculation of Azospirillum was done two days after germination of seeds. For this Azospirillum broth culture was mixed with water and this suspension of bacteria was poured at the base of the seedlings at the rate of 100 ml per plant.

Soil application of plant protection chemicals were done two weeks after planting. The treatments were same as that of the in vitro experiment. The quantity of the chemicals required to get the recommended doses for the total area were taken and mixed with water separately and were applied uniformly in soil in the corresponding plots.

3.8.1 Observations

3.8.1.1 Soil population of Azospirillum and Bradyrhizobium.

Soil population of Azospirillum and Bradyrhizobium were estimated by dilution plate technique using Okon's medium and YEMA medium respectively. One gram soil sample was added to 99 ml of sterile water in a conical flask and mixed well. From this soil suspension one ml was pipetted out to another 99 ml of sterile water in a conical flask using a sterile pipette so as to get a dilution of 10^{-4}. From this one ml was pipetted out to sterile petriplate using a sterile pipette and to this about 10-15 ml of the corresponding media was poured and swirlled to mix the

soil suspension with the media uniformly. After this the petriplates were incubated for observing the colonies of Azospirillum and Bradyrhizobium. Natural soil population of these two bacteria were estimated before sowing. The rhizosphere population of Azospirillum and Bradyrhizobium were estimated after inoculation of bacteria and seven, 21 and 35 days after the application of plant protection chemicals. For estimation of soil population of the bacteria in the rhizosphere, random samples were taken from each plot and mixed together to get composite samples and from these composite samples one g was taken for estimation of the bacteria.

3.8.1.2. Growth parameters of cowpea plant

Observations on the growth parameters of cowpea plant were taken on the 55th day after sowing. The following observations are recorded.

Plant height: Plant height was taken from the soil level to the tip of the plant.

Root length : Root length was measured from the collar region downwards.

Total fresh weight: Total fresh weight of the plant was taken immediately after uprooting.

Total dry weight: The plant samples were oven dried at 60°C to a constant weight. After this drying weights were taken.

Root dry weight: Root dry weights were taken after drying the samples in oven at 60°C for a constant weight.

Number of nodules: The number of nodules were recorded after uprooting the plants carefully

Nitrogen content: Nitrogen content was estimated from the dried plant samples.

3.8.1.3 Estimation of percentage nitrogen content of plant top (Jackson, 1967).

One hundred mg of powdered plant sample and one gram of digestion mixture (potassium sulphate, cupric sulphate and selenium powder in 10.0:1.0:0.1 ratio) were digested with five ml of concentrated sulphuric acid in a digestor till the solution becomes clear. After cooling to room temperature, the contents were transferred into a 100 ml volumetric flask and made up the volume with distilled water. Ten ml of the sample along with five ml of 40 per cent sodium hydroxide solutions were steam distilled in a Microkjeldahl distillation system. Ammonia liberated was collected in a receiver flask containing 20 ml of two per cent boric acid, three drops of 0.1 per cent methyl red indicator and six drops of bromocresol green solution.

The distillate was titrated against 0.01 N sulphuric acid, and from the titre value percentage nitrogen content was calculated using the following formula.

$$\text{Percentage nitrogen content} = \frac{V \times N \times V_1 \times 0.014 \times 100}{V_2 \times W}$$

Where

V - Titre value

N - Normality of sulphuric acid

V_1 - Volume of which the sample was made upto

V_2 - Volume of sample used for distillation

W - Weight of plant sample taken.

3.9 In vitro tolerance of Azospirillum and Bradyrhizobium to selected plant protection chemicals

The tolerance levels of Azospirillum and Bradyrhizobium to different doses of the selected plant protection chemicals were tested by paper disc method.

The experiment was conducted with the following chemicals each with five different doses.

Fungicides	II lower dose %	I lower dose %	Recommended dose %	I higher dose %	II higher dose %
Carbendazim	0.05	0.075	0.1	0.125	0.150
Thiram	0.05	0.10	0.2	0.3	0.4
Bordeaux mixture	0.25	0.50	1.0	2.0	3.0
Fytolan	0.1	0.2	0.3	0.4	0.5

Insecticides	II lower dose ppm	I lower dose ppm	Recommended dose ppm	I higher dose ppm	II higher dose ppm
Carbofuran	4.16	8.33	12.50	16.6	20.8
Phorate	1.25	2.5	5.0	6.25	7.5
HCH	5.0	7.5	10.0	12.5	15.0

The above concentrations of fungicides and insecticides were prepared in sterile distilled water. Sterile filter paper discs of 10 mm diameter were dipped in the prepared solutions and placed at the centre of the seeded medium in sterile petriplates. The seeded medium was prepared by adding 48 hour old cultures of Azospirillum and Bradyrhizobium in Okon's medium and YEMA medium respectively. The sterile filter paper discs dipped in sterile distilled water served as control. The test was done in triplicates and the plates were incubated at room temperature. Observations on the diameter of the inhibition zones were recorded after three days.

3.10 Statistical analysis

Statistical analysis of the data for the in vitro experiments was done as Completely Randomised Design. The analysis of the data obtained from the field experiment was done as factorial set up in Randomised Block Design (Das and Giri, 1979).

Results

RESULTS

The results of the experiments conducted are given below:

4.1 Isolation of *Azospirillum*:

Azospirillum was isolated from the roots of cowpea plants collected from vegetable garden of the College of Horticulture, Vellanikkara. The thin, white, subsurface pellicular growth in nitrogen free semisolid medium indicated the presence of *Azospirillum*. The isolate was Gram negative and vibrioid in shape. The cultural and physiological characters of the isolate are presented below.

4.2 Cultural characters

In Okon's medium, the isolate formed typical, thin, dry, slightly convex colonies with a granular wavy surface and undulate margin, when incubated at 37°C for five days. The colonies were found to be partially embedded in the medium. The colour of the medium changed to blue, initially as a halo surrounding the individual colonies which later coalasced and spread through the entire plate. In RC medium, the colonies formed were scarlet dry, round to irregular, with rugose surface and undulating edges. When

grown in Potato Infusion Agar, colonies of Azospirillum isolate formed were pink, round to irregular, dense and often wrinkled. Thus, the isolate showed typical characters of Azospirillum when grown in Okon's medium RC medium and Potato Infusion Agar.

4.3 Physiological characters

4.3.1 Acid production from glucose

The Azospirillum isolate produced acid from glucose under aerobic conditions. This was indicated by the change in colour of the inoculated medium from green to yellow after incubation at 37°C for four days.

4.3.2 Utilisation of carbon sources

The Azospirillum isolate had good growth in medium containing malate as the carbon source. Growth in media containing sugars such as glucose, lactose, sucrose and mannose was scanty.

4.3.3 Dissimilation of nitrate

When the Azospirillum isolate was grown in NFb medium containing ammonium nitrate, the shredding of agar block was observed, indicating the dissimilation of nitrate.

4.4 Isolation and Characterization of *Bradyrhizobium*

The suspension from the crushed nodules of cowpea when streaked on YEMA medium, formed, large, white, gummy, colonies of *Bradyrhizobium* after incubation at room temperature for four days. On microscopic examination the bacteria was found to be Gram negative and rod shaped.

On glucose peptone agar, the growth of *Bradyrhizobium* was scanty, without any change in colour of the medium even after 7 days of incubation.

4.5 *In vitro* effect of selected plant protection chemicals at the recommended doses and their combinations on the growth of *Azospirillum* and *Bradyrhizobium*

The results of the effect of selected plant protection chemicals on the native strains of *Azospirillum* and *Bradyrhizobium* are given in table 1.

From the study, it was found that the insecticides Carbofuran, Phorate and HCH when used alone were not inhibitory to *Azospirillum* at their recommended doses. Similarly, fungicides, Carbendazim, Bordeaux mixture and Fytolan were also not inhibitory to *Azospirillum* when used alone and in combination with all the three insecticides. The fungicide Thiram when used alone and also along with insecticides caused the inhibition of

Table 1. *In vitro* Inhibitory effect of fungicides, insecticides and their combinations on the growth of *Azospirillum* and *Bradyrhizobium*

Treatments	Diameter of inhibition zones (cm)	
	Azospirillum	*Bradyrhizobium*
F_0I_0	0	0
F_0I_1	0	0
F_0I_2	0	0
F_0I_3	0	0
F_1I_0	0	0
F_1I_1	0	0
F_1I_2	0	0
F_2I_0	3.1	2.4
F_2I_1	3.4	2.3
F_2I_2	2.7	2.5
F_2I_3	3.5	2.2
F_3I_0	0	3.0
F_3I_1	0	2.6
F_3I_2	0	2.4
F_3I_3	0	2.4
F_4I_0	0	2.4
F_4I_1	0	2.7
F_4I_2	0	2.5
F_4I_3	0	2.8

Plate I. Inhibition of Bradyrhizobium due to different fungicides

F_1 - Carbendazim

F_2 - Thiram

F_3 - Bordeaux mixture

F_4 - Fytolan

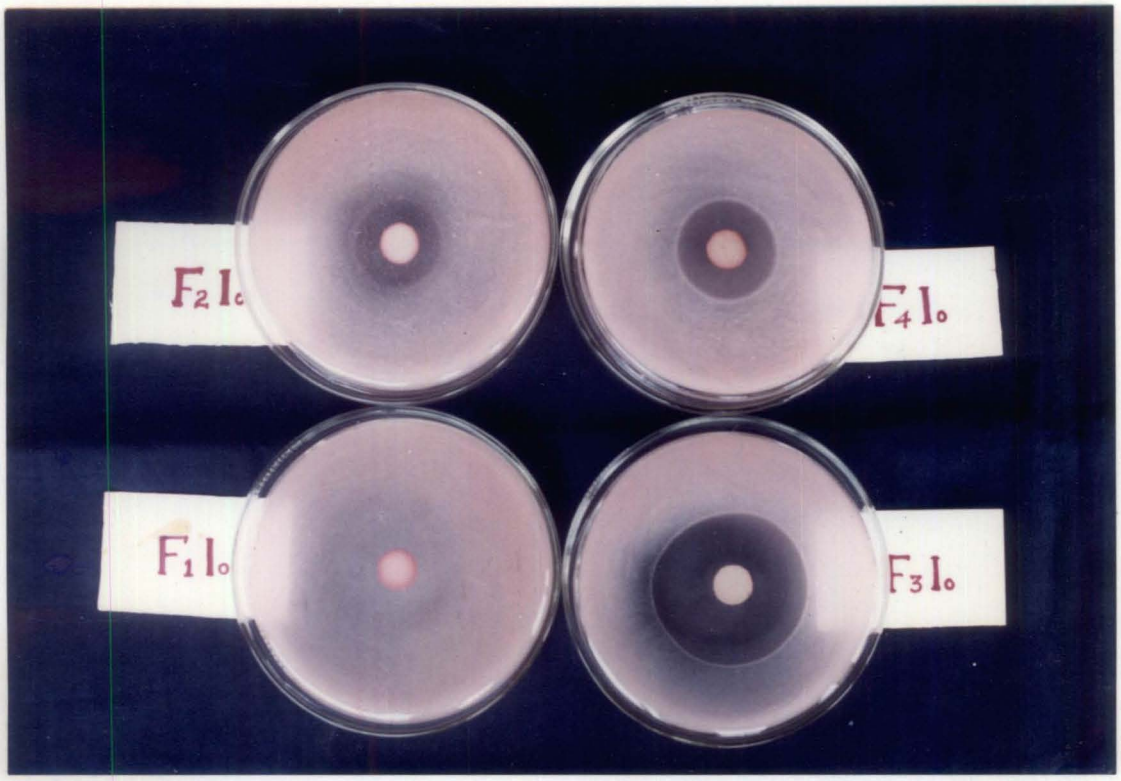
Plate II. Inhibition of Bradyrhizobium by the combination of Bordeaux mixture with different insecticides

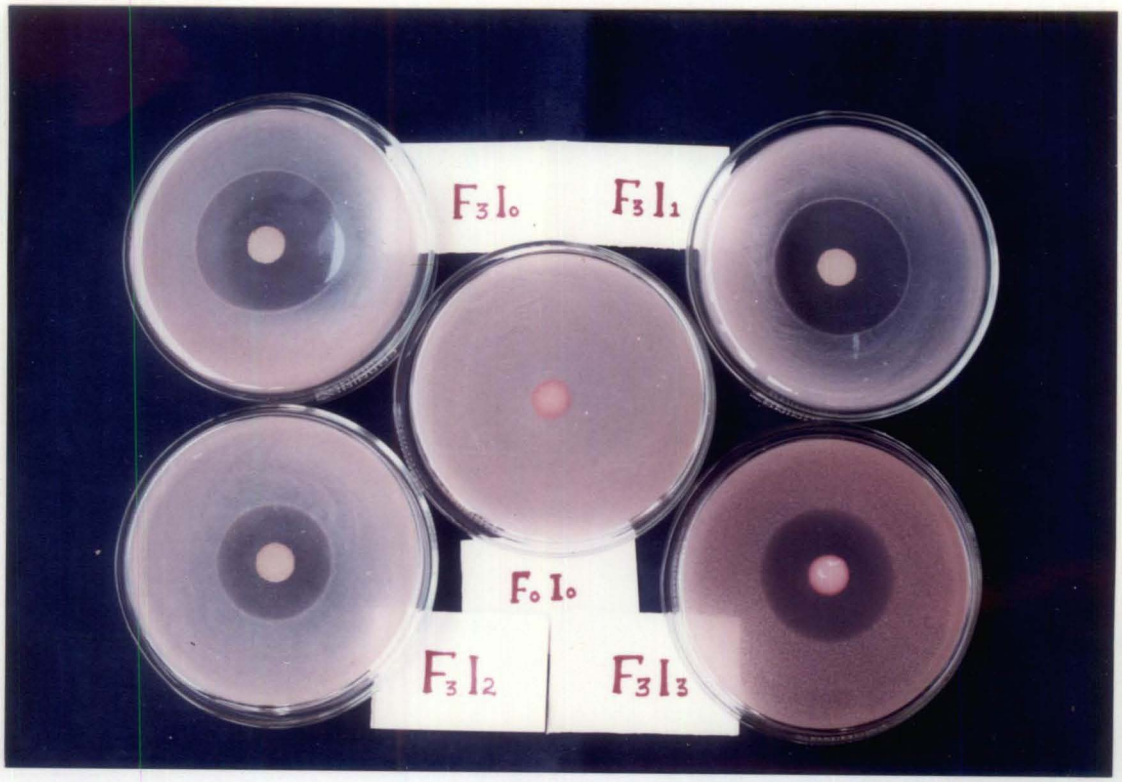
F_0I_0 - Control

F_3I_2 - Bordeaux mixture + Phorate

F_3I_1 - Bordeaux mixture

F_3I_3 - Bordeaux mixture + HCH


F2 I0
F4 I0
F1 I0
F3 I0


F3 I0
F3 I1
F0 I0
F3 I2
F3 I3

Azospirillum. Combination of Thiram with HCH showed the maximum inhibition while combination of Thiram with Phorate showed the minimum inhibition.

The insectides Carbofuran, Phorate and HCH were not inhibitory to Bradyrhizobium when used alone. Fungicide Carbendazim did not cause inhibition of Bradyrhizobium when used alone and in combination with all the three insecticides. The growth of Bradyrhizobium was inhibited by the fungicides Thiram, Bordeaux mixture and Fytolan when used alone and also when combined with insecticides (Plate I and II).

4.6 Effect of selected plant protection chemicals at the recommended doses and their combinations on Azospirillum and Bradyrhizobium in cowpea rhizosphere in the field

The results of the field experiment conducted to study the effect of selected plant protection chemicals at the recommended doses and their combinations on the population of Azospirillum and Bradyrhizobium in cowpea rhizosphere are given below

4.6.1. Soil population of Azospirillum

The natural soil population of Azospirillum in different plots was found to range from 1.5×10^4 to 3.5×10^4 cells per gram of soil. After the soil inoculation of Azospirillum, the population showed a general increase. The

population of Azospirillum seven days after inoculation ranged from 3.2×10^4 to 5.5×10^4 cells per gram of soil (Table 2).

In the control plots, where no plant protection chemicals were applied, gradual increase in Azospirillum population in the rhizosphere was noticed. Seven days after inoculation, the bacterial count was 3.7×10^4 cells per gram of soil and it was reached to 26.5×10^4 cells per gram of soil 42 days after inoculation.

In the rhizosphere of cowpea plants treated with insecticides Carbofuran 3% G (0.75 kg ai/ha), Phorate 10% G (1.5 kg ai/ha) and HCH 10% DP (2 kg ai/ha) also, the Azospirillum population showed a gradual increase as in the case of control plants.

Among the cowpea plants treated with fungicides, the rhizosphere population of Azospirillum showed gradual increase in Carbendazim, Bordeaux mixture and Fytolan treatments. Thiram treated plants showed a reduction in rhizosphere population of Azospirillum during the initial days. But later on the bacterial population increased.

When the combination of fungicides and insecticides applied in the soil no fluctuation from the increasing

Table 2. Population changes of *Azospirillum* in cowpea rhizosphere as influenced by fungicides, insecticides and their combinations.

Sl. No.	Treatments	Soil population of *Azospirillum* x 10^4 cells per gram of soil				
		Before inoculation	7 days after inoculation	7 days after application of plant protection chemicals	21 days after application of plant protection chemicals	35 days after application of plant protection chemicals
1.	F_0I_0	1.7	3.7	13.8	21.3	26.5
2.	F_0I_1	3.1	4.4	14.5	23.0	29.4
3.	F_0I_2	1.5	3.8	13.6	26.4	31.3
4.	F_0I_3	1.5	3.4	16.0	23.6	28.9
5.	F_1I_0	2.1	3.1	16.5	22.5	27.3
6.	F_1I_1	1.8	4.1	19.4	24.6	32.3
7.	F_1I_2	3.0	3.4	20.3	21.3	25.7
8.	F_1I_3	1.7	5.5	17.5	28.4	36.2
9.	F_2I_0	3.5	4.7	7.9	11.9	29.5
10.	F_2I_1	2.1	4.4	6.5	20.5	30.3
11.	F_2I_2	1.6	3.5	4.0	19.5	32.4
12.	F_2I_3	3.5	3.5	8.0	18.4	29.8
13.	F_3I_0	2.1	3.5	12.9	18.4	25.4
14.	F_3I_1	2.2	4.0	13.4	19.7	23.5
15.	F_3I_2	2.8	3.6	16.5	19.8	29.5
16.	F_3I_3	2.1	3.5	10.4	21.5	36.5
17.	F_4I_0	2.0	3.4	14.6	19.5	33.0
18.	F_4I_1	1.8	3.2	18.9	23.6	27.5
19.	F_4I_2	1.9	3.7	17.9	23.5	29.8
20.	F_4I_3	2.4	3.2	21.0	24.6	33.5

trend of Azospirillum population was noticed except in the combination of insecticides with Thiram.

4.6.2 Soil population of Bradyrhizobium

Natural soil population of Bradyrhizobium was ranging from 2.5×10^4 to 4.1×10^4 cells per gram of soil. After the sowing of Bradyrhizobium inoculated seeds the soil population increased and it ranged from 8.8×10^4 to 11.4×10^4 cells per gram of soil, 14 days after sowing. In the control plot, the rhizosphere population of Bradyrhizobium was 3.7×10^4 cells per gram of soil before sowing. This was increased to 9.2×10^4 cells after 14 days of sowing. Again this was increased to 22.2×10^4 cells per gram of soil after 49 days of sowing (Table 3.)

Among the plants treated with insecticides Carbofuran, Phorate and HCH a similar increase in the population of Bradyrhizobium was observed. In the rhizosphere of plants treated with fungicides, Carbendazim did not cause any reduction in bacterial population. But in Thiram, Bordeaux mixture and Fytolan treatments, there was an initial reduction in Bradyrhizobium population but later an increasing trend was noticed.

When the fungicides and insecticides were applied together in the soil, the rhizosphere population of

Table 3. Population changes of Bradyrhizobium in cowpea rhizosphere as influenced by fungicides, insecticides and their combinations.

Sl. No.	Treatments	Soil population of Bradyrhizobium x 10^4 cells per gram of soil: Before sowing	14 days after sowing	7 days after application of plant protection chemicals	21 days after application of plant protection chemicals	35 days after application of plant protection chemicals
1.	F_0I_0	3.7	9.2	14.2	21.8	22.2
2.	F_0I_1	3.3	10.6	16.8	20.3	23.8
3.	F_0I_2	3.5	11.0	15.4	18.2	22.0
4.	F_0I_3	3.8	9.3	14.8	19.7	26.9
5.	F_1I_0	2.8	9.6	18.3	18.6	24.3
6.	F_1I_1	2.6	10.1	17.5	23.5	26.4
7.	F_1I_2	2.8	10.4	16.4	18.3	27.3
8.	F_1I_3	3.0	11.4	18.9	19.5	21.4
9.	F_2I_0	4.1	10.1	8.6	12.3	21.7
10.	F_2I_1	3.8	9.9	9.0	14.6	26.5
11.	F_2I_2	3.3	11.0	7.4	18.5	31.0
12.	F_2I_3	3.7	8.8	8.0	11.0	27.3
13.	F_3I_0	2.9	9.5	8.3	18.9	29.0
14.	F_3I_1	3.1	10.0	7.4	23.4	27.6
15.	F_3I_2	3.7	11.2	11.0	15.0	31.3
16.	F_3I_3	2.7	10.4	9.0	17.5	30.6
17.	F_4I_0	2.7	9.8	8.5	19.0	26.0
18.	F_4I_1	2.5	9.4	9.0	20.3	31.0
19.	F_4I_2	2.8	11.4	6.5	14.5	20.5
20.	F_4I_3	3.2	11.2	7.4	18.3	21.2

Bradyrhizobium did not show any reduction in their number in plants treated with combinations of all insecticides with Carbendazim. But the combination of insecticides with Thiram, Bordeaux mixture and Fytolan generaly showed a reduction in the number of bacteria initially, later a gradual increase was noticed.

4.7 Growth parameters of cowpea plants as influenced by Azospirillum and Bradyrhizobium upon application of recommended doses of selected fungicides, insecticides and thier combinations

4.7.1 Total fresh weight

Effect of fungicides, insecticides, and thier combinations on Azospirillum in cowpea rhizosphere and in turn their effect on total fresh weight of cowpea plants are given in table 4. There was no statistical significance between the treatments.

Among the Azospirillum inoculated plants treated with fungicides, the highest total fresh weight was recorded in F_2 (112.184 g), followed by F_3 (105.942 g). The total fresh weight obtained in control was only 72.942 g. However, F_4 showed a lower total fresh weight than the control.

The highest total Fresh weight of Azospirillum inoculated plants treated with insecticides was recorded in I_3. (101.193 g). The total fresh weight in control was 84.667 g. The other treatments I_1 and I_2 also recorded

Table 4. Total fresh weight of Azospirillum inoculated cowpea plants treated with fungicides, insecticides and their combinations (g)

Fungicide treatments (F)	Insecticide treatments (I)				Mean
	I_0	I_1	I_2	I_3	
F_0	8.430 (71.667)	6.774 (43.233)	9.991 (100.867)	8.761 (77.00)	8.489 (72.942)
F_1	9.260 (86.467)	8.680 (78.067)	9.385 (102.767)	10.042 (103.167)	9.469 (92.617)
F_2	9.588 (97.033)	12.383 (154.267)	8.110 (72.767)	11.064 (124.667)	10.286 (112.184)
F_3	8.715 (81.700)	9.406 (94.767)	9.826 (97.600)	12.013 (149.700)	9.990 (105.942)
F_4	9.079 (86.467)	9.205 (91.000)	7.656 (60.333)	6.663 (51.433)	8.151 (72.308)
Mean	9.016 (84.667)	9.289 (92.067)	9.093 (86.867)	7.709 (101.193)	

Data transformed. Original values are given in parantheses

F Not significant

I Not significant

F x I Not significant

higher total fresh weights of 92.027 g and 86.867 g respectively.

Azospirillum inoculated plants treated with combination of fungicides and insecticides recorded highest total fresh weight of 154.267 g in F_2I_1. The treatment F_0I_1 recorded the lowest total fresh weight of 43.233 g. The treatments F_4I_2 and F_4I_3 also recorded lower total fresh weights than the control. The total fresh weight in control was 71.667 g.

Total fresh weight of cowpea plants inoculated with Bradyrhizobium in which fungicides, insecticides and their combinations were applied are presented in table 5. The data did not show any statistical significance among treatments.

Among the Bradyrhizobium inoculated plants treated with fungicides, highest total fresh weight of 63.117 g was observed in F_2 treated plants. It was followed by F_3 (63.000 g) and F_4 (57.067 g). The total fresh weight observed in control was 55.575 g. F_1 showed lower total fresh weight than that in the control.

Bradyrhizobium inoculated plants treated with insecticides recorded highest total fresh weight of 65.747 g in I_2. The total fresh weight in control was 59.840 g. The lowest fresh weight of 51.973 g was recorded in I_1.

Table 5. Total fresh weight of Bradyrhizobium inoculated cowpea plants treated with fungicides, insecticides and their combinations (g)

Fungicide treatments (F)	Insecticide treatments (I)				Mean
	I_0	I_1	I_2	I_3	
F_0	6.137 (38.00)	6.945 (50.133)	7.940 (64.433)	8.340 (69.733)	7.341 (55.575)
F_1	5.772 (33.733)	6.214 (39.967)	6.903 (47.900)	8.703 (88.333)	6.898 (52.483)
F_2	7.253 (59.833)	6.925 (49.433)	9.379 (90.667)	7.540 (57.533)	7.774 (63.117)
F_3	8.932 (83.200)	8.316 (72.433)	8.122 (72.167)	4.821 (24.200)	7.548 (63.000)
F_4	9.331 (89.433)	6.878 (47.900)	7.280 (53.567)	6.099 (37.367)	7.394 (57.067)
Mean	7.483 (59.840)	7.053 (51.973)	7.925 (65.747)	7.100 (55.433)	

Data transformed. Original values are given in parantheses

F Not significant

I Not significant

F x I Not significant

The total fresh weight of Bradyrhizobium inoculated plants treated with combination of fungicides and insecticides were greater than that of control except in F_1I_0, F_4I_3 and F_3I_3. The highest total fresh weight of 90.677 g was recorded by the plants treated with F_2I_2. The lowest total fresh weight of 24.200 g was recorded in F_3I_3.

4.7.2 Root fresh weight

Effect of fungicides, insecticides and their combinations on Azospirillum in cowpea rhizosphere and in turn their effect on root fresh weight of cowpea plants are given in the table 6.

Even though the treatments did not show any significant difference statistically, the maximum root fresh weight of Azospirillum inoculated plants treated with fungicides was recorded in F_2, (5.601 g). The root fresh weight of control was 4.383 g. Only the treatment F_4 showed a lower root fresh weight than the control.

The highest root fresh weight of 5.113 g was observed in I_1, among the Azospirillum inoculated plants treated with insecticides. The lowest value of 4.439 g was recorded in I_2.

Table 6. Root fresh weight of Azospirillum inoculated cowpea plants treated with fungicides, insecticides and their combinations (g)

Fungicide treatments (F)	Insecticide treatments (I) I_0	I_1	I_2	I_3	Mean
F_0	2.112 (4.533)	1.772 (3.106)	2.310 (5.367)	2.111 (4.467)	2.076 (4.383)
F_1	2.172 (4.733)	2.119 (4.600)	2.347 (5.766)	2.366 (5.833)	2.241 (5.233)
F_2	2.233 (5.235)	2.800 (8.167)	1.774 (3.438)	2.362 (5.600)	2.305 (5.601)
F_3	1.901 (3.800)	2.133 (4.833)	2.168 (4.733)	2.415 (6.067)	2.154 (4.858)
F_4	2.346 (5.633)	2.142 (4.800)	1.671 (2.900)	1.473 (2.367)	1.908 (3.925)
Mean	2.153 (4.786)	2.203 (5.133)	2.046 (4.439)	2.145 (4.867)	

Data transformed. Original values are given in parantheses

F Not significant

I Not significant

F x I Not significant

Azospirillum inoculated plants treated with F_2I_1 combination of fungicide and insecticides showed highest root fresh weight of 8.167 g. The lowest root fresh weight was recorded by the plants treated with F_4I_3 (2.367 g). The treatment F_0I_1, F_0I_3, F_2I_0, F_3I_0, F_4I_2 and F_4I_3 recorded root fresh weights lower than the control.

The root fresh weight of cowpea plants inoculated with Bradyrhizobium and treated with fungicides, insecticides and thier combinations are presented in table 7. There was no significant difference between treatments.

The maximum root fresh weight of 4.558 g was noticed in F_3, among the Bradyrhizobium inoculated plants treated with fungicides.

Bradyrhizobium inoculated plants treated with insecticides showed the highest root fresh weight in I_2 (4.187 g). The root fresh weight obtained in control was 4.040 g. I_1 and I_3 recorded lower root fresh weights than control.

The root fresh weight of Bradyrhizobium inoculated plants treated with combination of fungicides and insecticides was highest in F_3I_0 (5.800 g). The lowest root fresh weight of 2.267 g was observed in F_4I_3. The root fresh weight in control was 2.967 g.

Table 7. Root fresh weight of Bradyrhizobium inoculated cowpea plants treated with fungicides, insecticides and their combinations (g)

Fungicide treatments (F)	Insecticide treatments (I) I_0	I_1	I_2	I_3	Mean
F_0	1.710 (2.967)	1.880 (3.633)	1.824 (3.600)	2.130 (4.367)	1.886 (3.692)
F_1	1.760 (3.133)	1.715 (3.000)	1.380 (3.600)	2.204 (5.267)	1.889 (3.750)
F_2	2.026 (4.200)	1.496 (2.567)	2.036 (4.400)	2.081 (4.367)	1.960 (3.383)
F_3	2.398 (5.800)	2.047 (4.333)	2.215 (5.033)	1.692 (3.067)	2.088 (4.558)
F_4	1.797 (4.100)	1.762 (3.367)	2.051 (4.300)	1.497 (2.267)	1.826 (3.508)
Mean	1.978 (4.040)	1.779 (3.380)	2.001 (4.187)	1.921 (3.907)	

Data transformed. Original values are given in parantheses

F Not significant

I Not significant

F x I Not significant

4.7.3 Total dry weight

Total dry weight of Azospirillum inoculated plants treated with fungicides, insecticides and their combinations are given in table 8.

Even though there was no significant difference between treatments, among the plants inoculated with Azospirillum and treated with fungicides, F_3 showed the maximum total dry weight of 13.867 g. The total dry weight in control was 11.092 g. The lowest total dry weight was observed in F_4 (9.175 g).

Among the Azospirillum inoculated plants treated with insecticides, the highest total dry weight was observed in I_1 (13.173 g). The total dry weight in control was 11.073 g and this was the lowest value.

Total dry weight of Azospirillum inoculated plants treated with combinations of fungicides and insecticides was highest in F_3I_3 (19.067 g). Total dry weight in control was 12.200 g while the lowest value of 5.600 g was recorded by F_4I_2.

Total dry weight of Bradyrhizobium inoculated plants treated with fungicides, insecticides and their combinations are presented in table 9. This also showed no statistical significance.

Table 8. Total dry weight of Azospirillum inoculated cowpea plants treated with fungicides, insecticides and their combinations (g)

Fungicide treatments (F)	Insecticide treatments (I)				Mean
	I_0	I_1	I_2	I_3	
F_0	3.341 (12.200)	3.333 (11.335)	3.560 (12.800)	2.829 (8.033)	3.266 (11.092)
F_1	2.823 (8.067)	3.295 (11.400)	3.931 (15.000)	2.885 (8.433)	3.234 (10.725)
F_2	3.401 (12.600)	4.230 (17.900)	3.268 (11.500)	3.520 (12.867)	3.605 (13.717)
F_3	3.045 (10.000)	3.409 (12.500)	3.717 (13.900)	4.274 (19.067)	3.611 (13.867)
F_4	3.442 (12.500)	3.445 (12.733)	2.305 (5.600)	2.270 (5.867)	2.866 (9.175)
Mean	3.210 (11.073)	3.342 (13.173)	3.356 (11.760)	3.156 (10.853)	

Data transformed. Original values are given in parantheses

F Not significant

I Not significant

F x I Not significant

Table 9. Total dry weight of Bradyrhizobium inoculated cowpea plants treated with fungicides, insecticides and their combinations (g).

Fungicide treatments (F)	Insecticide treatments (I) I_0	I_1	I_2	I_3	Mean
F_0	2.280 (5.267)	2.880 (3.733)	3.101 (9.967)	3.050 (9.400)	2.580 (7.092)
F_1	2.293 (5.333)	2.595 (8.300)	1.984 (4.333)	3.300 (12.733)	2.543 (7.765)
F_2	2.617 (7.233)	2.098 (4.733)	2.328 (6.200)	2.620 (7.100)	2.416 (6.317)
F_3	3.778 (14.833)	3.114 (10.067)	2.565 7.433)	2.372 (6.000)	2.957 (7.853)
F_4	2.235 (10.800)	2.297 (5.867)	3.174 (10.167)	2.217 (5.067)	2.731 (7.975)
Mean	2.241 (8.693)	2.399 (6.540)	2.630 (7.620)	2.712 (8.060)	

Data transformed. Original values are given in parantheses

F Not significant

I Not significant

F x I Not significant

Among the Bradyrhizobium inoculated plants treated with fungicides, the maximum total dry weight of 7.975 g was recorded in F_4. In control, the total dry weight observed was 7.092 g. The lowest total dry weight was noticed in F_2 (6.317 g).

Bradyrhizobium inoculated plants treated with insecticides showed maximum total dry weight in control (8.693 g). The lowest value of 6.540 g was recorded in I_1.

Bradyrhizobium inoculated plants treated with combinations of fungicides and insecticides showed maximum total dry weight in F_1I_3 (122.733 g). Total dry weight in control was 5.267 g. The lowest total dry weight was recorded in F_1I_1 (3.733 g).

4.7.4. Root dry weight

Root dry weight of Azospirillum inoculated plants treated with fungicides, insecticides and the combination of fungicides and insecticides are given in table 10.

The root dry weight did not show any statistical significance. Among the plants treated with fungicides, maximum root dry weight of 1.200 g was recorded in F_2. The treatment F_3 showed a root dry weight of 1.159 g. The lowest root dry weight was recorded in F_4 (0.884 g).

Table 10. Root dry weight of Azospirillum inoculated cowpea plants treated with fungicides, insecticides and their combinations (g)

Fungicide treatments (F)	Insecticide treatments (I)				Mean
	I_0	I_1	I_2	I_3	
F_0	0.956 (1.000)	0.928 (0.967)	1.093 (1.200)	0.870 (0.767)	0.962 (0.984)
F_1	0.031 (1.067)	0.923 (0.900)	1.211 (1.467)	0.925 (0.867)	1.022 (1.075)
F_2	0.993 (1.067)	1.220 (1.500)	1.012 (1.133)	1.033 (1.100)	1.066 (1.200)
F_3	0.997 (1.000)	1.051 (1.167)	1.030 (1.067)	1.666 (1.400)	1.061 (1.159)
F_4	1.166 (1.400)	0.937 (0.967)	0.739 (0.567)	0.730 (0.600)	0.893 (0.884)
Mean	1.029 (1.107)	1.012 (1.100)	1.017 (1.087)	0.945 (0.947)	

Data transformed. Original values are given in parantheses

F Not significant

I Not significant

F x I Not significant

Among the Azospirillum inoculated plants treated with insecticides, maximum root dry weight was recorded in control (1.107 g). The lowest root dry weight of 0.947 g was observed in I_3. I_2 recorded root dry weight higher than that of control.

Azospirillum inoculated plants treated with combination of fungicides and insecticides the maximum root dry weight of 1.500 g was recorded in F_2I_1. The root dry weight in control was 1.000 g. The lowest root dry weight was recorded in F_4I_2 (0.567 g)

Root dry weight of plants inoculated with Bradyrhizobium treated with fungicides, insecticides and their combinations are presented in table 11.

There was no significant diffrence between treatments. The maximum root dry weight of fungicide treated plants was recorded in F_3 (0.867 g). The root dry weight in control was 0.750 g. The lowest root dry weight of 0.667 g was recorded in F_2.

Among the Bradyrhizobium inoculated plants treated with insecticides, the control plants recorded highest root dry weight of 0.907 g. I_1 treated plants showed lowest root dry weight of 0.660 g. I_2 and I_3 recorded root dry weights of 0.720 g and 0.780 g respectively.

Table 11. Root dry weight of Bradyrhizobium inoculated cowpea plants treated with fungicides, insecticides and their combinations (g)

Fungicide treatments (F)	Insecticide treatments (I)				Mean
	I_0	I_1	I_2	I_3	
F_0	0.816 (0.667)	0.629 (0.400)	0.910 (0.833)	1.043 (1.100)	0.850 (0.750)
F_1	0.882 (0.800)	0.782 (0.733)	0.648 (0.467)	0.954 (1.000)	0.816 (0.750)
F_2	0.848 (0.733)	0.693 (0.533)	0.683 (0.500)	0.941 (0.900)	0.791 (0.667)
F_3	1.530 (1.367)	0.868 (0.833)	0.849 (0.800)	0.648 (0.467)	0.879 (0.867)
F_4	0.749 (0.967)	0.816 (0.800)	0.981 (1.000)	0.654 (0.433)	0.850 (0.800)
Mean	0.929 (0.907)	0.758 (0.660)	0.814 (0.720)	0.848 (0.780)	

Data transformed. Original values are given in parantheses

F Not significant

I Not significant

F x I Not significant

The root dry weight of Bradyrhizobium inoculated plants treated with combination of fungicides and insecticides showed a maximum value of 1.367 g in F_3I_0. The root dry weight in control was 0.667 g. The lowest root dry weight of 0.400 g was observed in F_0I_1. The treatments F_1I_2, F_2I_1, F_2I_2, F_3I_3 and F_4I_3 also showed lower root dry weights than the control.

4.7.5 Plant height

Height of Azospirillum inoculated plants treated with fungicides, insecticides and their combinations are given in table 12. No significant differences between treatments was noticed.

Among the Azospirillum inoculated plants treated with fungicides, maximum height of 53.583 cm was recorded in F_2. The plant height recorded in control was 45.375 cm. The lowest height of 38.000cm was recorded in F_4 treated plants. The treatment F_1 also recorded plant height greater than that of control.

Azospirillum inoculated plants treated with insecticides showed maximum height of 48.433 cm in control. The treatment I_3 showed lowest value of plant height (43.467 cm).

Table 12. Height of Azospirillum inoculated cowpea plants treated with fungicides, insecticides and their combinations (cm)

Fungicide treatments (F)	Insecticide treatments (I) I_0	I_1	I_2	I_3	Mean
F_0	53.500	36.000	48.333	43.667	45.375
F_1	43.000	39.500	51.667	43.000	44.292
F_2	60.333	67.667	37.667	48.667	53.583
F_3	42.000	48.333	63.000	49.000	50.583
F_4	43.333	42.667	33.000	33.000	38.000
Mean	48.433	46.833	46.733	43.467	

F Not significant
I Not significant
F x I Not significant

Table 13. Height of Bradyrhizobium inoculated cowpea plants treated with fungicides, insecticides and their combinations (cm)

Fungicide treatments (F)	Insecticide treatments (I) I_0	I_1	I_2	I_3	Mean
F_0	48.333	37.333	41.167	46.167	43.250
F_1	44.667	40.333	36.500	41.667	40.792
F_2	36.667	39.667	42.333	40.667	39.833
F_3	43.667	44.000	43.667	34.667	41.500
F_4	48.333	41.000	45.333	45.667	45.083
Mean	44.333	40.467	41.800	41.767	

F Not significant
I Not significant
F x I Not significant

The plants treated with combinations of fungicides and insecticides showed maximum height of 67.667 cm in F_2I_1 and minimum in F_4I_2 and F_4I_3 (33.000 cm). The treatment F_2I_0, F_2I_1 and F_3I_2 applied plants showed heights higher than that observed in control.

The effect of fungicides, insecticides and their combinations on Bradyrhizobium and in turn their effect on plant height are given in table 13. There was no significant difference between treatments.

The plants treated with fungicides, showed maximum height in F_4 (45.083 cm). The control plant showed a height of 43.250 cm. The minimum plant height was recorded in F_2 (39.833 cm). The F_1 and F_3 treated plants also showed lower values than the control.

The insecticide treated plants showed maximum height of 44.333 cm in control. The treatment I_1 showed the lowest value (40.467 cm). The treatment I_2 and I_3 showed almost similar plant height of 41.800 cm and 41.767 cm respectively.

The plants inoculated with Bradyrhizobium, treated with the combination of fungicides and insecticides showed maximum height in F_4I_0 (48.333 cm) and in control. The lowest value of 34.667 cm was observed in F_3I_3.

4.7.6 Number of leaves

Number of leaves of Azospirillum inoculated plants treated with fungicides, insecticides and their combinations are presented in table 14. There was no significant difference between treatments in their effects on number of leaves. The maximum number of leaves among the plants treated with fungicides was recorded in F_2 (14.667). The number of leaves was minimum in control (10.500). F_1, F_3 and F_4 treated plants also showed more number of leaves than in control.

The Azospirillum inoculated plants treated with insecticides showed maximum number of leaves in I_1 (14.600). In control the number of leaves recorded was 10.600 and this was the lowest number of leaves observed.

Among the plants treated with combination of fungicides and insecticides, maximum number of leaves recorded was in F_2I_I (20.667), followed by F_4I_I (20.000). In control, the number of leaves observed was 9,000. The number of leaves observed was least in F_1I_0.

The number of leaves of Bradyrhizobium inoculated plants treated with fungicides, insecticides and their combinations are presented in table 15. There was no statistically significant difference in number of leaves.

Table 14. Number of leaves of of Azospirillum inoculated cowpea plants treated with fungicides, insecticides and their combinations

Fungicide treatments (F)	Insecticide treatments (I) I_0	I_1	I_2	I_3	Mean
F_0	2.937 (9.000)	3.007 (9.333)	3.555 (12.667)	3.255 (11.000)	3.201 (10.500)
F_1	2.570 (6.667)	3.260 (10.667)	3.709 (14.000)	3.050 (15.000)	3.347 (11.584)
F_2	3.613 (14.000)	4.495 (20.667)	2.922 (8.667)	3.884 (15.333)	3.728 (14.667)
F_3	3.493 (12.333)	4.463 (12.333)	3.350 (15.000)	3.520 (12.667)	3.581 (13.083)
F_4	3.256 (11.000)	4.043 (20.000)	3.073 (10.000)	4.149 (17.333)	3.630 (14.583)
Mean	3.184 (10.600)	3.654 (14.600)	3.422 (12.017)	3.732 (14.267)	

Data transformed. Original values are given in parantheses

F Not significant

I Not significant

F x I Not significant

Table 15. Number of leaves of of Bradyrhizobium inoculated cowpea plants treated with fungicides, insecticides and their combinations

Fungicide treatments (F)	Insecticide treatments (I)				Mean
	I_0	I_1	I_2	I_3	
F_0	2.393 (5.667)	2.854 (8.333)	2.914 (9.000)	3.152 (10.000)	2.823 (8.250)
F_1	2.449 (6.000)	2.979 (9.000)	2.627 (7.000)	3.998 (18.353)	3.013 (10.083)
F_2	3.380 (17.667)	2.483 (6.333)	3.705 (15.667)	2.304 (8.000)	3.268 (11.917)
F_3	3.497 (13.000)	3.234 (10.667)	3.713 (14.667)	2.215 (5.000)	3.165 (10.833)
F_4	2.371 (8.333)	3.521 (14.000)	3.087 (9.667)	2.570 (6.667)	3.012 (9.667)
Mean	3.014 (10.133)	3.014 (9.667)	3.249 (11.200)	2.948 (7.600)	

Data transformed. Original values are given in parantheses

F Not significant

I Not significant

F x I Not significant

The Bradyrhizobium inoculated plants treated with fungicides showed highest number of leaves in F_2 treatment (11.917). The number of leaves observed was minimum in control.

The insecticide treated plants showed highest number of leaves in I_2. I_3 treated plants showed lowest number of leaves (7.600).

The plants treated with combination of fungicide and insecticide showed highest number of leaves in $F_I I_3$ (18.333). In control the number of leaves observed was 5.667

4.7.7 Root length

Root length of Azospirillum inoculated plants treated with fungicides, insecticides and combination of fungicides and insecticides are presented in table 16. No statistical significance was observed for main effect of fungicides and insecticides.

Fungicide applied plants gave the maximum root length of 18.750 cm in F_2. In control the root length was 15.083 cm and it was the lowest.

Among the plants treated with insecticides maximum root length was noticed in control (17.600). The lowest value of 16.733 cm. was observed in I_1.

Table 16. Root length of *Azospirillum* inoculated cowpea plants treated with fungicides, insecticides and their combinations (cm)

Fungicide treatments (F)	Insecticide treatments (I)				Mean
	I_0	I_1	I_2	I_3	
F_0	14.000	12.000	21.000	13.333	15.083
F_1	15.667	21.667	17.333	17.000	17.917
F_2	23.000	16.333	12.333	23.333	18.750
F_3	16.333	13.667	18.333	19.333	16.917
F_4	19.000	20.000	16.000	14.000	17.250
Mean	17.600	16.733	17.000	17.400	

F - Not significant
I - Not significant
CD (0.05) for F x I 6.79

Table 17. Root length of *Bradyrhizobium* inoculated cowpea plants treated with fungicides, insecticides and their combinations (cm)

Fungicide treatments (F)	Insecticide treatments (I)				Mean
	I_0	I_1	I_2	I_3	
F_0	17.000	14.667	18.000	19.033	17.375
F_1	17.667	15.667	21.333	19.000	18.417
F_2	20.033	14.500	18.167	16.900	17.500
F_3	19.500	17.00	17.333	18.333	18.167
F_4	15.667	15.667	23.000	12.500	16.708
Mean	18.133	15.500	19.567	17.333	

F Not significant
I Not significant
F x I Not significant

There was significant difference between the treatments, when combination of fungicides and insecticides were applied. Maximum root length of 23.000 cm was recorded in F_2I_3. The lowest value of root length was recorded in F_0I_1 (12.000 cm). In control the root length was 14.000 cm.

Root length of Bradyrhizobium inoculated plants treated with fungicides, insecticides and their combination are given in table 17.

Among the plants treated with fungicides no significant difference between treatments was observed. Even though the maximum root length was observed in F_1 (18.417 cm), followed by F_3 (18.167 cm). In control, the root length was 17.375 cm.

There was significant difference among insecticide treatments in their effect on root length. Maximum root length of 19.567 cm was recorded in I_2. This was on par with I_3 and control. Root length of I_2 was significantly higher than I_1 (15.500 cm).

The Bradyrhizobium inoculated plants treated with combinations of fungicides and insecticides did not show any statistical significance. The maximum root length was

noticed in F_4I_2 (23.000). The lowest value of 12.500 cm. was recorded in F_4I_3. In control the root length was 17.000 cm.

4.7.8 Number of nodules

Number of nodules of Azospirillum inoculated plants treated with fungicides, insecticides and their combinations are given in table 18.

There was significant difference among fungicide treatments in their effect on number of nodules. Maximum number of nodules was recorded in control (41.750). This was on par with F_1 and significantly higher than the other three treatments. The lowest number of nodules was recorded in F_4 (34.250). The insecticide treatments also showed significant difference in their effect on nodule number in Azospirillum inoculated plants. Maximum nodule number of 40.133 was recorded in control. The lowest number of 36.067 was recorded in I_3. There was no significant difference between treatments when combinations of fungicides and insecticides were applied .

Number of nodules of Bradyrhizobium inoculated plants treated with fungicides, insecticides and their combinations are presented in table 19.

Table 18. Number of nodules on Azospirillum inoculated cowpea plants treated with fungicides, insecticides and their combinations.

Fungicide treatments (F)	Insecticide treatments (I) I_0	I_1	I_2	I_3	Mean
F_0	49.333	42.000	41.000	34.667	41.750
F_1	42.000	39.000	40.333	30.333	39.917
F_2	34.667	36.333	39.333	36.667	36.750
F_3	39.333	36.667	37.333	37.333	37.667
F_4	35.333	33.667	34.667	33.333	34.250
Mean	40.133	37.533	38.533	36.067	

CD (0.05) for F 3.15
CD (0.05) for I 2.82
F x I Not significant

Table 19. Number of nodules on Bradyrhizobium inoculated cowpea plants treated with fungicides, insecticides and their combinations.

Fungicide treatments (F)	Insecticide treatments (I) I_0	I_1	I_2	I_3	Mean
F_0	63.333	56.000	58.667	54.667	58.167
F_1	52.333	54.000	50.667	59.000	54.000
F_2	44.000	50.667	28.333	35.333	39.583
F_3	47.667	42.667	65.000	34.333	39.917
F_4	37.000	32.333	39.000	38.667	36.750
Mean	48.867	47.133	42.333	44.400	

CD (0.05) for F 6.09
I Not significant
F x I Not significant

Plants treated with fungicides showed significant difference in number of nodules. Maximum number of nodules was found in control (58.167). This was on par with F_1 and higher than the other three treatments. The lowest number of nodules was recorded in F_4.

The plants treated with insecticides did not show any significant difference between them. Highest number of nodules was recorded in control (48.867) followed by 47.133 in I_1. The lowest number of nodules recorded was in I_2.

The Bradyrhizobium inoculated plants treated with combination of fungicides and insecticides did not show any significant difference between them. Even though the maximum number of nodules was recorded in control (63.333). The minimum number of nodules recorded was in F_2I_2 (28.333).

4.7.9 Nitrogen content

Nitrogen content of Azospirillum inoculated plants treated with fungicides, insecticides and their combinations are presented in table 20.

There was no significant difference between fungicide treatment in their effect on nitrogen content of plant. However,maximum nitrogen content of 3.025 per cent was recorded in F_2. In control, nitrogen content was 2.823 per cent. The lowest nitrogen content was recorded in F_1 (2.353 per cent).

Table 20. Nitrogen content of Azospirillum inoculated cowpea plants treated with fungicides, insecticides and their combinations (per cent)

Fungicide treatments (F)	Insecticide treatments (I) I_0	I_1	I_2	I_3	Mean
F_0	2.800	2.220	2.707	2.564	2.823
F_1	2.567	2.987	2.753	3.127	2.353
F_2	3.300	2.860	3.033	2.707	3.025
F_3	3.127	3.267	2.707	2.473	2.893
F_4	3.173	3.407	2.987	2.240	2.952
Mean	3.033	3.148	2.837	2.623	

F Not significant
CD (0.05) For I 0.31
F x I Not significant

Table 21. Nitrogen content of Bradyrhizobium inoculated cowpea plants treated with fungicides, insecticides and their combinations (per cent)

Fungicide treatments (F)	Insecticide treatments (I) I_0	I_1	I_2	I_3	Mean
F_0	2.800	2.473	2.473	2.660	2.602
F_1	2.753	2.987	2.613	2.893	2.312
F_2	2.753	2.613	2.987	2.520	2.718
F_3	2.193	2.660	2.427	2.080	2.590
F_4	2.393	2.613	2.700	2.893	2.775
Mean	2.679	2.669	2.640	2.809	

F Not significant
I Not significant
F x I Not significant

Insecticide treatments showed significant difference between them in their effect on nitrogen content. The maximum nitrogen content of 3.148 per cent was recorded in I_1. This was on par with I_2 and control. The lowest content of nitrogen was recorded in I_3.

The plants treated with combination of fungicides and insecticides showed no significant difference in nitrogen content. The maximum content of nitrogen was recorded is F_4I_1 (3.407). The lowest content of nitrogen was in F_0I_1.

Nitrogen content of Bradyrhizobium inoculated plants treated with fungicides, insecticides and their combinations is presented in table 21. There was no statistical significance between treatments.

Among the plants treated with fungicides, the highest value of 2.775 per cent was recorded in F_4. The lowest content of nitrogen was recorded in F_1.

Bradyrhizobium plants treated with insecticides showed highest nitrogen content of 2.809 per cent in I_3. The lowest nitrogen content was observed in I_2.

The plants treated with combination of fungicides and insecticides showed maximum nitrogen content of 2.987 per cent in F_1I_1 and F_2I_2. In control, the nitrogen content

recorded was 2.800 per cent. The lowest value of 2.080 per cent was observed in F_3I_3.

4.8 *In vitro* tolerance of *Azospirillum* and *Bradyrhizobium* to different doses of selected plant protection chemicals

An *in vitro* experiment was conducted to find out the tolerance levels of *Azospirillum* and *Bradyrhizobium* to different doses of plant protection chemicals.

From the study, it was found that out of the four fungicides tested, *Azospirillum* was sensitive to Thiram only. Carbendazim, Bordeaux mixture and Fytolan did not show inhibition of this bacteria at all the five doses tested. Inhibition zone was minimum at the lowest dose of 0.05 per cent (2.0 cm). At the recommended dose of 0.2 per cent, the diameter of inhibition zone was 3.0 cm. At the two higher doses of Thiram, diameter of inhibition zone was 3.5 cm (Table 22).

In vitro tolerance of *Bradyrhizobium* to different doses of fungicides are presented in table 22.

Thiram showed inhibition of *Bradyrhizobium* in all the five doses tested. Inhibition of *Bradyrhizobium* increased with increase in concentration of Thiram. The diameter of inhibition zone at the lowest concentration of 0.05 per cent

Table 22. *In vitro* tolerance of *Azospirillum* and *Bradyrhizobium* to different doses of fungicides.

Fungicides	doses (per cent)	Diameter of inhibition zone (cm)	
		Azospirillum	*Bradyrhizobium*
Carbendazim	0.050	0	0
	0.075	0	0
	0.100	0	0
	0.125	0	0
	0.150	0	0
Thiram	0.050	2.0	2.4
	0.100	2.3	2.5
	0.200	3.0	2.7
	0.300	3.5	3.0
	0.400	3.5	3.1
Bordeaux mixture	0.250	0	0
	0.500	0	2.5
	1.000	0	3.4
	2.000	0	3.4
	3.000	0	3.5
Fytolan	0.100	0	2.8
	0.200	0	3.0
	0.300	0	3.3
	0.400	0	3.3
	0.500	0	3.4

was 2.4 cm. It was 2.7 cm at the recommended dose of 0.2 per cent. The maximum inhibition of 3.1 cm was recorded at 0.4 per cent.

Bordeaux mixture showed no inhibition at 0.250 per cent. The inhibition was minimum at 0.5 per cent (2.5 cm). At one per cent dose, the diameter of inhibition zone was 3.4 cm. The same amount of inhibition was noticed at 2 per cent also. At three per cent, the inhibition increased (3.5 cm).

Fytolan showed inhibition of Bradyrhizobium at all the five doses tested. The lowest dose of one per cent showed minimum inhibition of 2.8 cm. This was increased with increase in concentration and maximum zone of inhibition was noticed at 0.5 per cent (3.4. cm). At the recommended dose 0.3 per cent, the diameter of inhibition zone was 3.3 cm. At 0.4 per cent also the same amount of inhibition was noticed.

The three insecticides, Carbofuran, Phorate and HCH did not show any inhibition to Azospirillum and Bradyrhizobium at all the five doses tested.

Discussion

DISCUSSION

Biofertilizers play a vital role in modern agriculture. Of the several beneficial microorganisms Azospirillum has proved to be a potential organism and have received wider interest as biofertilizer. The beneficial inoculation effects of Azospirillum in many economically important crops have been reported by various workers (Sanoria et al., 1982; Pahwa and Patil, 1984; Singh et al., 1980; Sarig et al., 1986 and Bashan et al., 1989). Menon (1992) reported the beneficial effects of Azospirillum in cowpea in a pot culture. Bradyrhizobium cowpea symbiotic association is a well established phenomenon. The increase in yield, nodule number and nitrogen content in cowpea as a result of Bradyrhizobium inoculation was reported by many workers. (Rao, 1972; Sivaprasad and Shivappashetty, 1980; Sairam et al., 1989 and Beena et al., 1990).

Like fertilizers, plant protection chemicals are also important components of agriculture today. Large number of fungicides and insecticides are being used by farmers. There is every chance of contact between the beneficial microorganisms inoculated and the pesticides applied in various ways. The effect of plant protection chemicals on these beneficial microorganisms is of scientific and

practical importance. Informations in this regard is very less especially under field conditions. In this context this study was conducted to test the effect of selected plant protection chemicals on Azospirillum and Bradyrhizobium from cowpea under in vitro and field conditions

Native Azospirillum was isolated from the roots of cowpea plants. The isolate was found to be Gram negative and vibrioid in shape as described by Dobereiner and Day (1975). The isolate showed white subsurface pellicular growth in NFb medium which is the characteristic feature of Azospirillum as reported by Okon et al. (1976 a). In Okon's medium Azospirillum isolate produced thin, dry, slightly convex colonies with granular wavy surface and undulating margin. Formation of such colonies in Okon's medium has been reported by Okon et al. (1977). In RC medium the Azospirillum isolate showed scarlet red, dry round to irregular colonies with rugose surface and undulating margin. This character of Azospirillum was reported by Caceres (1982). Azospirillum isolate produced, pink round to irregular dense and wrinkled colonies in Potato Infusion Agar medium as documented by Dobereiner et al. (1976).

Azospirillum isolate produced acid from glucose in aerobic conditions and hence belong to group I, according to the classification of Tarrand et al. (1978).

Azospirillum isolate showed shredding of agar block when grown in medium containing ammonium nitrate, showing dissimilation of nitrate. Azospirillum isolate was nir^+ strain as described by Neyra et al. (1977) and Baldani and Dobereiner (1980).

Good growth of Azospirillum in medium containing malate as carbon source was observed. Where as it showed scanty growth on glucose, lactose and mannose. Thus sugars were found to be poor source of carbon for Azospirillum as reported by Okon et al. (1976 b).

All the morphological, cultural and physiological tests conducted, thus confirmed that the isolate used in the study was Azospirillum sp.

Bradyrhizobium was isolated from root nodules of cowpea using YEMA medium. Large gummy colonies with white colour was produced on this medium. The bacterium was Gram negative. These characters of Bradyrhizobium were described by Vincent (1977).

Using Azospirillum and Bradyrhizobium, an in vitro experiment was conducted to test the effect of selected

fungicides and insecticides at their recommended doses and their combinations on these bacteria.

From the *in vitro* experiment, it was found that the fungicide Thiram (0.2 per cent) was inhibitory to the growth of *Azospirillum*. The reduction in growth rate of *Azospirillum brasilense* by Thiram was reported by Gallori *et al*. (1992). Carbendazim (0.1 per cent) Bordeaux mixture (1 per cent) and Fytolan (0.3 per cent) were noninhibitory to *Azospirillum*.

Azospirillum was not inhibited by Carbofuran, Phorate and HCH under *in vitro* conditions at their recommended doses. The stimulatory effect of Carbofuran on nitrogen fixation and indole acetic acid production by *Azospirillum* sp. was reported by Jena *et al*. (1987). The inhibitory effect of organochlorine insecticide Dicofol at 250-1000 ppm and the stimulatory effect of same at 1 ppm was reported by Mano *et al*., 1988. Here, HCH, the organochlorine insecticide did not cause inhibition of *Azospirillum* at 10 ppm concentration.

Among the different combinations of fungicides and insecticides tested, only the combinations of the Fungicide Thiram with Carbofuran, Phorate and HCH caused inhibition of *Azospirillum*. This inhibitory effect may be mainly due to

Thiram, since the insecticides alone did not cause inhibition of Azospirillum.

Among the effect of different fungicides on Bradyrhizobium, Carbendazim (0.1 per cent) did not inhibit the growth. In an in vitro experiment conducted by Chakraborty et al. (1985), it was found that Carbendazim (0.2 per cent) did not inhibit the growth of Rhizobium sp. isolated from lentil, arhar, mung, groundnut and berseem. Patel et al. (1989) reported that Carbendazim (200 ppm) did not inhibit the growth of Rhizobium sp. in in vitro.

Thiram (0.2 per cent), Boradeaux mixture (1 per cent) and Fytolan (0.3 per cent) inhibited the growth of Bradyrhizobium. The inhibitory effect of Thiram on the growth of R. phaseoli, R. japonicum, R. trifolii and R. meliloti was reported by Mukewar and Bhide (1969). The in vitro inhibitory effect of copper fungicide, Blue copper (0.5 per cent) on Rhizobium isolates from lentil, arhar and berseem was reported by Chakraborty et al. (1985).

The insecticide Carbofuran, Phorate and HCH did not cause inhibition of Bradyrhizobium. The stimulatory growth of Rhizobium isolated from groundnut in culture by insecticide Carbofuran (15-30 ppm) and Phorate (4-10 ppm) was reported by Joshi and Kulkarni (1987). At the same time, the inhibitory effect of Carbofuran (1.5 ppm to 150 ppm) to

Rhizobium sp. isolated from redgram was reported by Oblisami et al. (1973). Here Carbofuran 12.5 ppm did not cause inhibition of Bradyrhizobium. This difference in the effect of same insecticide in the same concentration may be due to the differences in the isolates obtained from different plants.

The combinations of insecticides Carbofuran, Phorate and HCH along with the fungicides Thiram, Bordeaux mixture and Fytolan also inhibited the growth of Bradyrhizobium.

The fungicides and insecticides tested in vitro were also tested in the field. After the inoculation of Azospirillum and Bradyrhizobium, plant protection chemicals were applied in the soil and rhizosphere population of both bacteria were estimated periodically. Growth parameters of cowpea plants were also observed.

The rhizosphere population of Azospirillum showed a gradual increase in control plants, where no plant protection chemicals were applied . Among the plants treated with fungicides, the rhizosphere population of Azospirillum in Thiram treated plants showed a reduction in the initial days and later on, the bacterial count was found increased. The reduction in the rhizosphere population of Azospirillum in Pennisetum americanum by seed treatment with Thiram, was reported by Govindarajan and

Purushothaman (1988). In all the insecticide treated plants the rhizosphere population did not show any reduction.

There was no significant difference between fungicide treatments on growth parameters of cowpea plants. Even though the fungicide Thiram showed inhibition of Azospirillum under in vitro conditions, the inhibitory effect on the rhizosphere population decreased gradually. No adverse effect on the growth of cowpea plants was noticed in the field.

There was no deleterious effect of Carbendazim, Bordeaux mixture and Fytolan application on the rhizosphere population of Azospirillum. Even though no significant difference between treatments was observed in most of the growth parameters, Carbendazim treated plants showed better performance than the control in growth parameters such as total fresh weight, root fresh weight, root dry weight, number of leaves and root length. Bordeaux mixture and Fytolan treated plants showed significant reduction in number of nodules, but the nitrogen content did not show significant reduction. So the fungicides, Carbendazim, Bordeaux mixture and Fytolan at their recommended doses had no adverse effect on Azospirillum in laboratory and in the field conditions.

Under *in vitro* conditions, the insecticides Carbofuran, Phorate and HCH did not cause inhibition of *Azospirillum*. The rhizosphere population of *Azospirillum* was also not adversely affected by these insecticides. Cowpea plants treated with these insecticides did not show significant difference in most of the growth parameters, except in the case of HCH treated plants, where, significant reduction in nodule number and nitrogen content was found. Carbofuran treated plants performed better than that of control in many of the growth parameters including nitrogen content. The stimulatory effect of Carbofuran on *Azospirillum* in its ability to fix nitrogen under cultural conditions was reported by Jena *et al*. (1987).

When the fungicides and insecticides were applied together in the field, the combinations of insecticides with Thiram showed an initial reduction in rhizosphere population of *Azospirillum*. Later on the bacterial population increased. In all the other treatment combinations, the rhizosphere population of *Azospirillum* showed an increasing trend.

The rhizosphere population of *Bradyrhizobium* inoculated plants showed a gradual increase in control plot. In the rhizosphere of plants treated with fungicide Carbendazim, no reduction in rhizosphere population of *Bradyrhizobium* was

observed. In the *in vitro* study also no adverse effect of Carbendazim on *Bradyrhizobium* was noticed. The performance of cowpea plants treated with Carbendazim in their growth was similar to that of control. No significant reduction in number of nodules and nitrogen content was noticed. Ramadoss and Sivaprakasam (1989) reported that the cowpea seed treatment with Carbendazim was compatible with *Rhizobium* and had no inhibition of nodulation. In blackgram also no adverse effect of Carbendazim seed treatment on nodulation was noticed by Logiswaran *et al*. (1991).

Fungicide Thiram showed *in vitro* inhibition of *Bradyrhizobium*. The rhizosphere population of *Bradyrhizobium* showed a reduction initially in Thiram treated plants. The growth performance of cowpea plants treated with Thiram, did not show significant difference from that of control. But number of nodules showed significant reduction than control. Similar inhibitory effect of Thiram on the growth of *Rhizobium japonicum* and its nodulation on the roots of soybean plant was reported by Abd el-Monem and El-Sawah (1984).

Bordeaux mixture and Fytolan inhibited the growth of *Bradyrhizobium* under *in vitro* conditions. The rhizosphere population of this bacteria showed a reduction in their number initially, later on the population was found

increased. The growth parameters of cowpea plants did not show any significant difference from control plants. Even though the number of nodules showed reduction, no significant reduction in nitrogen content was noticed. Under laboratory conditions the fungicides Bordeaux mixture and Fytolan affected the growth of Bradyrhizobium at their recommended doses. No adverse effect on cowpea - Bradyrhizobium symbiosis was noticed in the field.

The insecticides Carbofuran, Phorate and HCH did not show inhibition of Bradyrhizobium in in vitro and the rhizosphere population of Bradyrhizobium also showed no reduction. The growth performance of cowpea plants treated with these insecticides was also not affected adversely.

An in vitro experiment was conducted to study the tolerance levels of Azospirillum and Bradyrhizobium to different doses of fungicides and insecticides. Carbendazim, Bordeaux mixture and Fytolan were not found to inhibit the growth of Azospirillum even at their highest doses tested. Thiram caused inhibition of Azospirillum even at the lowest dose of 0.05 per cent. The two lower doses, recommended doses and two higher doses of Carbendazim, Bordeaux mixture and Fytolan were tested and none of these caused inhibition of Azospirillum.

The insecticides Carbofuran, Phorate and HCH did not cause reduction in the growth of Azospirillum even at their highest doses tested. The inhibitory nature of insecticides, Endrin, Disulfoton and Carbofuran at varying levels on Rhizobium from redgram was reported by Oblisami et al. (1973). Carbofuran (1.5 - 150 ppm) did not cause inhibition of Rhizobium from redgram. But here the growth of Bradyrhizobium was not reduced by the insecticide Carbofuran (4.16 - 20.80 ppm).

The two lower doses, recommended doses and two higher doses of Thiram and Fytolan caused inhibition of Bradyrhizobium. Bordeaux mixture 0.25 per cent did not cause inhibition of this bacteria. But the higher concentrations caused inhibition of Bradyrhizobium .

The insecticides Carbofuran, Phorate and HCH did not cause inhibition of Bradyrhizobium even at their highest doses in laboratory conditions.

Based on the study it can be concluded that all the fungicides and insecticides tested can be safely used in cowpea fields at their recommended doses without causing any deleterious effects either to the crop or to the microorganisms.

Summary

SUMMARY

A study was conducted to find out the effect of selected plant protection chemicals at the recommended doses and their combinations on Azospirillum and Bradyrhizobium in cowpea rhizosphere at the College of Horticulture, Vellanikkara during the year 1992-94. The results of the experiment are summarized below.

The identity of Azospirillum and Bradyrhizobium was established using routine laboratory tests.

From the in vitro experiment conducted to find out the effect of selected fungicides and insecticides at their recommended doses and their combinations on Azospirillum and Bradyrhizobium, it was found that the insecticides Carbofuran, Phorate and HCH were not inhibitory to both Azospirillum and Bradyrhizobium. Among the fungicides tested, Carbendazim showed no inhibition of both the bacteria. Thiram was inhibitory to both Azospirillum and Bradyrhizobium. Bordeaux mixture and Fytolan were not inhibitory to Azospirillum, but these two fungicides inhibited the growth of Bradyrhizobium. When the combinations of insecticides with the fungicides were tested, only the combinations of all insecticides with Thiram caused inhibition of Azospirillum. Bradyrhizobium

was inhibited by the combination of all the insecticides with Thiram, Bordeaux mixture and Fytolan. The combinations of all the insecticides with Carbendazim did not cause inhibition of Azospirillum and Bradyrhizobium.

In the field experiment, the rhizosphere population of Azospirillum and Bradyrhizobium were estimated before and after the inoculation of the microorganisms and after the application of fungicides and insecticides. Growth parameters of cowpea plants were also recorded. The rhizosphere population of Azospirillum showed a gradual increase with the growth of cowpea plant. Similar trend in rhizosphere population of Azospirillum was observed in Carbendazim, Bordeaux mixture and Fytolan treated plots. Treatment with insecticides, Carbofuran, Phorate and HCH also showed a similar trend. Thiram caused a slight reduction in rhizosphere population of Azospirillum immediately after the application. But later on an increase in Azospirillum population was noticed. The combination of all the fungicides with all the insecticides showed no reduction in Azospirillum population except in the combination of all insecticides with Thiram.

The Bradyrhizobium population was not affected by the fungicide Carbendazim as well as insecticides Carbofuran, Phorate and HCH at their recommended field doses. Thiram, Bordeaux mixture and Fytolan caused reduction of

Bradyrhizobium population after the application, but later the bacterial count increased. Similar trend was noticed in the combination of insecticides with Thiram, Bordeaux mixture and Fytolan treated plants.

Most of the growth parameters of Azospirillum inoculated plants treated with fungicides, showed no significant difference from control plants. Even though the number of nodules in Thiram, Bordeaux mixture and Fytolan treated plants showed significant reduction, a corresponding reduction in nitrogen content was not noticed. Plants treated with Carbofuran, Phorate and HCH did not show significant difference in growth parameters. HCH treated plants showed significant reduction in nodule number and nitrogen content compared to control.

Growth characters of the Bradyrhizobium inoculated plants treated with Carbendazim was similar to that of control. Thiram, Bordeaux mixture and Fytolan treated plants showed significant reduction in number of nodules. But no reduction in nitrogen content was noticed. Growth performance of the plants treated with the insecticides were also similar to that of control. The insecticides Carbofuran, Phorate and HCH also caused no adverse affect on the growth of cowpea plants.

When the fungicides and insecticides were applied together, no adverse effect on growth of cowpea plants were noticed in the case of plants inoculated with Azospirillum as well as Bradyrhizobium.

The tolerance levels of Azospirillum and Bradyrhizobium to different doses of fungicides and insecticides were also tested. The fungicides, Carbendazim, Bordeaux mixture and Fytolan were not inhibitory to Azospirillum even at their highest doses of 0.15, 3.0 and 0.5 per cent respectively. Thiram caused inhibition of Azospirillum even at the lowest dose of 0.05 per cent. All the five doses of Carbendazim did not inhibit the growth of Bradyrhizodium. Thiram and Fytolan inhibited the growth of Bradyrhizobium, even at their lowest dose of 0.05 and 0.1 per cent respectively. Bordeaux mixture 0.25 per cent was non inhibitory, but the higher doses caused inhibition.

Both Azospirillum and Bradyrhizobium were tolerant to the three insecticides Carbofuran, Phorate and HCH even at their highest doses tested.

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Appendices

Appendix - I

Analysis of variance table - Growth parameters of cowpea plants inoculated with Azospirillum treated with fungicides, insecticides and their combinations.

Source	df	Mean square				
		Total fresh weight	Root fresh weight	Total dry weight	Root dry weight	Plant height
Fungicides	4	10.358	0.287	0.148	0.064	435.452
Insecticides	3	1.441	0.065	0.449	0.022	65.167
Combinations	12	6.433	0.334	0.831	0.059	259.538
Error	38	5.173	0.228	0.745	0.063	177.319

Appendix - II

Analysis of variance table. Growth parameters of cowpea plants inoculated with Azospirillum treated with fungicides, insecticides and their combinations.

Source	df	Mean square			
		Number of leaves	Root length	Number of nodules	Nitrogen content
Fungicides	4	0.566	22.433	100.600	0.076
Insecticides	3	0.916	2.283	43.867	0.798
Combinations	12	0.638	48.589	22.500	0.287
Error	38	0.415	16.897	14.606	0.182

Appendix - III

Analysis of variance table - Growth parameters of cowpea plants inoculated with Bradyrhizobium treated with fungicides, insecticides and their combinations.

Source	df	Mean square				
		Total fresh weight	Root fresh weight	Total dry weight	Root dry weight	Plant height
Fungicides		1.251	0.118	0.516	0.014	435.452
Insecticides		2.433	0.149	0.518	0.077	65.167
Combinations		6.269	0.196	0.945	0.077	259.538
Error		3.402	0.216	0.740	0.070	177.319

Appendix - IV

Analysis of variance table. Growth parameters of cowpea plants inoculated with Bradyrhizobium treated with fungicides, insecticides and their combinations.

Source	df	Mean square			
		Number of leaves	Root length	Number of nodules	Nitrogen content
Fungicides		0.345	22.433	125.308	0.121
Insecticides		0.263	2.283	125.528	0.085
Combinations		1.226	48.589	98.875	0.171
Error		0.768	16.897	54.382	0.249

EFFECT OF SELECTED PLANT PROTECTION CHEMICALS ON THE BENEFICIAL MICROORGANISMS IN COWPEA RHIZOSPHERE

By

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ABSTRACT OF A THESIS

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ABSTRACT

A study was conducted at the College of Horticulture, Vellanikkara, during 1992-94, on the effect of selected plant protection chemicals on beneficial microorganisms in cowpea rhizosphere. Four fungicides, three insecticides and their combinations were tested *in vitro* as well as in the field. The tolerance levels of *Azospirillum* and *Bradyrhizobium* to different doses of these chemicals were also tested.

The fungicide Thiram caused inhibition of *Azospirillum in vitro*. The adverse effect of Thiram on rhizosphere population of *Azospirillum* was seen only during initial days after application. The growth performance of cowpea plants were not affected adversely. The fungicides, Carbendazim, Bordeaux mixture and Fytolan and insecticides Carbofuran, Phorate and HCH at their recommended doses did not cause inhibition of *Azospirillum in vitro* and did not reduce the rhizosphere population of *Azospirillum*. The growth performance of cowpea plants was also not affected adversely by these fungicides and insecticides. The combinations of insecticides with Thiram caused inhibition of *Azospirillum* under *in vitro* conditions and caused reduction in rhizosphere population of the bacteria initially. But no adverse effect on growth of cowpea plant

was noticed. Bradyrhizobium was inhibited by the fungicides, Thiram, Bordeaux mixture and Fytolan in vitro. The rhizosphere population of Bradyrhizobium also showed reduction initially. The growth performance of plants was not affected adversely. The number of nodules showed significant reduction. No reduction in nitrogen content was noticed. Carbendazim and insecticides Carbofuran, Phorate and HCH did not show inhibition of Bradyrhizobium in in vitro. The rhizosphere population and growth of cowpea plants were also not affected adversely by these chemicals.

Under in vitro conditions the combination of insecticides with Thiram, Bordeaux mixture and Fytolan caused inhibition of Bradyrhizobium. The rhizosphere population showed reduction initially, later on it was increased. The combination of insecticides with Carbendazim did not inhibit the growth of Bradyrhizobium under in vitro conditions and also in the rhizosphere. The treatment combinations did not affect the growth of cowpea plants in the field adversely.

The tolerance levels of Azospirillum and Bradyrhizobium to different doses of fungicides and insecticides were also tested. The fungicides Carbendazim, Bordeaux mixture and Fytolan were not inhibitory to Azospirillum, even at their highest doses of 0.15, 3.0 and

0.5 per cent respectively. Similarly, the insecticides Carbofuran, Phorate and HCH were also not inhibitory to Azospirillum at their highest doses tested. The fungicide Thiram caused inhibition of Azospirillum even at the lowest dose of 0.05 per cent.

Bradyrhizobium was inhibited by Thiram and Fytolan even at their lowset doses of 0.05 and 0.1 per cent respectively. Bordeaux mixture 0.25 per cent did not cause inhibition, but the higher doses caused inhibition. Carbendazim and insecticides Carbofuran, Phorate and HCH did not inhibit the growth of Bradyrhizobium in all the five doses tested.