DETECTION OF SAFE DOSES OF DIFFERENT PESTICIDES FOR CHICKPEA *(Cicer arietinum* L.)-*Rhizobium* SYMBIOSIS

Thesis

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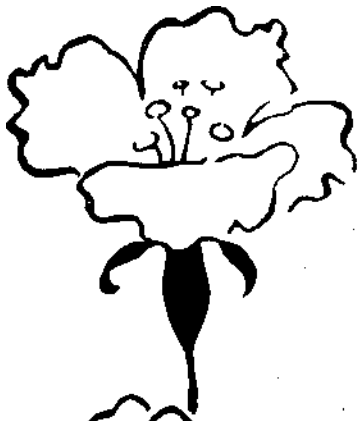

In the name of God, who is most merciful, created the earth and heavens, I feel proud to dedicate my valuable thesis to my beloved parents, Bhaiya and Bhabhi whose endless deeds proved to be flambeaubue showing me the right way towards my destination.

CERTIFICATE -I

This is to certify that the thesis entitled "DETECTION OF SAFE DOSES OF DIFFERENT PESTICIDES FOR CHICKPEA *(Cicer* ***arietinum*** **L.)-*RHIZOBIUM*** SYMBIOSIS" submitted in the partial fulfilment of the requirements for the degree of "Master of Science in **Agriculture"** (Soil Science) of the Indira Gandhi Agricultural University, Raipur (C.G.), is a record of the bonafide research work carried out by **SHRI PRASHANT** GUPTA under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma (certificate awarded etc.) or has been published/published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by him.

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CERTIFICATE-II

This is to certify that the thesis entitled **"DETECTION OF SAFE DOSES OF DIFFERENT PESTICIDES FOR CHICKPEA *(Cicer arietinum* L.)-*RHIZOBIUM* SYMBIOSIS"** submitted by **SHRI PRASHANT GUPTA** to the Indira Gandhi Agricultural University, Raipur (C.G.) in the partial fulfilment of the requirements for the degree of **M. Sc. (Ag.)** in the **Department of Soil Science** has been approved by the Student's Advisory Committee after oral examination in collaboration with the external examiner.

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Major Advisor and Chairman

Head of the Department

Director of Instructions

Date:

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Introduction

CHAPTER-I

INTRODUCTION

In the present era, the world population is increasing at rapid pace. This increasing population needs a large amount of food material for its nutrition. Thus, it is very essential that food crops should be grown in the equal proportion without loosing quality and quantity of produce. Pesticides are being used in a wide range to protect the crops. These pesticides pollute the air, water and soil also, and showing the detrimental effect. In the soil environment, microorganisms are affected by the chemicals, which is used in the form of pesticides. These pesticides kill/control the pest but it is not clearly found that their applied doses are harmful or beneficial for microbes like *Rhizobium*. Therefore, it is necessary to work out economically viable doses of pesticides in respect to pest control and BNF. Presently many pesticides are being used for cultivation of gram crop without knowing their direct and indirect effect on population of crop beneficial microbes. This is one of the reasons of reducing soil productivity per unit area of the country. In this connection, almost no work has been conducted on this aspect. Therefore, this study is planned with following objectives in order to select suitable/compatible doses of

pesticides on the basis of their cumulative effect on Gram-Rhizobium symbiosis.

1. Effect of different doses of pesticides on performance of chickpea
2. Effect of different doses of pesticides on population dynamics of Rhizobium
3. Influence of different doses of pesticides on biologically fixed amount of nitrogen.

Review of Literature

CHAPTER -II
REVIEW OF LITERATURE

Three experiments were conducted under controlled growth room conditions to detect the compatible/suitable doses of pesticides for effective *Rhizobium* symbiosis with chickpea *(Cicer arietinum* L.) in the Soil Microbiology Lab., Department of Soil Science, IGAU, Raipur,

The work pertaining to different aspects of chickpea-*Rhizobium* symbiosis and other work relevant to the present investigation has been covered in the review as follows:

I. Climatic conditions and *Rhizobium* legume symbiosis

The *rhizobial* population in soil varied to a greater extent by different edapho-climatic conditions like temperature, soil-moisture, humidity, concentration of oxygen and carbon dioxide in soil and air, soil organic matter, presence of different elements and chemicals in soil and due to other soil microbes which directly or indirectly affect the *rhizobial* growth.

Soil surface temperature range of Chhattisgarh region crosses to 60°C (air temperature reached up to 48°C and humidity drops up to 3 to 4 per cent) during summer season, destroying rhizobial population. Surface soils almost become sterile. This condition results in poor nodule formation in legumes grown

under such situations resulting in very low BNF. This is one of the major causes of low legume productivity in this region (Gupta *et al.*, 1995 and Anonymous, 1996).

There have been a number of investigations on the effect of root temperature on nodulation and infection process of temperate species of clovers (*Trifoliumspp.)* on agar slopes in environment controlled growth chambers (Gibson, 1963, 1965, 1966 and 1967). The results point out that below 10°C, root-hair infection by *Rhizobium* is retarded, whereas at 24°C and above, the rate of infection is enhanced. The most congenial range of temperature for bacteroid tissue formation in nodules appears to be 20-30°C, although nitrogen fixation remains unaltered in the range of 12-32°C. However, these results are dependent on variations between *Rhizobium* strains and host cultivars.

The effect of day temperature on root nodulation of soybean (*Glycine* max) and chickpea *(Cicer arietinum*) has been studied in pot trials in environment controlled growth cabinets (Dart *et al.*, 1975). In soybean, one of the strains of *Rhizobium* was most effective at 33°C, while others showed no difference in effectiveness at 21°C. In chickpea, none of the bacterial strains produced nodules at 21°C and at root temperatures beyond 32°C.

Gibson (1971) found that the maximum temperature for nodulation varied with *Phaseolus atropureus* and *Stylosanthes humilies* and had ability to nodulate more readily at 36°C. Further,

Nakul *et al.* (1993) observed that the strains were tolerant to intermittent heat treatment at 45°C for 10 days hence, were chosen for temperature tolerance. Temperature is a critical factor in the infection and nodulation in legumes. The process of infection, nodule development and fixation requires different maximum, optimum and minimum temperature. It may vary from species to species (Gibson, 1977). Dart *et al*. (1976) found evidence of difference in *Rhizobium* strains in adapting to different temperatures. The optimum temperature for nodulation of legumes is about 27-30°C for tropical areas.

Our country comes under tropical zone where the temperature sometimes shoots up very high. So the viability of *Rhizobium* is greatly reduced. A favorable temperature for multiplication of most species of *Rhizobium* is up to 40°C as reported by Bhriguvanshi and Gangwar (1984). Day *et al*. (1978) and Kritovich *et al*. (1981) reported that bacterial growth is optimum at 37°C to 42°C after which there is sharp decline. Similarly, Singh and Khurana (1992) have clearly mentioned that *Rhizobium* cells tended to loose their nodule inducting ability when inoculated at elevated temperatures. Moisture stress further added to the decline in survival and in the proportion of Nod^+ cells. Pareek *et al* (1990) have also reported that legume-*Rhizobium* symbiosis is highly affected by soil moisture and temperature in Tarai belt of U.P.

II. Method of inoculation and benefit

Agar based cultures are the quickest way to inoculate plants in small experiments. The surface growth of *Rhizobium* on agar is scraped with needle or scalpel and suspended in water, which is used to sprinkle seeds before sowing.

Carrier based cultures are mixed with minimum amount of water to form a slurry with the addition of 10 per cent sugar or 40 per cent gum arabic and the slurry is sprinkled on seeds. The seeds are allowed to dry in shade and sown.

Rhizobia were applied to seed because this was an easy, convenient way to establish the bacteria in the root zone of the developing seedling. The laborious task of spreading tons of soil to provide a few rhizobia could thus be by passed (Peppier and Perlman, 1979).

In USA, the normal rate of application is 4.4 gm of inoculum per kilogram of seed regardless of seed size. The amount of water is greatly reduced for the larger seeds. The big seeds naturally receive larger number of rhizobia per seed. According to Burton and Curley (1965), they need more rhizobia for effective nodulation. Nodulatory standards often specify a minimum of 10^3 viable rhizobia per seed regardless of size. An inoculant with 10^6 rhizobia per gram would meet this standard with peas (Peppier and Perlman, 1979)

In USA and Australia, pelleted seeds are often used to establish legumes in acid soils or to avoid the hazards of pesticides or fertilizers (Burton, 1979 and Brockwell, 1977). The usual method of pelleting involves additions of 40 per cent gum arabic or 5 per cent car boxy methyl cellulose to the incoculant slurry before application to seeds. Finely ground calcium carbonate capable of passing through 300 mesh sieve is added to freshly inoculated wet seeds in a container and mixed rapidly for 2 minutes until the seeds are coated. Besides lime, other forms of pelleting agents such as dolomite, gypsum, bentonite, rock-phosphate, talc, charcoal and basic slag have been used to establish soybean in problem soil (Chhonkar *et al.*, 1971).

Responses to legume inoculation have been demonstrated with major legumes such as pigeonpea (*Cajanus cajan*) and mungbean (*Vigna mungo*). The benefits to the farmer can be sizeable by proper *Rhizobium* inoculation as shown for some of the important grain legume.

Besides, peat-based inoculants other forms used in the USA are granular soil inoculants where marble, calcite grains or cores are wetted by peat based cultures using adhesives. Such granular inoculants could be broadcasted by air plane. Most of these improvements tend to be expensive and their possible use in developing countries is hence limited (Subba Rao *et al.*, 1993).

Three methods of inoculating flat pea (*Lathyrus sylvestris*) with *Rhizobium leguminosarum* were investigated to determine which would be effective for at least two weeks after seeding. Application of powdered peat to wet seeds was inadequate. Lime pelleting and granular soil implants were effective inoculants. Granular soil implants caused nodulation to a greater depth on the tap root, and lime pelleting caused plants to exhibit higher acetylene reduction activity (Wright, 1985).

III. Crop response to pesticides

Pandey and Rai (1995) evaluated the effect of application of aldrin, BHC (HCH), carbofuran, 2,4-D, fluchoralin and butachlor at the recommended rate (RR) or 2, 3 or 5 times of the RR on yield and N-uptake by soybean. The seed yield was highest with carbofuran (3.4 t ha^{-1}) and lowest with 2, 4-D (2.1 t ha^{-1}). Higher rates of pesticides decreased the yield.

The effect of pesticides on yield and N-uptake by chickpea was studied by Pandey and Rai (1996). The pesticides *i.e.* aldrin, BHC (HCH), 2,4-D, fluchoralin, carbofuran and butachlor were applied at recommended rate (RR) or 2, 3 or 5 times of RR. Application of higher doses of pesticides decreased yield in the case of aldrin. Nitrogen uptake was lowest with the highest pesticide application rate.

Trotus *et al.* (1996) conducted an experiment at the Secuieni Agricultural Research Station, Romani during 1992-95, in which

Phaseolus vulgaris seeds, were treated with insecticides Furadan 35 ST (carbofuran), Gaucho 70 W (imidacloprid), Cosmos 50 FS (unspecified) and Promot 400 CS (premetrin) in association with bacterization with bio-preparations of the FLO type (*Rhizobium spp.)*. Results revealed that these treatments inhibit germination, plant emergence and root growth.

Dastgheib *et al.* (1995) conducted an experiment to evaluate the effect of pre and post emergent application of herbicides to chickpea. Herbicides *i.e.* cyanazine, metribuzin, terbuthylazine and cyanazine+metribuzin gave an adequate level of control without phytotoxicity to chickpeas and resulted in seed yields comparable with the untreated control and alachlor, cyanazine, bentazone, clethodium, haloxyfop, pendimethalin, bentazone+ MCPB and cyanazine+raloxyfop resulted in severe damage to chickpeas.

Browde *et al.* (1994) conducted an experiment in 1989, 1990 and 1991 to quantity growth response to herbicide *i.e.* acifluorfen [5-{2-Chloro-4 (trifluoromethye)phenoxy}-2-nitrobenzoic acid] plus bentazone [3-(l-methyl ethyl)-(14)-2,1,3- benzothiadiozin –4(34)-one 2,2-dioxide]. They summarised that herbicides reduced conductance and caused visible injury each year, limited growth (plant height, leaf area, pod number and dry weight of leaf pod and stem plus petiole) in 1990 and 1991.

El-Masry *et al.* (1994) studied the effect of chlorflurenol on the anatomical, morphological and physiological characteristics of *O. crenata* and faba beans. Faba bean seeds were sown in plots with clay soil and *O. crenata* seeds were placed 5 cm below the soil surface. Chlorflurenol was foliarly sprayed at 50 and 100 ppm on the faba bean plants 30 days after sowing (DAS). Chlorflurenol significantly reduced the height, number of flowers and dry matter accumulation in *O. crenate* shoots.

Ozaur *et al.* (1993) performed an experiment in which soybean sown in spring and autumn of 1986 with the application of pendimethalin, isoproturer and oxadiaone @ 1.4, 1.5 and 3.0 kg ha-1, respectively. They reported that pendimethalin caused crop injury in terms of delayed and scattered emergence.

Trotus and Ghizdavu (1996) treated the seed of beans with furadan 35 ST (carbodfuran), carbodan 35 ST (carbofuran), Promet 400 CS (prometryn), gaucho 70 W (imidaclprid), talstar 20 ST (bufen thrin) and cosmos 50 FS (unspecified composition) insecticides. They found better yields from treated seeds than untreated seeds. Uradan 35 ST and corbodan 35 ST products had a negative influence on dry weight.

Diuron was tested by Zawozink *et al.* (1995) for toxicity to the bacteria *Rhizobium loti*, *Rhizobium meliloti* and *Bradyrhizobium japonieum.* Results indicated that growth of the *R. loti* strain assayed was affected by the addition to the culture medium. In

contrast, cultures of *R. meltoti* showed optical densities of 26, 42 and 58 pre cent lower than controls at 30,50 and 100 ppm of diuron. Leaf chlorophyll content was significantly reduced by 30 and 50 ppm pretreatment, while 100 ppm of herbicide completely inhibited plant growth.

Singh and Wright (1997) studied the effect of herbicides *i.e.* ter butryn/ter buthylazine (350/150 g ltr^{-1}) on the yield of peas and revealed that dry weight and yield of peas decreased with higher rates of application of both the herbicide treatments.

An experiment was conducted by Loc and Nguyen(1993) in which *Rhizobium* spp. was grown in culture media containing 2,4,5-trichloro phenoxyacetic acid and found that its growth was reduced in the presence of 0.10 and 0.25 mg cm^{-3} medium and almost completely inhibited with 0.5-1.00 mg cm^{-3} of the dry weight of 8 days old ground nut seedling grown in soil inoculated with *Rhizobium* was 5.2 gm with out 2,4,5-T and decreased with increasing concentrations of 2,4,5-T in the range of 0.02-1.00 mg kg^{-1} soil. Plant total N-content decreased with 3.44 per cent in the control to 1.82 per cent at the highest 2,4,5-T rate.

Jan *et al.* (1994) studied the effect of fertilization, *Rhizobuim* inoculation and carbofuran on yield of lentil. They inoculated the seed of lentil *cv.* Pak 18-12 with *Rhizobium* strain Le-19 or not inoculated with the application of N (100 kg ha^{-1}) and P_2O_5 (0 or 50 kg ha^{-1}) further carbofuran sprayed with 1 kg a.i. ha^{-1} or not

sprayed. The results exhibited that seed yields were highest in inoculated treatments. Seed yields were 2.18, 2.14, 2.32 and 2.18 t ha^{-1} with inoculation (I + P,I +carbofuran, I+P+carbofuran and N+P+carbofuran, respectively. Carborfuran alone did not increase seed yield above that control.

Amarante (1995) conducted an experiment in green house trails during 1992 in which seeds of soybean *cv.* Br-4 inoculated with *Bradyrhizobium japonicum* were sown in a brown latosol or a 50:50 soil: sand mixture and treated with 5 rates of metholacholr ranging from 0 to 6.0 kg a.i. ha^{-1} before emergence. At harvest, 52 days after emergence, increasing rates of metachlor significantly reduced dry weight of shoots leaves with no effect on root dry weight. For all the parameters affected, effect of metachlor were more pronounced on the soil/sand mixture.

Haider *et al.* (1995) conducted a pot experiment in which seeds of *V. faba cv.* Klein thrueringer were inoculated with *Rhizobium leguminosarium* and pre emergence sprays of 0, 50 ,100 and 200 per cent of the normal concentration of linuron or simazine (2 and1 kg ha^{-1}, respectively) were applied. The high concentration of herbicide reduced dry matter production as compared with control. Plant N-content was also reduced by the highest concentration of both herbicides.

The effect of pendimethalin (applied @ 1.5 and 2.50 kg ha^{-1}) and alachlor (@ 1.875 and 3.75 kg ha^{-1}) on plant growth and yield of soybean *cv.* IAC - 11 was studied by Nova *et al* (1996) In

treatments, there were two controls (inoculation with or without the *Brady rhizobuim japonicum* strain '5MS –463') and all other herbicide treated plants were inoculated. Results revealed that there was relative response to seed inoculation on plant growth.

A field experiment was conducted by Gurjar *et al.* (2001) in order to evaluate the effect of different herbicides on growth yield and economics of soybean. They observed that application of alachlor with its higher dose (@ 1.5kg a.i. ha^{-1}) recorded highest values for growth, yield attributing characters and yield, followed by pendimethalin @ 1.5 kg a.i. ha -. Alachlor @ 1.0 kg a.i. ha^{-1} and pendimethalin @ 1.o kg a.i. ha^{-1}.

Under pot condition, the effect of live seed treating fungicides of soybean on *Bradyrhizobium* Japonicum was tested by Jayasheela *et al.* (1998). The different fungicides *i.e.* Thiran, Difolation, Dilhane M-45, P CNB and Bavistin were recommended respectively per kg seed. No adverse effect in any of the fungicides tested. Seed treated with bavistin showed increased shoot dry weight and bean yield which differed significantly from all other treatments. Dithane M-45 was found as the next best fungicide for soybean seed treatment.

The chickpea with fluchloralin (0.5,1.0 and 1.5 kg ha^{-1}), metribuzin (0.75,0.50 and 0.75 kg ha^{-1}) and pendimethalin (0.75,1.0 and 1.75 kg ha^{-1}) was tested by Pahwa and Prakash (1990). They reported what herbicides either reduced the growth of shoots and roots or there was no effect.

IV. *Rhizobium* response to pesticides

The effect of application of aldrin BHC (HCH), carbofuran, 2, 4-D, fluchoralin and butachlor at the recommended rates (RR) or 2, 3 or 5 time the RR on nodulation was evaluated by Pandey and Rai (1995) reported that all the pesticides except butachlor increased the number of nodules plant^{-1} at RR but higher rates of all pesticide gave lower number of nodules plant^{-1} compared with control.

Pandey and Rai (1996) studied the effect of pesticides on nodulation of chickpea. The pesticides i.e aldrin BHC (HCH), 2, 4-D fiuchoralin carbofuran and butachlor were applied at recommended rate (RR) or 2, 3 or 5 times of RR. All pesticides applied at RR increased the nodulation but the application of higher doses decreased nodulation.

The result obtained by Trotus *et al.* (1996) at the Secuieni Agricultural Research Staion, Romania druing 1992-95 showed that *Phaseolus vilgaruris* seed treatment with insecticides furadan 35 ST (carbofuran), Gaucho 70 W (imidacloprid), cosmos 50 FS (unspecified) and Promet 400 CS (Prometrin) in association with bacterization with bio-preparations of the bio type (*Rhizobium* spp.) inhibit the root nodule formation.

While treating the seed of beans with different insecticides, Trotus and Ghizdavu (1996) reported that furadan 35 ST and carbodan 35 ST had a negative influence on the formation of nodules on plant roots. Jagadish *et al.* (1994) reported the same results.

Zawoznik *et al.* (1995) tested diuron for toxicity to the nodule forming bacteria *Rhizobium* loti, *Rhizobium meliloti* and *Bradyrhizobiumjaponicum* and to the nitrogen fixing symbiosis of these organism. Results revealed that nodulation percentage and nodule nitrogenase activity remained unaffected by diuron pretreatment but the number of nodules developed by alfalfa (Lucerne) roots was halved when inoculum was exposed to 50 and 100 ppm of diuron.

The nodulation ability of *B. japonicum* was significantly diminished with increasing herbicide concentration. Effectiveness of this strain measured as nodule nitrogenase activity, decreased when exposed to 50 ppm of diuron. While studying the effect of herbicides *i.e.* terbutryn/terbuthylazine (350/150 g ltr^{-1}), Singh and Wright (1997) summarised that nodulation of peas decreased with higher rates of application of both the herbicide treatment.

Loc and Nguyen (1993) conducted an experiment in which *Rhizobium spp.* was grown in culture media containing 2, 4, 5-T and found that its growth was reduced in the presence of 0.10-0.25 mg cm^{-3} medium and almost completely inhibited with 0.5-1.00 mg cm^{-3}. They had grown 8 days old groundnut seedling in soil inoculated with *Rhizobium* and exhibited that the number of effective nodules per plant was 13 without 2, 4,5-T and decreased from 5 to 1 as 2, 4, 5-T concentration increased from 0.02 to 0.25 mg kg^{-1}, nodules were absent at higher 2, 4, 5-T concentrations.

Nitrogenase activity decreased from 92.92 to 6.5 n mol C_2H_2 $plant^{-1}$ ha^{-1} as 2, 4, 5-T concentration increased from 0.02 to 0.50 mg kg^{-1} as compared with 128.22 n mol $plant^{-1}$ in the control.

Madhavi *et al.* (1994) studied the effect of 15 pesticides (monocrotophos, acephate, dichlorvos, metasystox, danitol, sumicidin, blitox, captan, captafol, dithianon, hexaconazole, zineb, butachlor, simazine and oxyflorfen) on nodulation, nitrogenase activity and sym plasmids in *Rhizobium spp.* IC 3342 nodulating *Cajanus cajan*, *Rhizobium leguminosasum* nodulating lentil and *Rhizobium meliloti* nodulating *Medicago sativa.* The results evaluated that 13 of the pesticides showed 55-100 per cent loss of nodulation and nitrogenase activity of *Rhizobium spp.* IC 3342. While, DDT and zineb showed a maximum of 16 per cent loss of both characteristics at the greatest concentration tested of the 6 insecticides only sumicidin showed a loss of nodulation and nitrogenase activity with *Rhizobium leguminosarum*, whereas fungicides showed losses of 20-40 per cent and herbicides 35-75 per cent in both cases. Simicidin was the only insecticide effective in decreasing nodulation and nitrogenase activity (by 35%) in *Rhizobium meliloti*, while fungicide and herbicides decreased both the properties by 20-100 per cent. All cultures which lost symbiotic properties showed losses of plasmid.

Jan *et al* (1994) conducted an experiment in 1985-86 at the Agricultural Research Station, Serainaurang, Bannu in which

seeds of lentil *cv*. Pak 10-12 were inoculated with *Rhizobium* strain Le-19 or not inoculated with the application of N (100 kg ha^{-1}) and P_2O_5 (0 or 50 kg P_2O_5 ha^{-1}) further, carbofuran sprayed @ 1 kg a.i. ha^{-1} or not sprayed and reported that inoculation alone and in combination with carbofuran decreased nodule, number of branches and number of nodules per 10 plants.

In green house trails during 1992, Amarante (1995) inoculated the seeds of soybeans *cv*. BR-4 with *Bradyrhizobium japonicum* which were sown in brown latosol or 50:50 soil:sand mixture and treated with 5 rates of metolachlor ranging from 0 to 6.0 kg a.i. ha^{-1} before emergence. At harvest, 52 days after emergence increasing rates of metachlor significantly reduced the dry weight of nodules with no effect on nodule number. The effects of metolachlor were more pronounced on the soil/sand mixture.

The effect of imazethapyr on pea *cv*. Frilene *Rhizobium* symbiosis was studied by Gonzalez *et al.* (1996). They observed that symbiotic plants were damaged by imazethapyr concentrations higher than 1.73 μM, applied pre emergence. The number of nodules per plant was affected more than nodules size, suggesting a direct imazethapyr effect of nodule initiation rather than on nodule development. However, imaxethapyr did not directly affect *Rhizobium,* but doses higher than 0.34 mM were required to cause slight effects on *Rhizobium* growth in a defined medium. Also, the nodulation ability of bacteria treated with

imazethapyr was not affected. These results suggest that imazethapyr inhibits the growth of symbiotic plants rather than having a direct effect on bacteria.

An experiment was carried out under green house conditions by Silva *et al.* (1998) in order to evaluate the effect of soil application of different herbicides on nodulation and nitrogen fixation in cowpeas. Treatments included a control (without herbicide) and following herbicides were applied at the recommended rates and at double recommended rates trifluration at 1.8 and 3.6 litres ha^{-1}, EPTC at 5.0 and 10.0 litres ha^{-1}, pendimethalin at 2.0 and 4.0 litres ha^{-1}, metribuzin at 1.1 and 2.2 litres ha^{-1}, linuron at 2.4 and 4.8 liters ha^{-1} and imazaquin at 1.0 and 2.0 litres ha^{-1} . All the treatments were inoculated with *Bradyrhizobium,* strain NBF 09. Results revealed that cowpea nodulation was reduced by trifluralin (1.8 litres), linuron (2.4 litres) and imazaquin (1.0 liters) but the effects occurred only at the earlier stages of cowpea growth. Total nitrogenase activity was reduced in the soil treated with herbicides.

Singh *et al.* (1994) evaluated the effect of herbicides (methabenztheazuron at 1.31 kg ha^{-1} liniuron at 0.75 kg and pendimethalin at 0.75 kg ha^{-1}) on soil microorganism dynamics and *Rhizobium* legume symbiosis in an experiment conducted on loamy sand soil at Ludhiana during 1989-90. They summarized that all herbicide treatment reduced the number of soil bacteria

and fungi 1 *day after* treatment and number of bacteria 5 days after treatment. However, only linuron reduced the number of soil fungi 5 days after treatment. As observed 45 days after sowing all herbicide treatments reduced the number of nodules/plant and nitrogenase activity (μmol C_2H_4/hour g^{-1} of nodule DW) from untreated control values 53.8 and 55.7 respectively to 35.0-43.7 and 25.9-36.1 respectively.

Glover and Schapaugh (1997) conducted an experiment on soybean and exhibited stem breakage, lodging, reduced nodulation and enlarged root tips after treatment with pendimethalin.

In an experiment, Saraf *et al.* (1994) treated *Bradyrhizobum japonicum* PR-2002 and *B. japoniaum* PR-PSR-2 with corbaryl (150 and 60 mug ml^{-1} respectively) and 2,4-D (500 and 400 mug ml^{-1}, respectively) and reported that the nitrogenase and uptake hydrogenase activities decreased at approximately sublethal concentration and increased at very low concentration respectively, of each chemical. It was concluded that in addition to their bacteriocidal activities, carbaryl and 2, 4-D also acted as electron donors, thereby affecting the oxidative metabolic pathway.

Fajiri (1996) studied the effects of a range of pre emergence herbicides on nodulation of annual medics and subterranean clover. The smallest reduction in biomass and nodule numbers was found in caliph and sava treated with simazine at 1.01 + imazethapyr at 150 ml and the largest reduction was recorded for some of the cultivars with simazine at 2.01.

A pot experiment conducted by Haider *et al.* (1995) in which seeds of *V. faba cv.* Klein Thueringer were inoculated with *Rhizobium leguminosarum* and per emergence sprays of 0, 50, 100 and 200 per cent of the normal concentration of linuron or simazine(2 and 1 kg ha^{-1} respectively) were applied. Nodulation and nodule DM were reduced by the high concentration of linuron compared with the control, whereas simazine was less effective. Nodule activity was also reduced by the highest concentration of both herbicides.

Nova *et al.* (1996) studied the effect of pendimethalin (applied @ 1.5 and 2.50 kg ha^{-1} and alachlor @ 1.875 and 3.75 kg ha^{-1}) on nodulation and nitrogen fixation of soybean *cv.* IAC-11. In treatments, there were two controls (inoculation with or without the *Bradyrhizabium japonicum* strain 'SNS-463') and all other herbicide treated plants were inoculated. Results revealed that there was negative response to seed inoculation on nodulation but nitrogenase activity was improved.

Pahwa and Prakash (1990) treated chickpea with fluchloralin (0.5,1.0 and 1.5 kg ha^{-1}) metribuzin (0.25,0.50 and 0.75 kg ha^{-1}) and pendimethalin (0.75,1.0 and 1.75 kg ha^{-1}). They showed that nodulation was not adversely affected by lower doses of herbicides and there was significant increase in leghaemoglobin content of nodules. Nitrogen fixation activity of nodules in treated plants was more up to 60 DAS.

An experiment was conducted by Martiensson and Nilsson (1989) to investigate the effect of chlorosulfuron on symbiotic performance of legume bacteria. They revealed that nodulation occurred in plants grown in soil containing $2x10^{-3}$, $3x10^{-6}$ and 2g ha^{-1} chlorosulfuron but nitrogenase activity of the nodules of these plants was less than for control plants. Plants grown in soil containing 2 g ha^{-1} of chlorosulfuron developed normally after an initial growth inhibition of 5 to 6 weeks. The inhibition of nodulation and nitrogenase activity of nodules grown in the presence of chlorosulfuron is probably due to adverse effects of herbicide on plant growth and development rather than rhizobia.

Singh *et al.*, (1990) conducted an experiment to evaluate the effect of insecticides on nodulation in soybean. Treatments included three doses of carbofuron 25 STD (seed treating dust) @ 10, 15 and 20 g kg^{-1} seed, monocrotophos 36EC and methyl demeton 25 EC each @ 5ml kg^{-1} seed and carbofuron 3g and phorate 10 g each @ 1 kg a.i. ha^{-1} by applying in furrow, given at the time of sowing with one control. Results showed that carbofuran recorded significantly more nodules weight (100.85 mg $plant^{-1}$) than the remaining insecticides and the control. Phorate was next effective in increasing the nodules weight and was significantly superior to the remaining insecticides except monocrotophos. The 60 days old crop showed more number of nodules and weight of nodules and shoot than 30 days old crop in

all treatments and the control. Monocrotophos was highly toxic to *Rhizobium* because it recorded the least number of nodules at 30 and 60 days and shoot weight at 60 days carbosulfan (20 g kg^{-1} seed) recorded significantly higher number and weight of nodules than the other treatments and the control.

While studying the effect of *Rhizobium* association with granular insecticides on nodulation in soybean, Kundu and Trimohan (1989) reported that there was virtually no nodulation in preflowering or early pod formation stage in the uninoculated plants. There were no significant differences in nodule number between treatments with *Rhizobium* alone and with *Rhizobium* in association with insecticides but maximum number of nodules were recorded with *Rhizobium* in association with quinalphos. A significant increase in nodule and fresh weight was recorded with *Rhizobium* + quinalphos at the preflowering stage and with *Rhizobium* + phorate at early pod formation stage.

Soam and Agrawal (1988) evaluated the effect of dimethoate on nodulation and nitrogenase activity of nodules in pea and reported that dimethoate (0.01 %) reduced the nodulation by 4 per cent in black gram and 6 per cent in pea compared with control. At 1 per cent concentration, it reduced nodulation by 63 per cent in black gram and 64 per cent in pea. Bacterial region was also less in nodules from the treated plants than in those from the untreated plants of both the crops. The detrimental effect of the

highest dose of pesticide on different symbiotic activities and nodulation was observed.

The pre-emergence effect of dinitro aniline herbicide flucloraline and a botanical pesticides neem granules was tested against two strains of fast growing *Rhizobium fredii and* two strains of slow growing *Bradyrhizobium japonicum.* The study revealed that fast growing strains were sensitive at 10 ppm a.i. concentration of the herbicide where as the slow growers showed high level of intrinsic tolerance on the other hand, non of the 4 strains were significantly suppressed by recommended doses of Neem granules.

Singh and Mathur (1989) studied the effect of phorate on nodulation and activity of enzymes of nitrogen metabolism *viz.*, nitrogenase (N2-ase), glutamine synthetase (GS), glutamate synthate (GOGAT) and glumate dehydrogenase (GDH) in the root nodules of black gram. They reported that phorate stimulated number of nodules per plant and activity of these enzymes at lower concentration and decreased their activity at higher concentration. Apparent Km values of N2-ase, GS, GOGAT and GDH were similar in the control and the treated plants, indicating that the treatment affected the synthesis of these enzymes rather than their activity.

Prasad (1985) observed the effects of spraying metasystox (0.05, 0.08, 0.10 and 0.015 %) and cuman-L (0.25, 0.50, 0.75 and

1.00 %) on in vitro nitrogenase activity in detached root nodules of *Phaseolus mungo.* The enzyme activity was inhibited by 20 per cent with 0.15 per cent of metasystox on recovery after the growth. On the other hand, treatments of cuman-L had little or no effect on the enzyme activity.

Gupta *et al.* (1988) evaluated the effect of pre and post inoculation of seed treatment with fungicides on nodulation of soybean. Maximum Rhizobial population per g of seed was observed with soybean inoculum alone ($1.97x10^6$), followed by inoculum and post treatment of thiram ($1.80x10^6$). Thiram gave maximum number of nodules (33.50), oven dry weight of nodules (48 mg), high crude protein (42.20 %). The total bacterial count of soybean under rhizosphere was highest *Rhizobium* inoculum alone and its combination with thiram. The total bacterial count of soybean rhizosphere increased up to 17^{th} day, then decline at harvest. The significant reduction in fungal population was observed in soil treated with thiram (pre and post treatment), followed by difolaton.

Martensson and Nilsson (1989) conducted an experiment to investigate the effect of chlorosulfuron on growth infective ability of legume bacteria. They summarized that bacterial growth in pure culture was unaffected by the addition of 0.55 and 5.5μM chlorosulfuron. Early root hair infections of alfalfa by bacteria were inhibited by chlorosulfuron at 0.28 pM not at 0.0028 pM in

the root media. Early emergence and growth of alfalfa plants in soil supplemented with 2 X 10^{-6}, 2 X 10^{-3}, 2, 4 and 8 g ha^{-1} of chlorosulfuron were unaffected. However, 5 to 8 days after emergence plants grown in soil supplemented with 4 or 8 g ha^{-1} of chlorosulfuron were severely damaged.

Kundu and Trimohan (1989) studied the effect of *Rhizobium* association with granular insecticides on yield in soybean. Results revealed that *Rhizobium* inoculated seeds recorded an increase of 19.40 per cent yield over uninoculated seeds but significantly higher yield (43.20%) was recorded when *Rhizobium* inoculated seeds were sown in association with phorate. A significant increase in height was however, recorded only when *Rhizobium* was in association with phorat, quinolphos or mephosulfon insecticides. At early pod formation stage, no significance difference in height was observed.

The effect of pre and post inoculation seed treatment with fungicides on grain yield of soybean was evaluated by Gupta *et al.* (1988). Though the thiram was found to inhibit the growth of *R. japonicum*, it gave maximum grain yield (4.09 g) per plant. The significant reduction in fungal population was observed in the soil treated with Thiram (pre and post treatment), followed by difolatan.

The combined influence of application of aldicarb, alone and along with N and P was studied by Yein *et al.* (1979) and they

found that there was significant increase in dry weight of plants of *Vigna radiata*. Captan had a masking effect on the effects of aldicarb while endosulfan had little effect. Treatments involving the use of aldicarb gave significantly higher, although differences among the treatments involving the use of aldicarb were non significant.

Nova *et al.* (1998) conducted an experiment in which the effect of herbicides *viz.*, fomesafen (250 g ha^{-1}), lactofen (192 g ha^{-1}), fluazifop-P-butyl (187 g ha^{-1}), haloxyfop-methyl (240 g ha^{-1}) and fonesafen (250 g ha^{-1}) + fluazifop-P-butyl (187 g ha^{-1}) on the seed of pea-nuts inoculated with recommended nitrogen fixing bacterial strains. No effect of herbicides was observed on root dry weight. Shoot dry weight was higher when lactofen and fomesafen were applied pea-nuts grain yield was affected by herbicides.

Prabhakaran and Ramassamy (1990) conducted a pot experiment at National Pulse Resarch Centre, TamilNadu in which seeds of *Vigna radiata* and *Vigna mungo* were treated @ 5 ml kg^{-1} seed with Carbosulfan (Marshal), Chlopyrifos (Corobon), Phosphamidon (Dimeroon),Metasystox (Demeton-S-methyol), Dimethote (Rogor) and Monocrotophos (Nuvacron) and then inoculated with *Rhizobium*. Seed treatment reduced *Rhizobium* population by 63.33-99.00 percent in *Vigna radiata* and by 95.73-.99.76 in *Vigna mungo*. Nodulation and growth were highest with *Rhizobium* inoculation alone.

Severe phytotoxicity was observed by Yadav *et al.* (1998) when the four cluster bean (Cyamopsis tetragonolobla treated with pendimethalin, fluchloralin and trifluralin 1.0 and 2.0 kg a.i. ha^{-1} respectively. However, less injury to all varieties was found with fluchloralin, particularly at the lowest concentration. Similarly, root and shoot length was reduced significantly.

An experiment was carried out by Silva *et al.* (1998) under green house conditions in order to evaluate the effects of soil applications of different herbicides on nodulation and nitrogen fixation in cowpea. Treatments included a control (without herbicide) and application of the following herbicides at the recommended rates and at double these rates, trifluralin @ 1.8 and 3.6 ltr ha^{-1}, EPTC @ 5.0 and 10 ltr ha^{-1}, pendimethalin @ 2.0 and 2.4 ltr ha^{-1} and metribuzin @ 1.1 and 2.2 liters ha^{-1} , linuron @ 2.4 and 4.8 ltr ha^{-1} and imazaquin @ 1.0 and 2.0 ltr ha^{-1}. All treatments were inoculated with *Bradyrhizobium*, strain NBF 0.9. The data showed that cowpea nodulation was reduced by trifluraalin (1.8 ltr.), linuron (2.4 ltr.), and imzaquin (1.0 ltr.). Total nitrogenase activity was reduced in the soil treated with herbicides. Metribuzin and linuron showed a large degree of phytotoxicity to the crops inhibiting its vegetative growth.

Mendes *et al.* (1994) conducted field trial in 1988-89 on a dark red Latosol in Brasilia with an established population of *Bradyrhizobium japonicum.* Plots were treated with 0 or 890 g a.i.

tritifluralin ha^{-1} before sowing seed of soybean *cv*. Doko or Cristalina untreated or inoculated with 1 of 2/3 *japonicum* strains and reported that inoculation and application of herbicide had no significant effect on nodulation 12 days after emergence and at flowering. Response to inoculation was independent of applied trifluralin and varied accordingly to serological composition of indigenous *B. japonicum*. When serogroup 29 W and 587 occurred in >70 per cent of nodules of untreated plants, inoculation with strains CPAC-17 and CPAC-15 increased occurrence of the inoculated strain to a greater degree than on soil where these serogroups were present in< 50 per cent of nodules.

While working at Hisar, Pahwa and Prakash (1992) reported that higher herbicide concentrations generally reduced shoot and root dry weight recorded at 60-75 days after sowing (DAS). At 75 DAS nodule number was decreased by all treatment except 0.5 kg fluchloralin. Nodule dry weight was increased by the lowest fluchloralin concentration whereas decreased by higher rates of all herbicides and most adversely by high rates of pendimethalin.

Working at Finland, Miettinen and Echegoyen (1996) reported that high concentrations of Gaucho insecticide (imidacloprid) and Vitavax-300 fungicides (carboxin and captan) inhibited the growth of root nodule bacterium under laboratory conditions.

Gupta and pandey (1992) evaluated the effect of herbicides (pendimethalin, alachlor, fluchloralin, dalapon, isoproturon and 2,4-D Na) on *Rhizobium spp.* in soil and on the germination of black gram (*Vigna mungo*). The minimum inhibitory concentration (MIC) of the herbicides in relation to *Rhizobium* varied from 7000 to 15000 ppm. The *Rhizobium* was most resistant to dalapon (MIC 15000 ppm) .In contrast, black gram was more sensitive to herbicides particularly 2,4-D Na

Madhavi *et al.* (1994) studied the influence of 15 pesticides on nodulation and nitrogenase activity and they found that thirteen of the pesticides showed 55- 100 per cent loss of nodulation and nitrogenase activity of *Rhizobium spp.* Of the 6 insecticides, only sumicidin showed a loss of nodulation and nitrogenase activity with *R. leguminosarum*, while herbicides showed losses of 35-75 per cent in both cases. Sumicidin was the only insecticide effective in decreasing nodulation and nitrogenase activity (by 35%) in *R. melilotim.* However, herbicides decreased both properties by 20-100 per cent.

Eberach and Douglas (1989) conducted an experiment in Australia, and noted that nodulation decreased linearly as amitrole, diclofopmethyl and glyphosate concentration in the rooting environment increased from 0 to 20 mg a.i. ltr^{-1}. This decrease indicate that these herbicides had a physiological effect on the nodulating potential of *Rhizobium.*

Field experiment was conducted in Poland by Sawicka and Selwet (1998), where they studied the effect of two herbicide active ingredients (imazethapyr and linuron) on symbiotic nitrogen fixation activity and microorganisms under legume crops. The studies indicated that both imazethapyr and linuron could cause a decrease in root-nodule bacteria nitrogenase activity.

Nguyen (1996) conducted an experiment during 1998-1990 at Hanoi (Vietnam) and 1992-1994 at Szczecin (Poland) in order to evaluate the effect of different concentration of 2,4,5-T on the growth and ability to fix nitrogen by the *Rhizobium*. 2,4,5-T acid was a negative effect on the growth of these bacteria. Doses of 0.5 and 1.0 mg cm^{-3} caused destruction of 99.9 per cent. *Rhizobium spp.* cell in relation to the control. 2,4,5-T had a negative effect on nitrogen fixation ability of the *Rhizobium spp.* at doses from 0.25 to 1.0 mg kg^{-1} of soil. Heavy dosage of 2,4,5-T reduced the length of plants (from 25.9 to 58.0%) and their dry matter content (from 23.1 to 55.7%) in comparison with the control. Whereas, lower doses had no negative effect.

In pot, Jaggi and Oberholzer (1990) reported that excessive doses of herbicides decreased root nodule number. N-fixation was badly affecting the increasing concentration of the herbicide doses. Double the rate of dinoseb-acetate resulted in 50 per cent reduction of N-fixation.

The effect of chlosulfuron on growth and symbiotic performance of legume bacteria was investigated by Martesson and Nilsson (1989) and summarized that growth in pure culture was unaffected by addition of 0.55 and 5.5 μM chlosulfuron. Early emergence and growth of *M. sativa* plants in soil supplemented with $2x10^{-6}$, $2x10^{-3}$, 2, 4 and 8 g ha^{-1} of chlorsulfuron were unaffected. However, 5-8 days after emergence plants grown in soil supplemented with 4 or 8 g ha^{-1} of chlorsulfuron were severely damaged, with no nodules developed. Nodulation occurred in plants grown in soil containing $2x10^{-3}$, $3x10^{-6}$ and 2 g ha^{-1} chlorosulfuron, but the nitrogenase activity of the nodules of these plants was comparatively less than the control plants. Inhibition of nodulation and nitrogenase activity of nodules growth due to higher chlorosulfuron doses.

While working at Canada, Sprout *et al.* (1992) found that metribuzin had a significant negative effect on plant weight, number of nodules, and taproot growth when sprayed at 8 days. Five to ten days after spraying, the plants began to recover from the inhibitory effects. The *R. leguminosarum* strains used as inoculant affected the degree of inhibition of lentil growth and the rate of plant recovery. Less than 0.2 per cent of foliarly applied metributzin had translocated to the root. Thus, the detrimental effects of metribuzin application to lentils were mainly due to the direct effect on the plants, which results in indirect effects on nodulation and nitrogen fixation.

Greenhouse experiments were conducted at Hyderabad, Madhavi *et al.* (1993) to determine the effect of insecticides (monocrotophos, acephate, dichlorovos, DDT, fenpropathrin and fenvalerate) and herbicides (butachlor,simazine and oxyfluorfen) on the growth and symbiotic properties of *Rhizobium spp.* The result revealed that plants infected with pesticide treated *Rhizobium* had deceased DW and total N content (TNC) due to reduced growth and N-fixing capacity. This was attributed to the loss of nodulation and nitrogenase activity in the *Rhizobium spp.* Herbicides had stronger effects or DW and TNC followed by insecticides.

Field studies were carried out during 1995 by Sawicka *et al.*(1996) in Poland to examine the influence of imazethapyr and linuron on microorganisms occurring in soil under legume crops (*Pisum sativa, Vicia faba, Lupinus albus, Lupinus luteus* and *Glysine max*). Results indicated that both herbicides stimulated the development of bacterial and Actinomycetales while inhibited the growth of fungi. They also caused a decrease in bacterial root-nodule nitrogenase activity.

Nova *et al.* (1996) conducted an experiment with peanuts (groundnuts) *cv.* Tatu on a dark red latosol at, Ribeirao Preto, SP,Brazil. The effect of seed inoculation with recommended nitrogen fixing bacterial strains was studied and with in each plot herbicides like fomesafen (250 g ha^{-1}), lactofen (192 g ha^{-1}),

fluazifop–p butyl (187 g ha^{-1}),haloxyfop methyl (240 g ha^{-1}) and fonesafen (250 g ha^{-1})+fluazifop-p-butyl (187 g ha^{-1}) mixture applied as post emergence. Inoculation treatment affected nitrogenase activity which was higher with inoculated seed treatment. Symbiotic nitrogen fixation decreased for 4 hours after herbicidal treatment, with the effects of fluazifop-p-butyl and haloxyfop-methyl being the most pronounced.

Fajri *et al.* (1996) analysed the influence of a range of pre emergence herbicides on nodulation of 5 annual medics and 7 subterranean clover. The smallest reduction was found in caliph and sava treated with simazine at 1.01 + imazethapyr at 150 ml and the largest reduction was recorded for the same cultivars with simazine at 2.01.

Wall (1994) conducted an experiment in 1992 and 1993 to investigate the response of flax and lentil to Spring application of herbicides *viz.*, ethalfluralin, pendimethalin and trifluralin. The herbicides were applied to preplant soil incorporation at 1.1 kg a.i. ha^{-1} in the Spring. He analysed that ethalfluraline, pendimethalen and trifluralin reduced flax population density. In case of flax, pendimethalin and trifluralin reduced flax population density. In case of lentil, pendimethalin reduced the lentil population density, dry weight and seed yield and ethalfuralin and triflurolin reduced lentil population density and seed yield in one year only.

Materials and Methods

CHAPTER-III

MATERIALS AND METHODS

A set of experiments under sterilised and unsterilised conditions were conducted in Soil Microbiology Lab., Department of Soil Science, IGAU, Raipur (C.G.) during 2001-2002 to detect safe and suitable doses of the different pesticides for effective Gram- *Rhizobium* symbiosis. In this connection experiments were conducted with Gram *(Cicer arietinum* L.), variety JG-11 by using unsterilised and sterilised Vertisol. Sand culture experiment devoid of mineral nitrogen source was also conducted under growth room conditions to fulfill above mentioned objective.

I. Geographical situation

Raipur lies at 21° 16' N latitude and 81° 36' E longitude with an altitude of 298.56 m above the mean sea level.

II. Climate

Raipur, the place of investigation comes under dry- sub humid to semi arid agroclimatic region under rice zone of the state. Out of the mean annual rainfall of 1200-1300 mm, about 85 per cent is received during third week of June to mid September.

III. Experimental details

This study was aimed at the detection of safe, compatible and suitable doses of different pesticides for improving chickpea-

Rhizobium symbiosis in Chhattisgarh region. In this connection, experiments were conducted by using unsterilised and sterilised Vertisol. Mineral nitrogen free sand culture experiment was also conducted under growth room conditions. In all the three experiments, four selected pesticides with three doses were tested under completely randomized design. Each treatment was replicated six times.

(I). **Treatments**

Table-1: The following treatments were set up for growth room

Sterilised / Unsterilised Vertisol		Sterilised sand culture	
T_1	Control (Uninoculated)	T_1	Control (Uninoculated)
T_2	Inoculated with *Rhizobium*	T_2	Inoculated with *Rhizobium*
T_3	T2 + Soil application with Metasystox @ 1 ltr ha^{-1}	T_3	T_2 + Seed treatment with Metasystox @ 2.5 ml kg^{-1} seed
T_4	T2 + Soil application with Metasystox @ 2 ltr ha^{-1}	T_4	T_2 + Seed treatment with Metasystox @ 5 ml kg^{-1}seed
T_5	T2 + Soil application with Metasystox @ 3 ltr ha^{-1}	T_5	T_2 + Seed treatment with Metasystox @ 7.5 ml kg^{-1}seed
T_6	T_2 + Soil application with Chloropyrifos @ 1 ltr ha^{-1}	T_6	T2+Seed treatment with Chloropyrifos @ 2.5 ml kg^{-1}seed
T_7	T2 + Soil application with Chloropyrifos @ 2 ltr ha^{-1}	T_7	T_2 + Seed treatment with Chloropyrifos @ 5 ml kg^{-1}seed
T_8	T2 + Soil application with Chloropyrifos @ 3 ltr ha^{-1}	T_8	T_2 + Seed treatment with Chloropyrifos @ 7.5 ml kg^{-1} seed
T_9	T_2 +Soil application with Pendimethalin @ 0.75 ltr a.i. ha^{-1}	T_9	T_2 + Seed treatment with Pendimethalin @ 5 ml kg^{-1} seed
T_{10}	T2+ Soil application with Pendimethalin @ 1.5 ltr a.i. ha^{-1}	T_{10}	T_2 + Seed treatment with Pendimethalin @ 10 ml kg^{-1} seed
T_{11}	T2+ Soil application with Pendimethalin @ 2.25 ltr a.i. ha^{-1}	T_{11}	T_2 + Seed treatment with Pendimethalin @ 15 ml kg^{-1} seed
T_{12}	T_2 + Soil application with Alachlor @ 1 ltr a.i. ha^{-1}	T_{12}	T_2 + Seed treatment with Alachlor @ 5 ml kg^{-1}seed
T_{13}	T2 + Soil application with Alachlor @ 2 ltr a.i. ha^{-1}	T_{13}	T2 + Seed treatment with Alachlor @ 10 ml kg^{-1}seed
T_{14}	T2 + Soil application with Alachlor @ 3 ltr a.i. ha^{-1}	T_{14}	T_2 + Seed treatment with Alachlor @ 15 ml kg^{-1}seed

(II) Experiments with Vertisol and sand

Experiments under growth room conditions were conducted by using unsterilised and sterilised Vertisol. For this purpose, sterilised and unsterilised Vertisol (Free from aggregate/clump) was filled in disposable cup of 250 g capacity. Similarly, fine graded sterilised river sand was also taken in the individual disposable cups.

(a) Surface sterilisation of seeds

Healthy seeds of chickpea (var. JG-11), were taken for experiments. Uniform sized seeds were rinsed with 95 per cent ethanol and then immersed for 4 minutes in 0.1 per cent mercuric chloride solution. The seeds were then washed thoroughly with double distilled sterilised water for at least 5 times.

(b) Sowing, handling and irrigation

In sterilised sand culture, certified healthy chickpea (*Cicer arietinum* L.) seeds variety JG-11 were treated before sowing with different pesticides as mentioned in treatments Table No.1, then inoculated by using measured quantity of YEM-rhizobial suspension. Neutralized gum arabic and lignite were used as sticking and wetting agent, respectively. The seeds of control cup received same amount of YEM broth without rhizobial population. Amount of mature YEM-rhizobial suspension was fixed to ensure at least 10^4 viable cells received by every seed (Nambiar, 1985). Three seeds were sown in each cup (Sowing date 10-06-2002). The

cups were, then properly tagged and labeled. Timely and uniform irrigation were provided to all the cups by N-free Mcknight seedlings nutrient solution as and when required.

Composition of Mcknight's seedling N-free nutrient solution

Solution A	mg **100ml^{-1}** Distilled water
Boric acid (H_3BO_3)	2.86
Manganese Sulphate (MnSO4.4H2O)	1.54
Zinc Sulphate ($ZnSO_4.7H_2O$)	0.22
Copper Sulphate ($CuSO_4$. 5H2O)	0.08
Molybdic acid (H_2MoO_4)	0.09

Solution B	mg **100ml^{-1}** Distilled water
Ferric Chloride ($FeCl_3$)	16.8
EDTA	2.0

Solution C	gm **ltr^{-1}** Distilled water
Calcium Sulphate ($CaSO_4$)	24.0
Magnesium Sulphate ($MgSO_4$)	4.0
Potassium Dihydrogen Phosphate(KH2PO4)	4.0
Potassium Chloride (KC1)	6.0

To the 960 ml of solution C, 20 ml each of solution A and B were added and the whole solution was diluted 20 times. pH of the nutrient solution was adjusted to 7.0. For sterilisation, the nutrient solution was autoclaved at 15 lb/inch^2 pressure for 30 minutes (Katre *et al.,* 1997).

Similarly, experiments with sterilised and unsterilised Vertisol, seeds were inoculated with YEM-rhizobial suspension as discussed earlier. However, doses of different pesticides were applied in to soil after sowing but before germination.

Table -2: Characteristics of experimental soil

Soil	Vertisol
pH (1:2.5)	7.56
E.C.(dsm^{-1})	0.26
Available N (kg/ha)	219.50
Available P (kg/ha)	11.6
Available K (kg/ha)	365.70
O.C.(%)	0.48
Mechanical analysis	
a. Sand %	21.40
b. Silte (%)	27.15
c. Clay (%)	51.45
Rhizobial population	3.2×10^3 g^{-1} soil

(c) Fertilizer application

Fertilizer solution urea (NH_2CONH_2), tricalcium phosphate (Ca_3PO_4) and potassium chloride (KC1) were prepared for application to the cups containing Vertisol.

IV. Observation recorded

1. Nodulation study at 35 and 60 DAS
2. Plant height at 8,15,30,45 and 60 DAS
3. Biomass accumulation at 35 and 60 DAS
4. Plant N-content at 35 and 60 DAS
5. N-uptake at 35 and 60 DAS
6. *Rhizobium* population dynamics study

V. Harvesting

Three replications of growth room grown chickpea plants were uprooted after 35 DAS and remaining three replications were uprooted after 60 DAS .The plant samples were oven dried at 60 to 65° C up to the attainment of constant weight. The weight of dry samples was recorded. The oven dried plant samples were grind in stainless steel grinder for subsequent chemical analysis. The grind samples were stored in envelopes and redried before analysis.

VI. Physico-chemical and microbial analysis

1. Physical analysis

Moisture content of soil

Soil samples were weighed, oven dried at 105° C and weighed again for determination of moisture content by using gravimetric method (Singer and Munns, 1992).

$$\text{Gravimetric water content} = \frac{\text{Weight loss}}{\text{Dry weight}}$$

2. Physico chemical analysis of soil

Soil samples (0-15 cm) were air dried, finely powdered in a wooden mortar with wooden pestle and passed through 2 mm sieve for physico-chemical analysis work. While about 25 gm of each soil sample was kept as such in sealed polythene bag to prevent the moisture loss and was properly stored in refrigerator for quantitative analysis of initial microbial population.

(a) Soil pH

The pH of the soil was determined in 1:2.5 soil water suspension, using glass electrode in Elico pH meter.

(b) Electrical conductivity

Electrical conductivity was determined in soil water suspension (1:2.5) by conductivity bridge as described by Jackson (1973).

3. Chemical analysis of soil

(a) Available N

Available N was determined by alkaline $KMnO_4$ method of Subbiah and Asija (1965). Twenty gram soil sample was taken in one litre boiling flask and 200 ml distilled water, 100 ml of 0.32 per cent KMnO4 and 100 ml of 2.5 per cent NaOH were then added in sequence. The flask was connected to the condenser immediately after adding NaOH and the content was boiled on heater to collect about 150 ml distillate in 10 ml boric acid solution containing mixed indicator (Bremner,1965). Ammonium-N in distillate was determined by titrating against 0.005 N (Bremner, 1965).

(b) Available P

Soil phosphorus was extracted by 0.5 M $NaHCO_3$ as described by Olsen *et al.* (1954) and phosphorus in the extract was determined by ascorbic acid method of Watanabe and Olsen (1965).

(c) Available K

Five gm of soil was shaken with 25 ml neutral 1 N Ammonium acetate (CH3COONH4 pH 7.0) for 5 minutes and filtered. Potassium was estimated by Flame Photometer (Hanway and Heidel, 1952).

4. Chemical analysis of plant

Nitrogen: The nitrogen content in the plant sample was estimated by micro-kjeldahl method as described by Jackson (1973) using Gerhardt auto digestion and distillation system (Vapodest-30).

5. Microbial analysis of soil

Microbial analysis: Microbial analysis of soil was done by serial dilution plating method (Subba Rao, 1988). Soil and sand samples up to 10 cm depth were drawn out with the help of sterilised spoon from each cup (pot) at different stages of the crop growth.

The sampling of soil and sand from the growth room experiments was done at the following stages of the crop growth.

1. Sampling at 35 DAS
2. Sampling at 60 Das

Soon after sampling, the soil and sand samples were kept in polythene bags to prevent the moisture loss and properly tagged, sealed and stored in refrigerator for quantitative estimation of *Rhizobium*.

Microbiological estimations with respect to rhizobial count in the soil and sand were done by dilution plate method (Subba Rao, 1988). For rhizobial counting, serial dilution of soil and sand samples were done by taking 1 gm of soil in 9 ml double distilled sterilised water in a dilution tube (Tuladhar, 1983) and it was shaken on a shaker for 30 minutes. After shaking, the dilution tube (No. 1) was kept for 30 minutes to allow the soil and sand particles to settle down, in this way 10^1 dilution of the soil/sand sample was obtained. Now 1.0 ml of the rhizobial suspension from dilution tube No. 1 was drawn out with the help of autopipette and transferred to another dilution tube No. 2 containing 9 ml double distilled sterilised water resulting 10^2 dilution. It was again kept on rotary shaker for 5 minutes. Again 1.0 ml suspension was drawn from dilution tube No. 2 for 10^3 dilution and this way serial dilution of a soil sample was carried out up to desirable dilution and finally *a* complete set of desirable dilution of each soil sample was obtained. Similarly, population density of *Rhizobium* in mature YEM broth was also determined.

Yeast Extract Mannitol Agar Media (YEMA) for rhizobia (Subba Rao, 1988).

Composition of the medium

Mannitol	10.0 gm
K_2HPO_4	0.5 gm
$MgSO_4.7H_2O$	0.2 gm
NaCl	0.1 gm
Yeast Extract	1.0 gm
$CaCO_3$	3.0 gm
Agar	15.0 gm
Distilled water	1000.0 ml
Congo red solution (1%)	2.5 ml
pH	7.0

About 20 ml of the appropriate sterilised and partially cooled agar media was poured into the sterilised Petriplates containing 1 ml aliquot of appropriate dilution at the bottom which was drawn out from the dilution tube with the help of a sterilised tips of autopipette and the plates were incubated at 28 °C in the incubator. Counting of rhizobial colonies was done after 24 hours of incubation. Counted colonies were marked with an instant marker to avoid repeated counting and the process of counting continued up to 7 days of incubation. Colony counting was done on the colony counter.

Plating of each sample was done in duplicate and mean values were worked out for each sample. One control was also

incorporated with each set of plating. After counting of colonies, rhizobial population was calculated on the basis of per gm of dry soil/sand using following formula (Schmidt and Caldwell, 1967). Rhizobial population density in the YEM broth was also estimated by using the same formula.

Number of rhizobia per gm of oven dry soil

$$\frac{\text{No. of colony forming units (CFU) x dilution}}{\text{Dry weight of one gm moist soil sample x Aliquot taken}}$$

Number of rhizobia/ml of matured YEM broth

$$\frac{\text{No. of colony forming units (CFU) x dilution}}{\text{Aliquot taken}}$$

The operation of making serial dilution, setting of plates, inoculation with appropriate media was done in sterilised atmosphere of Laminar Air Flow.

VII. Statistical analysis

All the pre and post harvest observations were recorded and tabulated in a systematic manner. The final observations were statistically analysed by completely randomized design (Panse and Shukhatme, 1978).

Results

CHAPTER IV
RESULTS

The results of present investigation entitled "Detection of safe doses of different pesticides for chickpea (*Cicer arietinum* L.)-*Rhizobium* symbiosis" given in the following heads and description of various treatments applied during the course of study is given in chapter 3 (Table-1).

I. Unsterilised soil conditions

The observations for plant height, fresh weight and dry weight of shoot, nodule number, *Rhizobium* population, N-content and uptake were taken under unsterilised soil conditions at successive stages of chickpea and presented in the following heads.

a. Effect of different treatments on plant height of chickpea

Evaluation of different pesticides was performed and data presented in Table 3 and Fig. 1 based on their effect on plant height of chickpea.

The data presented in Table 3 revealed that the different treatments under unsterilised soil conditions failed to produce any significant variation in plant height of chickpea at 8, 15 and 30 DAS. However, height of plants raised from seeds inoculated with *Rhizobium* (T_2) was found higher than plants raised with other

Table: 3 Effect of different doses of pesticides on height of growth room grown chickpea under unsterilised soil conditions

S. No.	Treatments	Plant height (cm)				
		8 DAS	15 DAS	30 DAS	45 DAS	60 DAS
T_1	Control (Uninoculated)	7.87	11.12	17.38	23.50	27.43
T_2	Inoculated with *Rhizobium*	9.16	13.92	19.67	28.91	32.52
T_3	T2+ Metasystox @ 1 ltr ha^{-1}	8.72	13.37	18.50	27.60	31.49
T_4	T2 + Metasystox @ 2 ltr ha^{-1}	8.63	12.92	18.12	26.10	30.12
T_5	T_2+ Metasystox @ 3 ltr ha^{-1}	7.53	13.87	17.96	23.57	27.36
T_6	T_2 + Chloropyrifos @ 1ltr ha^{-1}	8.54	13.47	18.93	28.76	31.50
T_7	T_2 ++ Chloropyrifos @ 2 ltr ha^{-1}	8.12	12.96	18.67	27.86	30.27
T_8	T_2 + Chloropyrifos @ 3 ltr ha ^{1}	8.15	13.15	18.20	24.37	27.98
T_9	T_2 + Pendimethalin @ 0.75 ltr a.i. ha^{-1}	8.79	12.92	18.12	27.29	30.10
T_{10}	T_2 + Pendimethalin @ 1.5 ltr a.i. ha^{-1}	8.12	12.50	17.97	26.19	28.43
T_{11}	T_2 + Pendimethalin @ 2.25 ltr a.i. ha^{-1}	7.69	11.97	17.50	22.53	24.17
T_{12}	T_2 + Alachlor @ 1 ltr a.i. ha^{-1}	8.15	12.87	18.27	28.47	30.97
T_{13}	T_2 + Alachlor @ 2 ltr a.i. ha ^{1}	8.15	12.50	18.00	27.91	29.76
T_{14}	T_2 + Alachlor @ 3 ltr a.i. ha^{-1}	7.19	12.30	17.60	23.63	25.27
CD	(5%)	NS	NS	NS	3.31	2.30

All the pesticides applied in soil

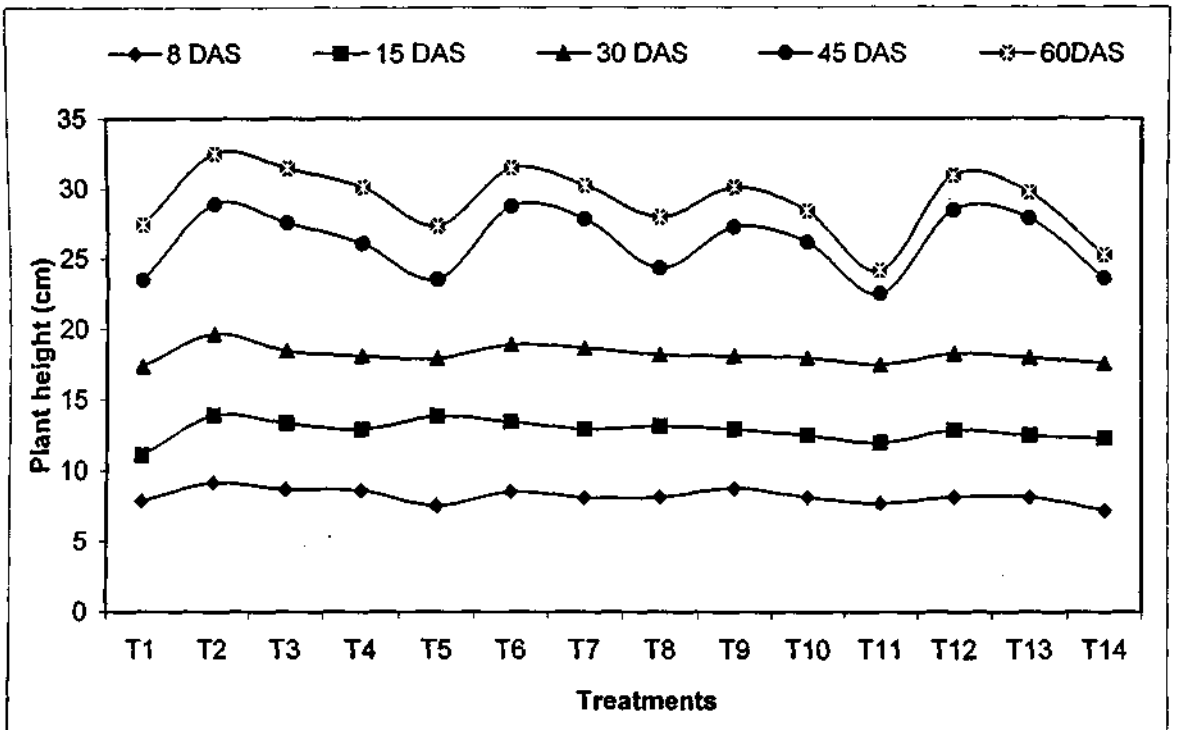


Fig. 1 : Effect of different doses of pesticides on height of growth room grown chickpea under unsterilised soil conditions

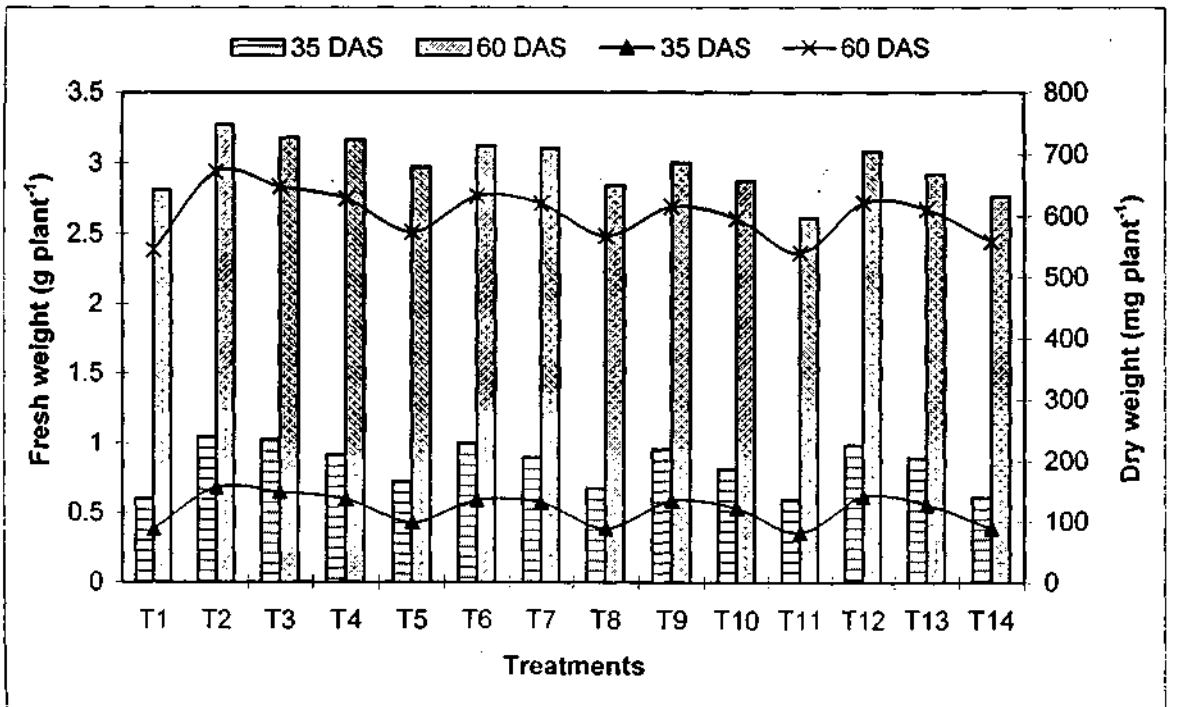


Fig. 2 : Effect pf different doses of pesticides on bio-mass accumulation by growth room grown chickpea under unsterilised soil conditions

treatments. The significantly tallest plant of chickpea was registered under the treatment T2 at 45 DAS followed by treatment T_{12} (T_2 + Soil application with Alachlor @ 1 ltr a.i. ha^{-1}) and T_3 (T2 + Soil application with Metasystox @ 1 ltr ha^{-1}). The treatment T4 (T2 + Soil application with Metasystox @ 2 ltr ha^{-1}), T_6 (T_2 + Soil application with Chloropyrifos @ 1 ltr ha^{-1}), T_7 (T2 + Soil application with Chloropyrifos @ 2 ltr ha^{-1}), T_9 (T2 + Soil application with Pendimethalin @ 0.75 ltr a.i. ha^{-1}) and T_{10} (T_2 + Soil application with Pendimethalin @ 1.5 ltr a.i. ha^{-1}) also produced comparable plant height of chickpea. The shortest plant height of chickpea at 45 DAS was observed under treatment T_{11} (T_2 + Soil application with Pendimethalin @ 2.25 ltr a.i. ha^{-1}). At 60 DAS significantly tallest plant of chickpea was observed under treatment T2 followed by T_6 (T_2 + Soil application with Chloropyrifos @ 1 ltr ha^{-1}), T_3 (T2 + Soil application with Metasystox @ 1 ltr ha^{-1}), T_{12} (T2 + Soil application with Alachlor @ 1 ltr a.i. ha^{-1}) and T_7 (T_2 + Soil application with Chloropyrifos @ 2 ltr ha^{-1}), which are statistically at par with each other. The significantly smallest plant observed under treatment T_{11} (T_2 + Soil application with Pendimethalin @ 2.25 ltr a.i. ha^{-1}) at 60 DAS. The minimum plant height was recorded in the treatments *i.e.* T_5, T_8, Tn and T_{14} where maximum doses of pesticides were applied as compare to the lower doses (T_3, T_6, T_9 and T_{12}) of each pesticide at 45 DAS. Similar trend was recorded at 60 DAS.

b. Effect of different treatments on fresh and dry weight of chickpea shoot

Effect of different doses of pesticides on fresh weight and dry weight of chickpea shoot is presented in Table 4 and showed in Fig. 2.

The significantly maximum fresh weight of chickpea shoot at 35 DAS recorded under treatment T_2 (inoculated with *Rhizobium*) followed by T_3 (T_2 + Soil application with Metasystox @ 1 ltr ha^{-1}), T_6 (T2 + Soil application with Chloropyrifos @ 1 ltr ha^{-1}), T_{12} (T_2 + Soil application with Alachlor @ 1 ltr a.i. ha^{-1}) and T_9 (T_2 + Soil application with Pendimethalin @ 0.75 ltr a.i. ha^{-1}), which were found statistically at par with each other. The significantly minimum fresh weight of chickpea shoot recorded under treatments T_{11} (T2 + Soil application with Pendimethalin @ 2.25 ltr a.i. ha^{-1}). At 60 DAS, the significantly higher fresh weight of chickpea recorded under treatment T_2, followed by T_3, T_6, and T4. The significantly less fresh weight of chickpea shoot produced by treatment Tn.

The higher level of pesticides T_5 (T2 +Soil application with Metasystox @ 3 ltr ha^{-1}), T_8(T_2 +Soil application with Chloropyrifos @ 3 ltr ha^{-1}), Tn (T_2 +Soil application with Pendimethalin @ 2.25 ltr a.i. ha^{-1}) and T_{14} (T_2 +Soil application with Alachlor @ 3 ltr a.i. ha^{-1}) inhibited the fresh weight of chickpea shoot as compared to low level of pesticides at 35 and 60 DAS (Table 4).

Table: 4 Effect of different doses of pesticides on bio-mass accumulation by growth room grown chickpea under unsterilised soil conditions

S. No.	Treatments	Plant Fresh Weight (g plant^{-1})		Plant Dry Weight (mg plant^{-1})	
		35 DAS	60 DAS	35 DAS	60 DAS
T_1	Control (Uninoculated)	0.605	2.81	88	545
T_2	Inoculated with *Rhizobium*	1.046	3.27	156	672
T_3	T_2+ Metasystox @ 1 ltr ha^{-1}	1.022	3.18	149	648
T_4	T_2 + Metasystox @ 2 ltr ha^{-1}	0.918	3.16	137	628
T_5	T_2+ Metasystox @ 3 ltr ha^{-1}	0.726	2.97	99	574
T_6	T_2 + Chloropyrifos @ 1 ltr ha^{-1}	0.999	3.12	135	632
T_7	T_2 ++ Chloropyrifos @ 2 ltr ha^{-1}	0.897	3.10	131	620
T_8	T_2 + Chloropyrifos @ 3 ltr ha^{-1}	0.679	2.84	89	568
T_9	T_2 + Pendimethalin @ 0.75 ltr a.i. ha^{-1}	0.957	3.00	134	615
T_{10}	T_2 + Pendimethalin @ 1.5 ltr a.i. ha^{-1}	0.816	2.87	122	596
T_{11}	T_2 + Pendimethalin @ 2.25 ltr a.i. ha^{-1}	0.599	2.61	82	540
T_{12}	T_2 + Alachlor @ 1 ltr a.i. ha^{-1}	0.987	3.08	141	621
T_{13}	T_2 + Alachlor @ 2 ltr a.i. ha^{-1}	0.895	2.92	128	611
T_{14}	T_2 + Alachlor @ 3 ltr a.i. ha^{-1}	0.612	2.76	89	558
CD	(5%)	0.09	0.14	30	49

* All the pesticides applied in soil

The significantly greater dry weight of chickpea shoot was found under treatment T2. The treatment T_3, T_6, T_7, T_9 and T_{12} produce comparable dry weight of chickpea shoot with treatment T_6. The significantly less dry weight of chickpea shoot recorded under T_{11} at 35 DAS.

There was a negative relationship found between dry weight and different high levels of pesticides. Dry weight increased with reduction in pesticides level, whereas decreased with the increase in pesticides levels.

c. Number of nodules and *Rhizobium* population as influenced by different treatments

Comparison of effect of different doses of pesticides on number of nodules and *Rhizobium* population is illustrated in Table 5 and presented graphically in Fig. 3.

The number of nodules influenced significantly by the different pesticides and their doses. The data presented in the Table 5 showed that, the significantly more number of nodules in chickpea recorded under treatment T2 at 35 DAS. The treatments T_6, Ty, T_9 and T_{12} produced comparable number of nodules under former treatment T_2. The less number of nodules of chickpea recorded under treatment Tn. The treatment T2 produced significantly more number of nodules in chickpea closely followed by treatment T_6 and treatment T12. The treatment T7 and treatment T2 +Soil application with Pendimethalin @ 0.75 ltr a.i.

Table: 5 Effect of different doses of pesticides on *Rhizobium* population dynamics and nodulation in growth room grown chickpea under unsterilised soil conditions

8. No.	Treatments	Effective Nodules Plant^{-1}		Rhizobium population (x 10^4 soil g^{-1})	
		35 DAS	60 DAS	35 DAS	6O DAS
T_1	Control (Uninoculated)	11.00	20.11	1.42	2.28
T_2	Inoculated with *Rhizobium*	16.78	27.11	1.72	2.78
T_3	T_2+ Metasysyox @ 1 ltr ha^{-1}	15.89	27.00	1.69	2.63
T_4	T_2 + Metasysyox @ 2 ltr ha^{-1}	15.33	25.00	1.61	2.57
T_5	T_2+ Metasysyox @ 3 ltr ha^{-1}	12.88	21.00	1.50	2.32
T_6	T_2 + Chloropyrifos @ 1ltr ha^{-1}	15.78	26.22	1.71	2.67
T_7	T_2 ++ Chloropyrifos @ 2 ltr ha^{-1}	15.11	24.00	1.67	2.61
T_8	T_2 + Chloropyrifos @ 3 ltr ha^{-1}	12.22	20.33	1.53	2.49
T_9	T_2 + Pendimethalin @ 0.75 ltr a.i. ha^{-1}	15.67	25.67	1.62	2.67
T_{10}	T_2 + Pendimethalin @ 1.5 ltr a.i. ha^{-1}	13.78	23.67	1.53	2.51
T_{11}	T_2 + Pendimethalin @ 2.25 ltr a.i. ha^{-1}	10.11	15.00	1.34	2.18
T_{12}	T_2 + Alachlor @ 1 ltr a.i. ha^{-1}	15.78	25.89	1.68	2.65
T13	T_2 + Alachlor @ 2 ltr a.i. ha^{-1}	14.78	23.78	1.59	2.53
T14	T_2 + Alachlor @ 3 ltr a.i. ha^{-1}	11.44	20.22	1.46	2.31
CD		1.80	2.60	0.13	0.15

* All the pesticides applied in soil

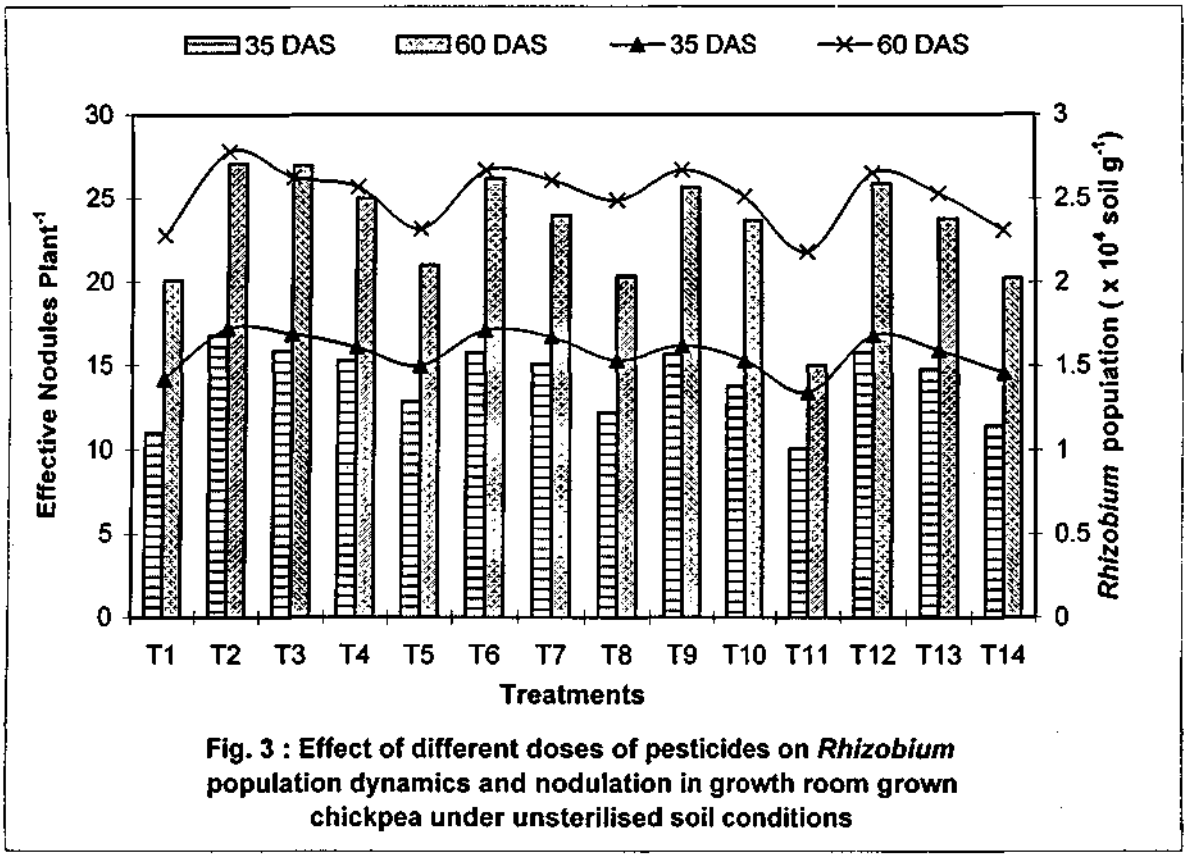


Fig. 3 : Effect of different doses of pesticides on *Rhizobium* population dynamics and nodulation in growth room grown chickpea under unsterilised soil conditions

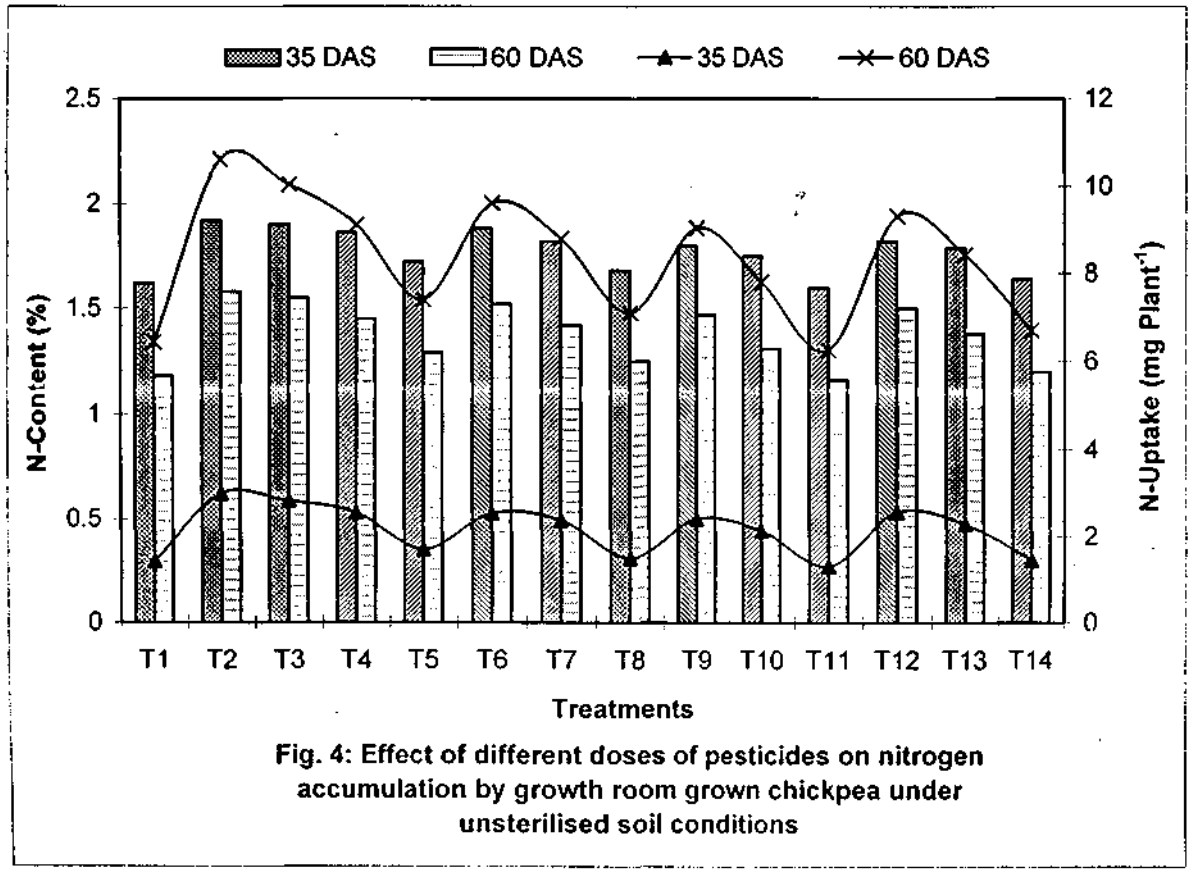


Fig. 4: Effect of different doses of pesticides on nitrogen accumulation by growth room grown chickpea under unsterilised soil conditions

ha^{-1} (Tg) also produced comparable number of nodules under former treatment T_2. The significantly less number of nodules recorded under treatment T_{11} at 60 DAS (Table *5)*.

The *Rhizobium* population was significantly affected by different pesticides and their levels at *35* and 60 DAS. The significantly maximum *Rhizobium* population counted under treatment T2 over all other treatments. However, remaining treatments maintained *Rhizobium* population at par under the treatment T2, except Tn that was recorded least *Rhizobium* population at *35* DAS.

Similarly at 60 DAS treatment T_2 counted significantly maximum *Rhizobium* population over treatment Tn (T2 +Soil application with Pendimethalin @ 2.25 ltr a.i. ha^{-1}). The remaining treatments maintained comparable *Rhizobium* population. Data presented in Table *5* revealed that number of nodules increased under reduction in pesticidal doses T_3 (T2 +Soil application with Metasystox @ 1 ltr ha^{-1}), T_6 (T2 +Soil application with Chloropyrifos @ 1 ltr ha^{-1}), T_9 (T_2 +Soil application with Pendimethalin @ 0.75 ltr a.i. ha^{-1}) and T_{12} and it decreased as pesticidal doses increased T_5 (T_2 +Soil application with Metasystox @ 3 ltr ha^{-1}), T_8 (T_2 +Soil application with Chloropyrifos @ 3 ltr ha^{-1}), T4 (T_2 +Soil application with Pendimethalin @ 2.25 ltr a.i. ha^{-1}) and T_{14} (T_2 +Soil application with Alachlor @ 3 ltr a.i. ha^{-1}).

d. N-content and uptake as influenced by different treatments

Data were recorded and presented in Table 6 to assess the effect of different doses of pesticides on N-content and uptake at 35 DAS and 60 DAS. Fig. 4 is the graphical representation of data recorded for N- content and uptake.

It is easy to conclude from the table that maximum N-content was recorded with control (inoculated with *Rhizobium*). Other treatments such as T_3, T4, T_6 and T_7 were also given comparable results and at par with each other at 35 DAS. Minimum N-content was recorded with T_{11} (T2 +Soil application with Pendimethalin @ 2.25 ltr a.i. ha^{-1}).

Statistical analysis as presented in Table 6 showed that at 60 DAS, T_2 was found to be superior over other treatments as it gave highest N-content followed by T_3 (T2 +Soil application with Metasystox @ 1 ltr ha^{-1}), T4 (T_2 +Soil application with Metasystox @ 2 ltr ha^{-1}) and T_6 (T_2 +Soil application with Chloropyrifos @ 1 ltr ha^{-1}). However, minimum N-content was observed with Tn (T_2 +Soil application with Pendimethalin @ 2.25 ltr a.i. ha^{-1}).

II. Sterilized soil condition

The observations for plant height, fresh weight and dry weight of shoot, nodule number, *Rhizobium* population, N-content and uptake were taken under sterilized soil condition at successive stages of chickpea and presented in the following heads.

Table: 6 Effect of different doses of pesticides on nitrogen accumulation by growth room grown chickpea under unsterilised soil conditions

8. No.	Treatments	N-Content (%)		N-Uptake (mg plant^{-1})	
		35 DAS	60 DAS	35 DAS	60 DAS
T_1	Control (Uninoculated)	1.62	1.18	1.43	6.43
T_2	Inoculated with *Rhizobium*	1.92	1.58	3.00	10.62
T_3	T_2+ Metasysyox @ 1 ltr ha^{-1}	1.9	1.55	2.83	10.04
T_4	T_2 + Metasysyox @ 2 ltr ha^{-1}	1.86	1.45	2.55	9.11
T_5	T_2+ Metasysyox @ 3 ltr ha^{-1}	1.72	1.29	1.70	7.40
T_6	T_2 + Chloropyrifos @ 1ltr ha^{-1}	1.88	1.52	2.54	9.61
T_7	T_2 ++ Chloropyrifos @ 2 ltr ha^{-1}	1.82	1.42	2.38	8.80
T_8	T_2 + Chloropyrifos @ 3 ltr ha^{-1}	1.68	1.25	1.50	7.10
T_9	T_2 + Pendimethalin @ 0.75 ltr a.i. ha^{-1}	1.8	1.47	2.41	9.04
T_{10}	T_2+ Pendimethalin @ 1.5 ltr a.i. ha^{-1}	1.75	1.31	2.14	7.81
T_{11}	T_2 + Pendimethalin @ 2.25 ltr a.i. ha^{-1}	1.6	1.16	1.31 %	6.26
T12	T_2 + Alachlor @ 1 ltr a.i. ha^{-1}	1.82	1.5	2.57	9.32
T13	T_2 + Alachlor @ 2 ltr a.i. ha^{-1}	1.79	1.38	2.29	8.43
T14	T_2 + Alachlor @ 3 ltr a.i. ha^{1}	1.64	1.2	1.46	6.70
CD		0.13	0.13	0.83	1.51

* All the pesticides applied in soil

a. Effect of different pesticide doses on plant height of chickpea

Table 7 showed the relationship between plant height observed corresponding to different doses of pesticides applied under sterilized soil condition. Comparison of above mentioned data was also shown in Fig. 5.

Plant height of chickpea affected significantly by the different pesticides and their doses. At 8, 15 and 30 DAS the different treatments failed to produce any significant variation on plant height of chickpea. However, treatment T2 produced taller plant than other treatments at 8, 15 and 30 DAS. At 45 DAS treatment T_2 was found to be most effective fors attaining maximum plant height whereas, treatments T_3, T_6, T_{12}, T_9, T_7, T_{10} T4 and T_{13} produced comparable height of chickpea plant under former treatment T_2. The smallest plant of chickpea was observed under treatment T_{11}. The treatment T2 also produced significantly tallest plant of chickpea at 60 DAS and it was at par with plant height of chickpea of treatment T_3, T_4, T_6, Ty, T_9 and T_{12}. The smallest plant at 60 DAS was recorded under treatment T_{11} (Table 8).

It may be concluded from observations that high doses of pesticides T2 +Soil application with Metasystox @ 3 ltr ha^{-1}(T_5), T2 +Soil application with Chloropyrifos @ 3 ltr ha^{-1}(T_8), Tn and T_2 +Soil application with Alachlor @ 3 ltr a.i. ha^{-1}(T_{14}) were caused to

Table: 7 Effect of different doses of pesticides on height of growth room grown chickpea under sterilised soil conditions

S. No.	Treatments	Plant height (cm)				
		8 DAS	15 DAS	30 DAS	45 DAS	60 DAS
T_1	Control (Uninoculated)	8.97	14.33	19.33	25.64	29.74
T_2	Inoculated with *Rhizobium*	11.42	15.87	21.89	30.87	34.79
T_3	T2+ Metasystox@ 1 ltr ha^{-1}	11.37	15.63	21.76	30.40	34.57
T_4	T_2 + Metasystox @ 2 ltr ha^{-1}	10.98	15.10	21.30	29.69	33.20
T_5	T2+ Metasystox @ 3 ltr ha^{-1}	10.74	14.64	20.84	26.35	30.84
T_6	T2 + Chloropyrifos @ 1ltr ha^{-1}	11.69	15.91	21.80	30.20	33.88
T_7	T2++Chloropyrifos @ 2 ltr ha^{-1}	11.20	15.47	21.50	29.10	32.97
T_8	T2 + Chloropyrifos @ 3 ltr ha^{-1}	10.95	14.79	21.10	26.30	29.18
T_9	T2 + Pendimethalin @ 0.75 ltr a.i. ha^{-1}	11.28	15.34	19.81	29.39	32.91
T_{10}	T2 + Pendirnethalin @ 1.5 ltr a.i. ha^{-1}	10.84	14.59	19.10	28.61	31.58
T_{11}	T2 + Pendirnethalin @2.25 ltr a.i. ha^{-1}	9.27	13.92	18.94	25.40	27.73
Tl2	T2 + Alachlor @ 1 ltr a.i. ha^{-1}	11.79	15.50	20.82	29.98	33.10
Tl3	T_2 + Alachlor @ 2 ltr a.i. ha^{-1}	10.93	14.71	20.10	28.68	32.46
Tl4	T_2 + Alachlor @ 3 ltr a.i. ha^{-1}	9.89	14.10	19.69	25.84	28.35
CD	(5%)	NS	NS	NS	3.05	2.20

* All the pesticides applied in soil

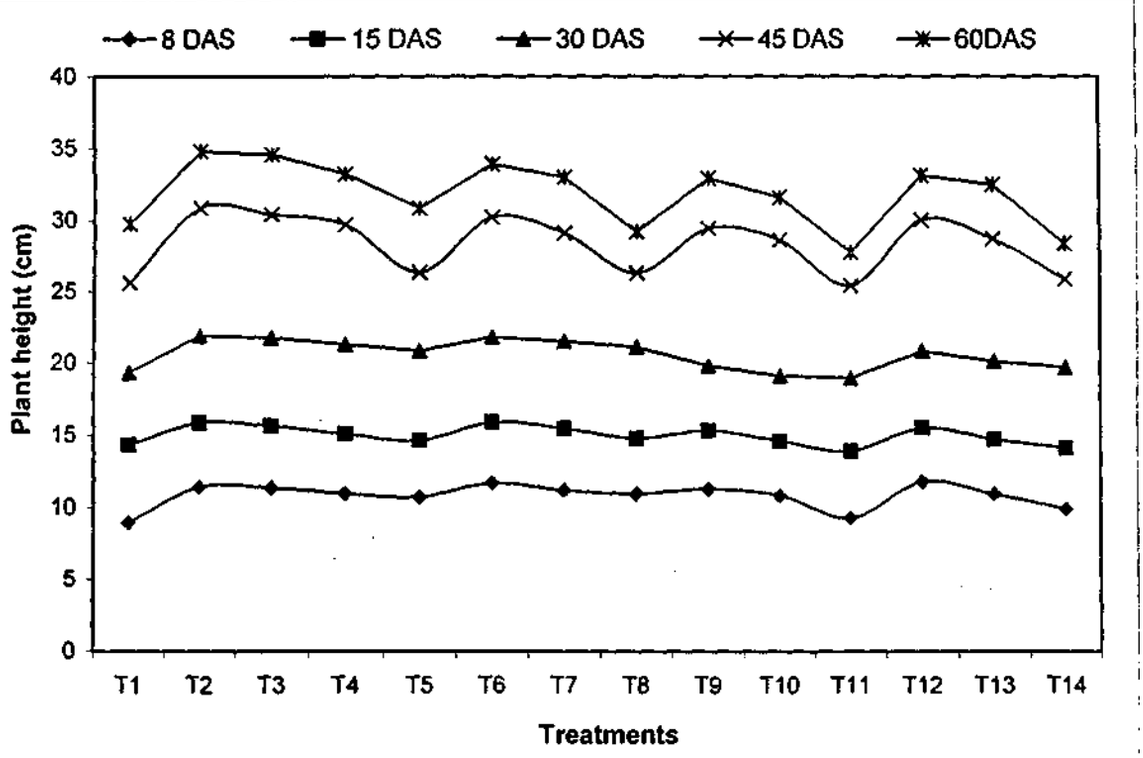


Fig.5 : Effect of different doses of pesticides on height of growth room grown chickpea under sterilised soil conditions

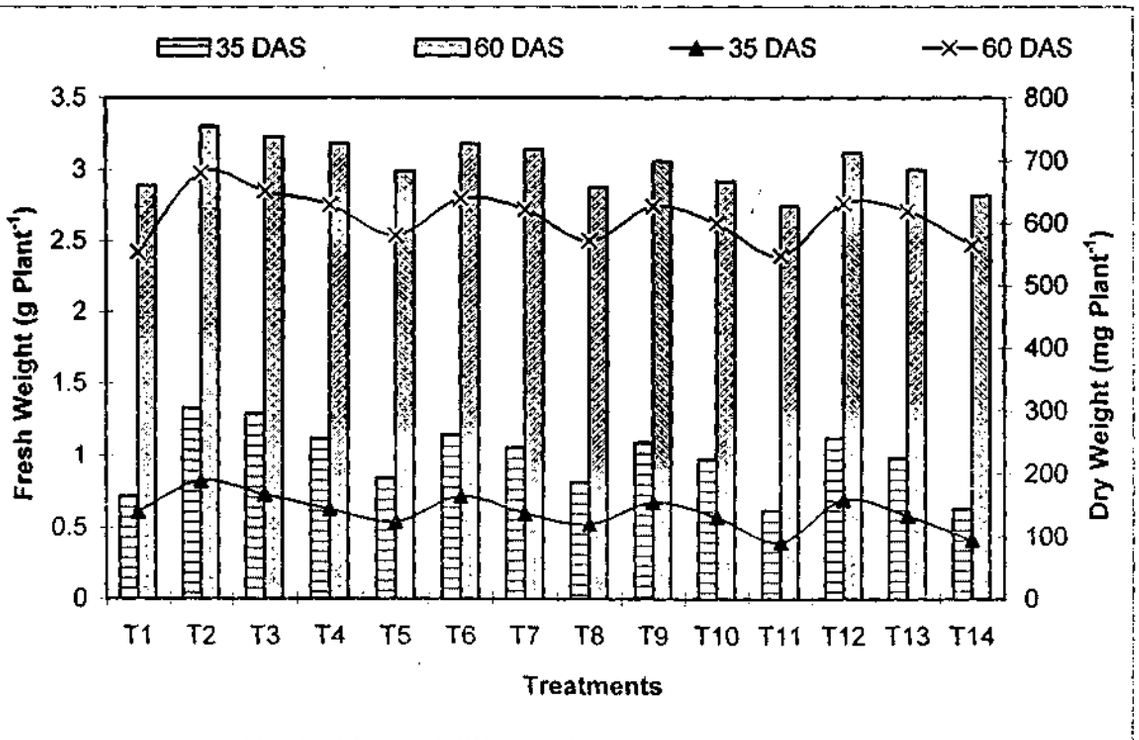


Fig. 6 : Effect of different doses of pesticides on bio-mass accumulation by growth room grown chickpea under sterilised soil conditions

decrease the plant height significantly, whereas low doses of pesticide T_3, T_6, T_9 and T_{12} increased plant height at 45 and 60 DAS.

b. Effect of different treatments on fresh and dry weight of chickpea shoot

Effect of different doses of pesticides on fresh weight of chickpea shoot is presented in Table 8 and showed Fig. 6.

The data presented in Table 8 revealed that the fresh weight of chickpea at 35 and 60 DAS influenced significantly by different pesticides ant their doses of application. The significantly higher fresh weight of chickpea shoot recorded under treatment T2 followed by treatment T_3, *Tg,* T4 and T_7, and found at par with each other at 35 DAS. The significantly less fresh weight of chickpea shoot observed under treatment T_{11}. Treatment T2gave highest fresh weight at 60 DAS also. Other treatments such as T_3, T4, T_6, T_7, T_9, T_{12} and T2 +Soil application with T_{13} also recorded comparable fresh weight of chickpea shoot with former treatment T2. The significantly less fresh weight of chickpea shoot recorded under treatment T_{11}.

Table 8 further revealed that higher concentration of different pesticides T_5, T_8, T_{14} and Tn negatively affected the fresh weight of chickpea at 45 DAS. Similar, trend was also observed in 60DAS.

Table: 8 Effect of different doses of pesticides on bio-mass accumulation by growth room grown chickpea under sterilised soil conditions

S. No.	Treatments	Plant Fresh Weight (g plant^{-1})		Plant Dry Weight (mg plant^{-1})	
		35 DAS	6O DAS	35 DAS	60 DAS
T_1	Control (Uninoculated)	0.723	2.89	140	554
T_2	Inoculated with *Rhizobium*	1.327	3.302	189	681
T_3	T_2+ Metasystox @ 1 ltr ha^{-1}	1.286	3.23	167	652
T_4	T2 + Metasystox @ 2 ltr ha^{-1}	1.119	3.18	144	630
T_5	T2+ Metasystox @ 3 ltr ha^{-1}	0.845	2.99	124	581
T_6	T2 + Chloropyrifos @ 1ltr ha^{-1}	1.142	3.18	164	640
T_7	T2 ++ Chloropyrifos @ 2 ltr ha^{-1}	1.057	3.14	137	623
T_8	T_2 + Chloropyrifos @ 3 ltr ha^{-1}	0.817	2.88	120	572
T_9	T2 + PendimethaUn @ 0.75 ltr a.i. ha^{-1}	1.092	3.06	155	628
T_{10}	T_2 + PendimethaUn @ 15 ltr a.i. ha^{-1}	0.975	2.92	131	600
T_{11}	T2 + Pendimethalin @ 2.25 ltr a.i. ha^{-1}	0.621	2.75	91	548
T12	T_2 + Alachlor @ 1 ltr a.i. ha^{-1}	1.123	3.12	159	632
T13	T_2 + Alachlor @ 2 ltr a.i. ha^{-1}	0.983	3.00	133	620
T14	T_2 + Alachlor @ 3 ltr a.i. ha^{-1}	0.634	2.82	95	565
CD	(5%)	0.28	0.16	20	48

All the pesticides applied in soil

The dry weight of shoot showed the significant variation by the different pesticides and their dose of application at *35* and 60 DAS. The significantly maximum dry weight of chickpea shoot observed under treatment T2 ver other treatments at *35* DAS (Table 8). The significantly less dry weight of chickpea shoot was observed under treatment T_{11}. At 60 DAS, treatment T2 also produced significantly higher dry weight of chickpea shoot than other treatments. The significantly less dry weight of chickpea shoot noted under treatment Tn at 60 DAS.

Data presented in Table 8 indicated that dry weight was reduced with application of high doses of different pesticides (T_5, T_8, Tn and T_{14}). While, lower doses of different pesticides (T_3, T_6, T_{12} and T_9) enhanced the dry weight of chickpea shoot.

c. Number of nodules and *Rhizobium* population

Data presented in Table 9 and Fig. 7 based on their effect on number of nodules per plant and *Rhizobium* population of chickpea.

The number of nodules was counted at *35* and 60 DAS. The data showed in Table 9 revealed that the significantly highest nodules number were observed under treatment T2 at 35 DAS, the remaining treatments T_3, T4, T_6, T_9 and T_{12} produced comparable number of nodules and significantly superior than remaining treatments. The similar trend was observed at 60 DAS. The control treatment T_1 produced no nodules in chickpea while, treatment Tn

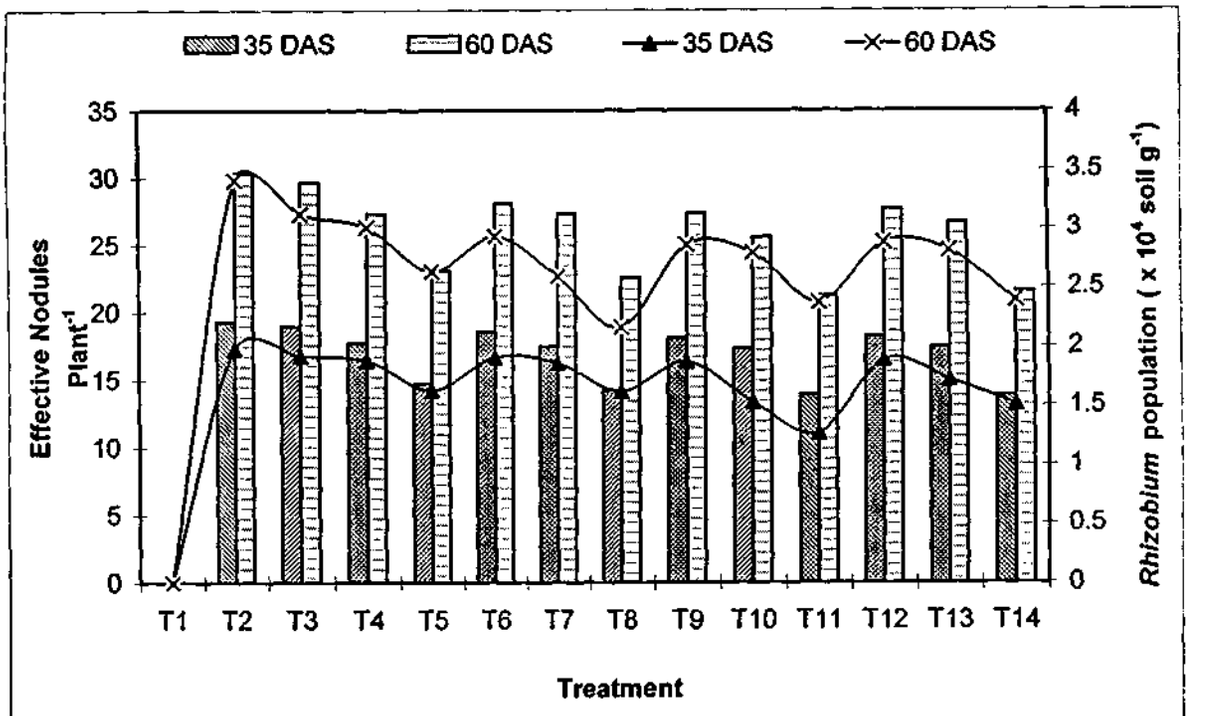


Fig. 7 : EEffect of different doses of pesticides on *Rhizobium* dynamics and nodulation in growth room grown chickpea under sterilised soil conditions

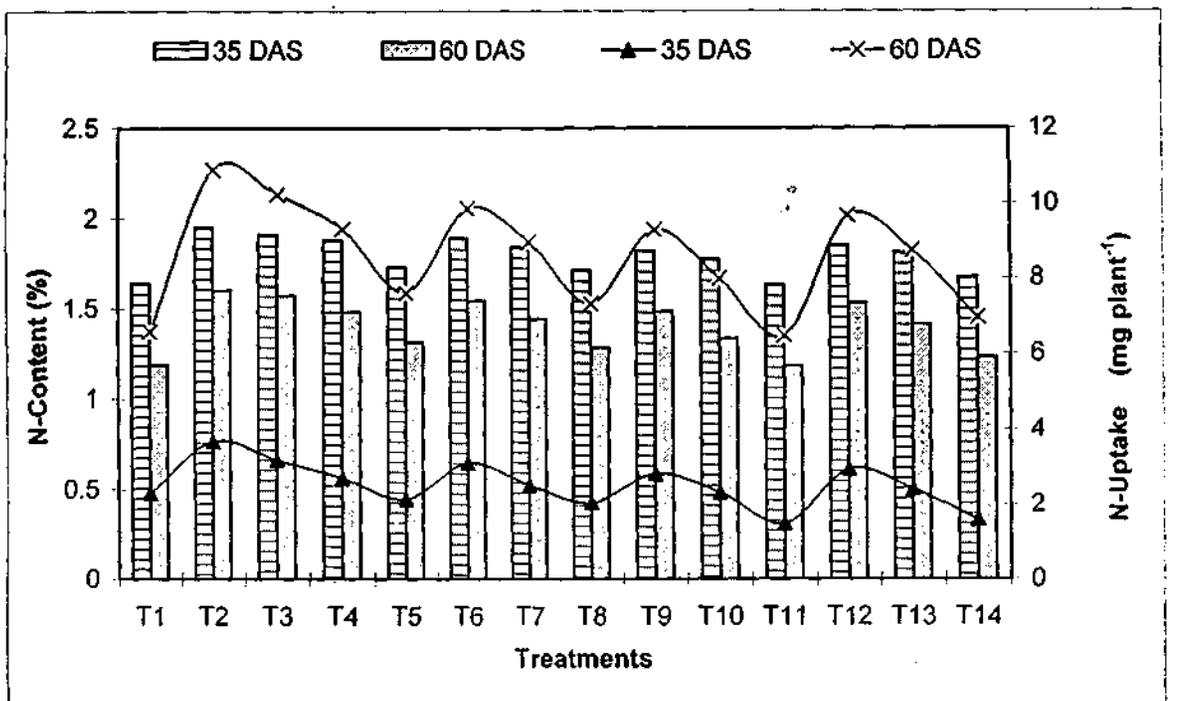


Fig. 8 : Effect of different doses of pesticides on nitrogen accumulation by growth room grown chickpea under sterilised soil conditions

significantly less number of nodules in chickpea at 35 and 60 DAS.

Table 9 indicated that high level of different pesticides T_5, T_8, T_{14} and T_{11} inhibited the number of nodules per plant of chickpea at 35 DAS. However, low level of different pesticides T_3, T_6, T_{12} and T_9 increased the number of nodules as compared to high level. Similar trend was also observed at 60 DAS.

Rhizobium population under different treatments were counted at 35 DAS and 60 DAS and presented in Table 9 and illustrated in Fig. 7 The result showed that the significantly higher *Rhizobium* population were observed under treatment T2. The treatments T_3 and T4 were found at par with each other. While, the minimum count of *Rhizobium* population noted under treatment T_{11}. At 60 DAS, the significantly maximum *Rhizobium* population was observed under treatment T2. Whereas treatment T3 and T4 found to be at par with the other treatments and significantly superior over rest of the treatments. No *Rhizobium* population was observed under treatment T_1.

Table 9 further revealed that *Rhizobium* population reduced by higher doses of pesticides (T_5, T_8, T_{11} and T_{14}) decreased the *Rhizobium* population however, lower doses of pesticides (T_3, T_6, T_{12} and T_9) increased the *Rhizobium* population but it was lesser from T_2.

Table: 9 Effect of different doses of pesticides on *Rhizobium* dynamics and nodulation in growth room grown chickpea under sterilised soil conditions

S. No.	Treatments	Effective Nodules Plant^{-1}		Rhizobium population (x 10^4 soil g^{-1})	
		35 DAS	6O DAS	35 DAS	60 DAS
T_1	Control (Uninoculated)	0.00	0.00	0.00	0.00
T_2	Inoculated with *Rhizobium*	19.33	30.33	1.98	3.41
T_3	T2+ Metasysyox @ 1 ltr ha^{-1}	19.00	29.67	1.92	3.12
T4	T2 + Metasysyox @ 2 ltr ha^{-1}	17.78	27.33	1.88	3.01
T_5	T2+ Metasysyox @ 3 ltr ha^{-1}	14.78	23.11	1.63	2.63
T_6	T2 + Chloropyrifos @ 1ltr ha^{-1}	18.56	28.11	1.91	2.93
T_7	T2 ++ Chloropyrifos @ 2 ltr ha^{-1}	17.50	27.33	1.86	2.59
	T2 + Chloropyrifos @ 3 ltr ha^{-1}	14.22	22.56	1.62	2.16
T_9	T2+Pendimethalin@ 0.75 ltr a.i. ha^{-1}	18.11	27.33	1.87	2.86
	T_2 + Pendimethalin @ 1.5 ltr a.i. ha^{-1}	17.33	25.56	1.53	2.79
T_{11}	T2 + Pendimethalin @ 2.25 ltr a.i. ha^{-1}	13.89	21.22	1.27	2.37
T_{12}	T2 + Alachlor @ 1 ltr a.i. ha^{-1}	18.22	27.67	1.89	2.88
T_{13}	T_2 + Alachlor @ 2 ltr a.i. ha^{-1}	17.44	26.67	1.72	2.81
T_{14}	T_2 + Alachlor @ 3 ltr a.i. ha^{-1}	13.89	21.56	1.51	2.39
CD	(5%)	1.99	2.05	0.16	0.12

All the pesticides applied in soil

d. N-content and uptake as influenced by different treatments

Data were recorded and presented in Table 10 to assess the effect of different doses of pesticides on N-content and uptake at 35 DAS and 60 DAS. Fig. 8 is the graphical representation of data recorded for N- content and uptake.

It is easy to conclude from the table that maximum N-content was recorded with. T2 inoculated with *Rhizobium*. Other treatments such as T_3, T4 , T_6 and T_7 were also given comparable results and at par with each other at 35 DAS. Minimum N-content was recorded with T_{11}.

Statistical analysis as presented in Table 10 showed that at 60 DAS, T2 was found to be superior over other treatments as it gave highest N-content followed by T_3, T4 and T_6. However, minimum N-content was observed with T_{11}.

III. Sterilized sand culture

The observations for plant height, fresh weight and dry weight of shoot, nodule number, *Rhizobium* population, N-content and uptake were taken under sterilized sand culture at successive stages of chickpea and presented in the following heads.

a. Effect of different pesticide doses on plant height of chickpea

Observations were taken at the different stages of study *viz.*, *8*, 15, 30, 45, and 60 DAS. Data observed for plant height of chickpea considering the effect of different doses of pesticides

Table: 10 Effect of different doses of pesticides on nitrogen accumulation by growth room grown chickpea under sterilised soil conditions

S. No.	Treatments	N-Content (%)		N-Uptake (mg plant^{-1})	
		35 DAS	60 DAS	35 DAS	60 DAS
T_1	Control (Uninoculated)	1.64	1.19	2.30	6.59
T_2	Inoculated with *Rhizobium*	1.95	1.6	3.69	10.90
T_3	T2+ Metasysyox @ 1 ltr ha^{-1}	1.91	1.57	3.19	10.24
T_4	T2 + Metasysyox @ 2 ltr ha^{-1}	1.88	1.48	2.71	9.32
T_5	T2+ Metasysyox @ 3 ltr ha^{-1}	1.73	1.31	2.15	7.61
T_6	T2 + Chloropyrifos@ 1ltr ha^{-1}	1.89	1.54	3.10	9.86
T_7	T2 ++ Chloropyrifos @ 2 ltr ha^{-1}	1.84	1.44	2.52	8.97
T_8	T_2 + Chloropyrifos @ 3 ltr ha^{-1}	1.71	1.28	2.05	7.32
T_9	T_2 + Pendimethalin @ 0.75 ltr a.i. ha^{-1}	1.82	1.48	2.82	9.29
T_{10}	T_2 + Pendimethalin @ 1.5 ltr a.i. ha^{-1}	1.77	1.33	2.32	7.98
T_{11}	T_2 + Pendimethalin @ 2.25 ltr a.i. ha^{-1}	1.63	1.18	1.48	6.47
T_{12}	T_2 + Alachlor @ 1 ltr a.i. ha^{-1}	1.85	1.53	2.94	9.67
T_{13}	T_2 + Alachlor @ 2 ltr a.i. ha^{-1}	1.81	1.41	2.41	8.74
T_{14}	T_2 + Alachlor @ 3 ltr a.i. ha^{-1}	1.67	1.23	1.59	6.95
CD	(5%)	0.12	0.15	0.79	1.56

All the pesticides applied in soil

under sterilized sand culture are presented in Table 11 and shown graphically in Fig. 9.

It is easy to understand from the table that plant height of chickpea under different treatments was found statistically non-significant at 8, 15 and 30 DAS. Observation taken at 45 DAS revealed that maximum plant height was recorded with T2 followed by T_3 and T_6 while, minimum plant height was recorded with T_{11}.

Similar trend was observed at 60 DAS, as maximum plant height was recorded with T2. Other treatment such as T_3, T_6, T4 and T_7 were found to be at par with T2. Minimum plant height was recorded with T_{11}.

It may be concluded from observations that high doses of pesticides T_5, T_8, Tn and T_{14} were caused to decrease the plant height significantly, whereas low doses of pesticide T_3, T_6, T_9 and T_{12} increased plant height at 45 and 60 DAS.

b. Fresh weight and dry weight.

Data recorded for plant fresh weight influenced by different concentrations of pesticides are given in Table 12 and showed in Fig. 10.

It is clear from the table that at 35 DAS the treatment T2 significantly increased fresh weight, however other treatments such as T_3, T4 and T_6 were found to be at par with T_2. Lowest fresh weight was observed with Tn Observation recorded at 60 DAS revealed that the maximum height was observed with T_2 followed by T_3 and minimum was recorded with treatment T_{11}.

Table 11 Effect of different doses of pesticides on height of growth room grown chickpea under sterilised sand culture conditions

S. No.	Treatments	Plant height (cm)				
		8 DAS	15 DAS	30 DAS	45 DAS	60 DAS
T_1	Control (Uninoculated)	5.12	9.36	14.93	20.00	23.10
T_2	Inoculated with *Rhizobium*)	7.20	11.67	16.15	24.95	29.50
T_3	T2+ Metasystox @ 2.5 ml kg^{-1} seed	6.50	11.50	16.00	23.76	28.75
T_4	T2 + Metasystox @ 5 ml kg^{-1} seed	6.00	11.18	15.80	23.50	27.60
T_5	T_2+ Metasystox @ 7.5 ml kg^{-1} seed	5.87	10.75	15.20	21.00	24.10
T_6	T_2 + Chloropyrifos @ 2.5 ml kg^{-1} seed	6.38	10.93	15.90	23.10	27.98
T_7	T_2 +Chloropyrifos @ 5 ml kg^{-1} seed	5.92	10.50	15.35	22.10	27.10
Tg	T_2 + Chloropyrifos @ 7.5 ml kg^{-1} seed	5.20	9.86	14.92	19.00	23.67
T_9	T_2 + Pendimethalin @ 5 ml kg^{-1} seed	5.15	8.00	14.90	21.60	25.50
T_{10}	T_2 + Pendimethalin @ 10 ml kg^{-1} seed	4.70	7.61	14.30	18.50	24.30
T_{11}	T_2 + Pendimethalin @ 15 ml kg^{-1} seed	4.10	6.90	13.86	15.10	20.25
T_{12}	T_2 + Alachlor @ 5 ml kg^{-1} seed	5.00	8.,54	15.40	22.60	26.70
T_{13}	T_2 + Alachlor @ 10 ml kg^{-1}seed	5.,10	8,,00	14.50	19.36	25.10
T14	T_2 + Alachlor @ 15 ml kg^{-1} seed	4.86	7.,63	13.75	16.10	22.00
CD	(5%)	NS	NS	NS	3.27	2.65

* In all the treatments seeds treated with pesticides

Table: 12 Effect of different doses of pesticides on bio-mass accumulation by growth room grown chickpea under sterilised sand culture conditions

S. No.	Treatments	Plant Fresh Weight (g plant^{-1})		Plant Dry Weight (mg plant^{-1})	
		35 DAS	6O DAS	35 DAS	60 DAS
T_1	Control (Uninoculated)	0.345	2.76	45	540
T_2	Inoculated with *Rhizobium*	0.892	3.43	132	669
T_3	T2+ Metasystox @ 2.5 ml kg^{-1} seed	0.790	3.31	109	646
T_4	T2 + Metasystox @ 5 ml kg^{-1} seed	0.685	3.21	100	626
T_5	T2+ Metasystox @ 7.5 ml kg^{-1} seed	0.457	2.92	63	571
T_6	T2 + Chloropyrifos @ 2.5 ml kg^{-1} seed	0.737	3.22	107	629
T_7	T2 +Chloropyrifos @ 5 ml kg^{-1} seed	0.648	3.16	93	617
T_8	T_2 + Chloropyrifos @ 7.5 ml kg^{-1} seed	0.401	2.90	56	566
T_9	T2 + Pendimethalin @ 5 ml kg^{-1} seed	0.697	3.13	95	612
T_{10}	T2 + Pendimethalin @ 10 ml kg^{-1} seed	0.597	2.89	87	564
T_{11}	T_2 + Pendimethalin @ 15 ml kg^{-1} seed	0.276	2.75	37	537
T12	T_2 + Alachlor @ 5 ml kg^{-1} seed	0.710	3.16	104	618
T_{13}	T_2 + Alachlor @ 10 ml kg^{-1} seed	0.620	3.11	88	608
T14	T_2 + Alachlor @ 15 ml kg^{-1} seed	0.368	2.84	50	554
CD	(5%)	0.25	0.13	47	26

* In all the treatments seeds treated with pesticides

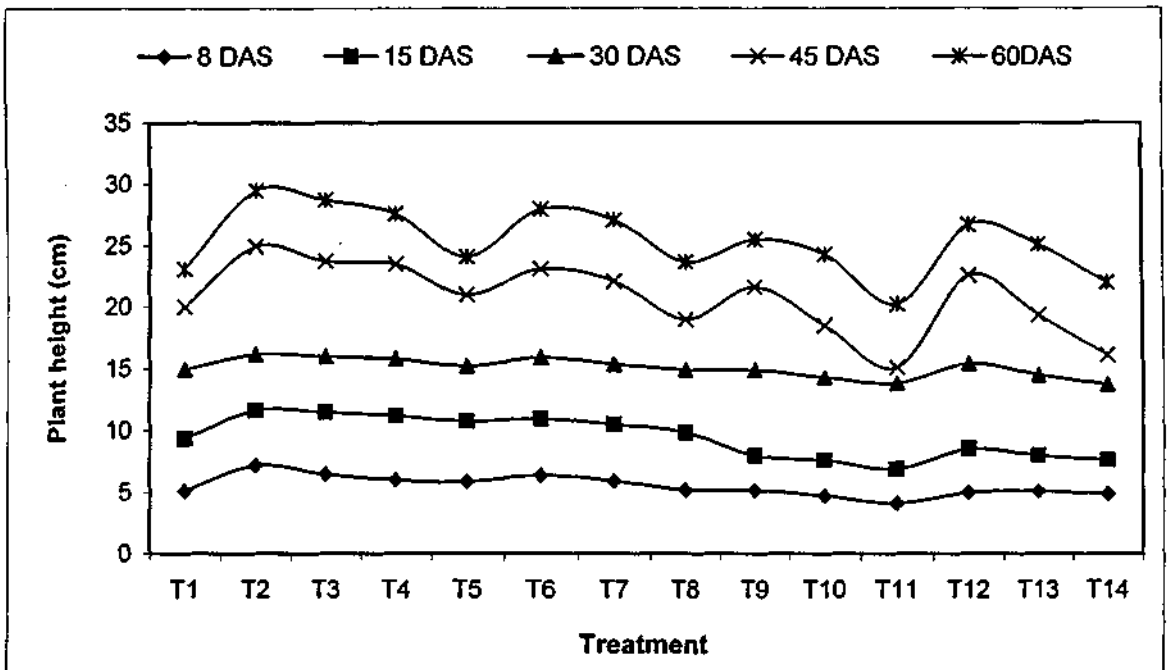


Fig. 9: Effect of different doses of pesticides on height of growth room grown chickpea under sterilised sand culture conditions

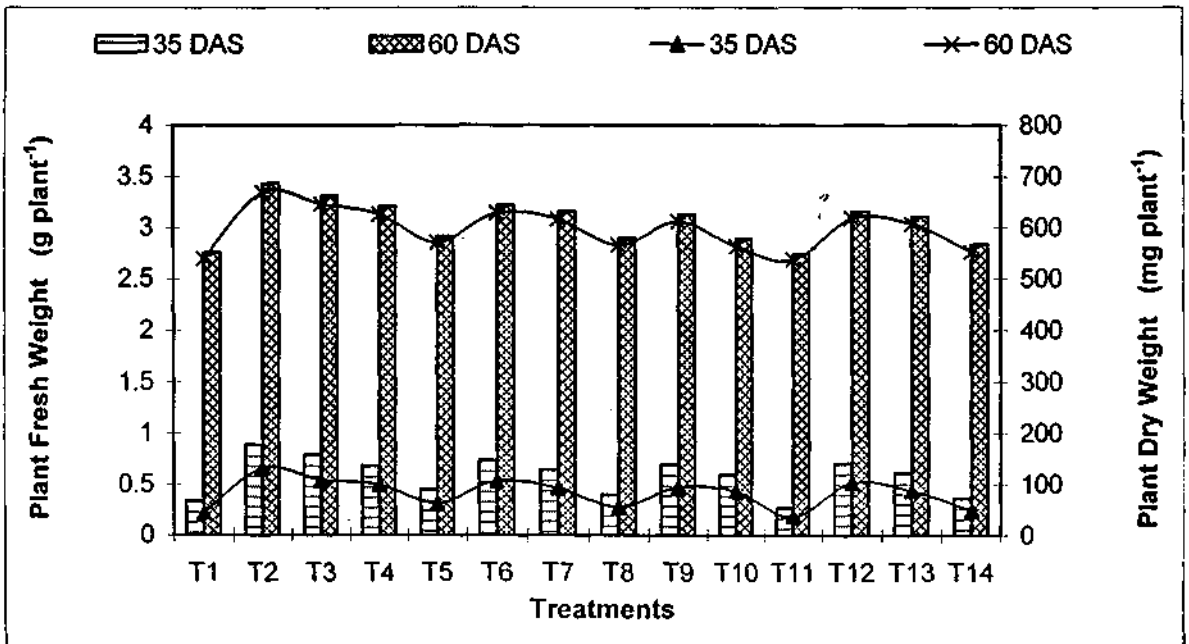


Fig. 10 : Effect of different doses of pesticides on bio-mass accumulation by growth room grown chickpea under sterilised sand culture conditions

Dry weight of chickpea plant was also recorded at 35 DAS and 60 DAS. Data were recorded at 35 DAS inferred that treatment T_2 significantly increased dry weight and found to be significantly superior over rest of the treatments. Lowest dry weight recorded with T_{11}. Similar trend was found at 60 DAS as maximum dry weight was recorded with T_2 and lowest dry weight observed with Tn.

There was a negative relationship found between weight of plant (Fresh and dry weight) and different high levels of pesticides T_5, T_8, Tn and T_{14} as fresh and dry weight increased with reduction in pesticides level, whereas fresh and dry weight decreased with high levels T_3, T_6, T_9 and T_{12} of pesticides.

c. Nodule number and *Rhizobium* population

Data observed at 35 DAS and 60 DAS for number of nodules and *Rhizobium* population of chickpea plant are given in Table 13 and graphically presented in Fig. 11.

Table 13 revealed that the maximum number of nodules was counted with T_2 followed by T_3, T_4, and T_{12} at 35 DAS. However, the lowest number of nodules per plant was counted with Tn.

Data recorded at 60 DAS inferred that maximum number of nodules was counted with T_2. Treatments T_3, T_6 and T4 were also produced satisfactory results and statistically at par with each other. The lowest number of nodules was counted with Tn.

Table: 13 Effect of different doses of pesticides on *Rhizobium* dynamics and nodulation in growth room grown chickpea under sterilised sand culture conditions

S. No.	Treatments	Effective Nodules Plant^{-1}		Rhizobium population (x 10^4 soil g^{-1})	
		35 DAS	60 DAS	35 DAS	60 DAS
T_1	Control (Uninoculated)	0.00	0.00	0.00	0.00
T_2	Inoculated with *Rhizobium*	9.78	14.00	1.89	2.59
T_3	T2+Metasystox@2.5ml kg^{-1} seed	8.33	11.89	1.60	2.52
T_4	T2 + Metasystox@5ml kg^{-1}seed	7.78	11.00	1.51	2.41
T_5	T2+Metasystox@7.5ml kg^{-1}seed	6.22	9.11	1.38	2.24
T_6	T2 + Chloropyrifos @ 2.5 ml kg^{-1} seed	8.00	11.44	1.55	2.48
T_7	T2 +Chloropyrifos@ 5 ml kg^{-1}seed	7.33	10.67	1.48	2.38
T_8	T_2 + Chloropyrifos @ 7.5 ml kg^{-1}seed	6.00	9.00	1.32	2.12
T_9	T2 + Pendimethalin@5 ml kg^{-1}seed	7.78	1Q.00	1.40	2.22
T_{10}	T2 + Pendimethalin@10 ml kg^{-1}seed	6.87	8.78	1.31	2.28
T_{11}	T_2 + Pendimethalin@15 ml kg^{-1}seed	5.11	6.22	1.20	1.26
TI2	T_2 + Alachlor@5 ml kg-^{1}seed	7.89	10.89	1.49	2.29
	T_2 + Alachlor @ 10 ml kg^{-1} seed	6.89	9.56	1.38	2.30
T_{14}	T_2 + Alachlor@15 ml kg^{-1}seed	5.22	7.11	1.23	1.89
CD		1.50	1.01	0.15	0.16

In all the treatments seeds treated with pesticides

Observation taken for *Rhizobium* population at 35 DAS showed that T2 was found to be best over other treatment. T_2 gave highest *Rhizobium* population over rest of the treatments, whereas, the lowest *Rhizobium* population was observed with T_{11}. Similar trend was also recorded for *Rhizobium* population at 60 DAS as T2 gave highest *Rhizobium* population and found to be superior among all other treatments.

The maximum doses of pesticides *i.e.* T_5, T_8, Tn and were recorded minimum number of nodules and *Rhizobium* population as compared to lower doses of each pesticide *i.e.* T_3, T_6, T_9 and T_{12} at 45 DAS. Similar trends were also recorded at 60 DAS.

d. N-content and uptake

Data related to N-content and uptake are illustrated in Table 14 and presented graphically in Fig. 12.

It is easy to conclude from the Table 14 that treatment T2 recorded highest N-content and it was statistically at par with T_3, T_6 and T4 at 35 DAS, whereas, minimum N-content was observed with Tn.

From the data given in Table 14, it was inferred that N-content recorded with T2 was highest among other treatments followed by T_3, T_6 and T_4 at 60 DAS. The lowest N-content was recorded with Tn.

Table: 14 Effect of different doses of pesticides on nitrogen accumulation by growth room grown chickpea under sterilised sand conditions

S. No.	Treatments	N-Content (%)		N-Uptake (mg plant^{-1})	
		35 DAS	60 DAS	35 DAS	60 DAS
T_1	Control (Uninoculated)	1.00	0.85	0.45	4.59
T_2	Inoculated with *Rhizobium*	1.69	1.36	2.23	9.09
T_3	T2+Metasystox@2.5ml kg^{-1}seed	1.67	1.33	1.82	8.59
T_4	T2 + Metasystox @ 5 ml kg^{-1} seed	1.64	1.21	1.64	7.57
T_5	T2+Metasystox@7.5ml kg^{-1}seed	1.49	1.07	0.94	6.11
T_6	T2 + Chloropyrifos@ 2.5 ml kg^{-1}seed	1.66	1.31	1.78	8.22
T_7	T2 +Chloropyrifos @ 5 ml kg^{-1}seed	1.59	1.18	1.48	7.28
T_8	T2 + Chloropyrifos @ 7.5 ml kg^{-1}seed	1.45	1.03	0.81	5.83
T_9	T2 + Pendimethalin@5ml kg^{-1}seed	1.57	1.24	1.49	7.59
T_{10}	T2+Pendimethalin@10 ml kg^{-1}seed	1.53	1.08	1.33	6.09
T_{11}	T_2 + Pendimethalin @ 15 ml kg^{-1}seed	1.38	0.94	0.51	5.05
T_{12}	T2 + Alachlor @ 5 ml kg^{-1} seed	1.60	1.27	1.66	7.85
T13	T2 + Alachlor @ 10ml kg^{-1} seed	1.56	1.16	1.37	7.05
T14	T_2 + Alachlor @ 15 ml kg^{-1} seed	1.47	0.97	0.73	5.37
CD	(5%)	0.08	0.12	0.63	1.03

* In all the treatments seeds treated with pesticides

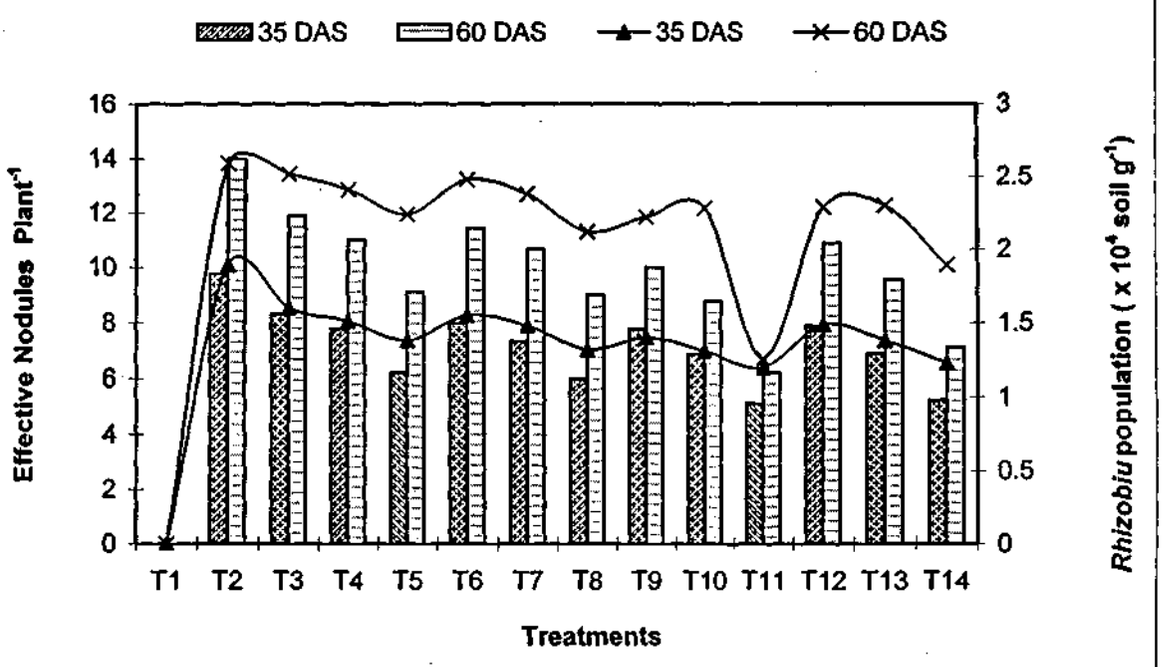


Fig. 11: Effect of different doses of pesticides on *Rhizobium* dynamics and nodulation in growth room grown chickpea under sterilised sand culture conditions

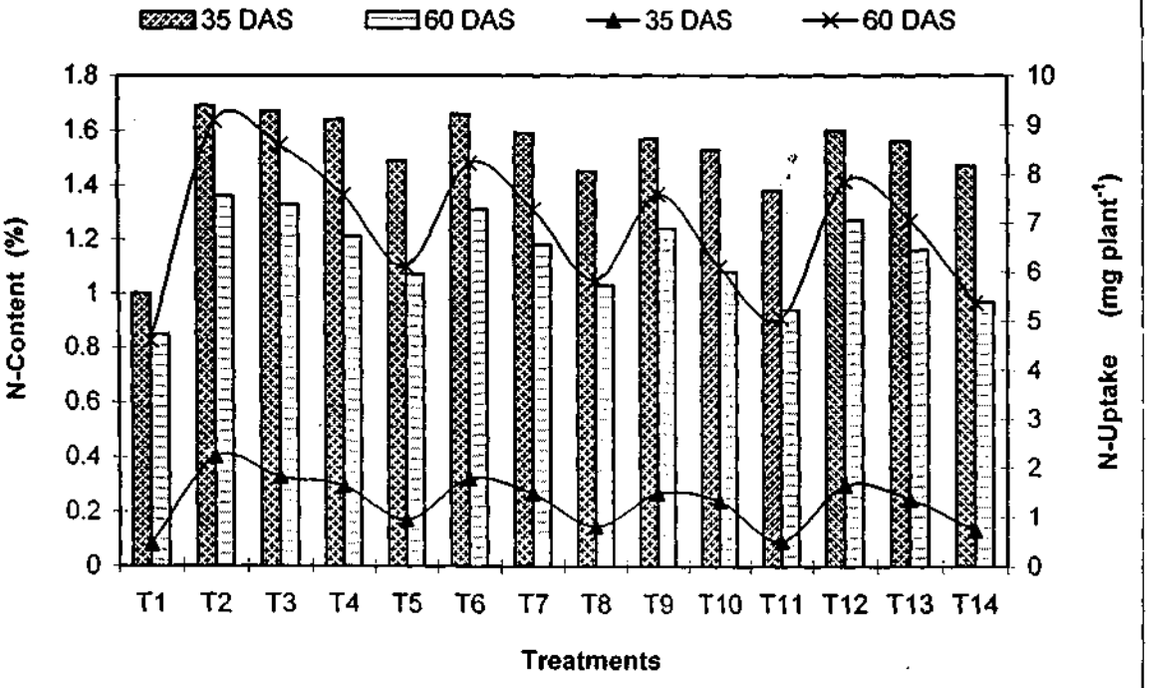
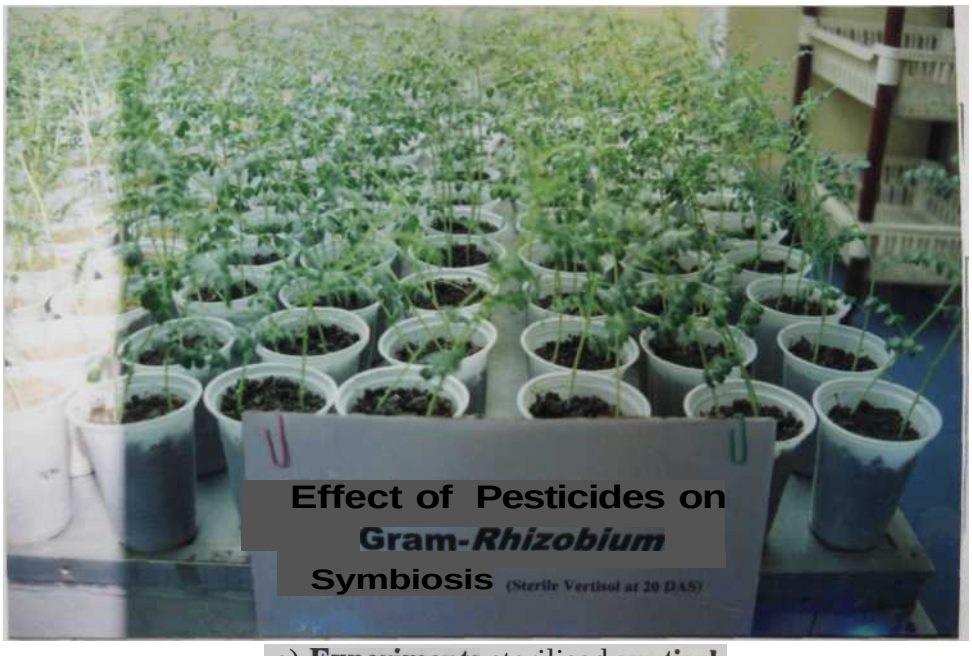


Fig. 12 : Effect of different doses of pesticides on nitrogen accumulation by growth room grown chickpea under sterilised sand culture conditions

Data revealed that treatment T_2 gave best results for N-uptake in chickpea plant and significantly superior over other treatments at 35 DAS. Treatments T_6, T_3, T4 gave comparable N-uptake in chickpea plant. Minimum N-uptake was recorded with T_{11}.

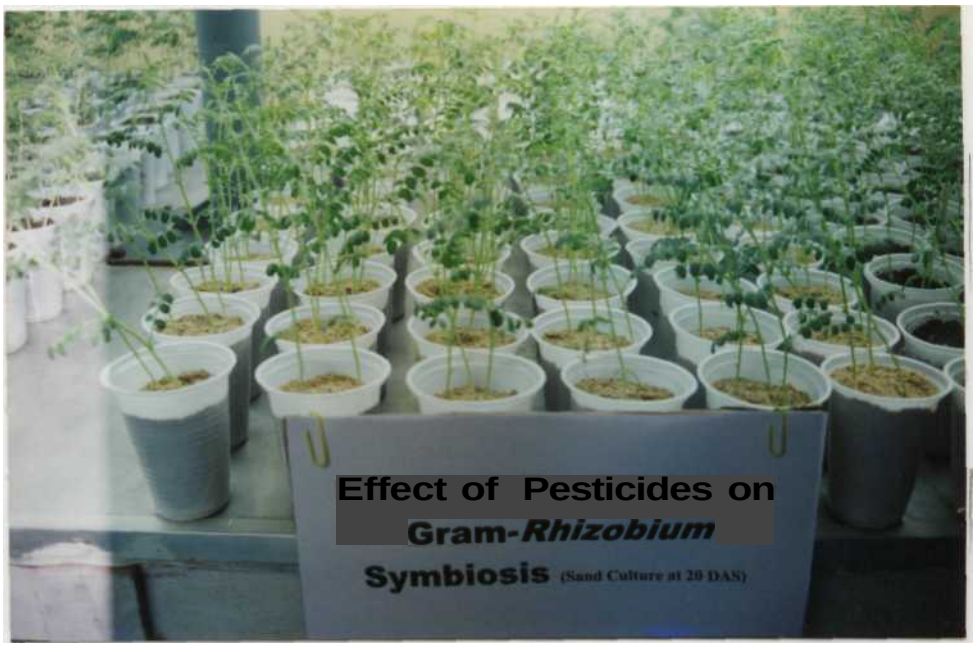
Results obtained at 60 DAS were inferred that T_2 gave maximum N-uptake followed by T_3, T_6 and T_4. Minimum N-uptake was recorded with Tn.

Plate 1: General view of growth room experiments under sterilised and **unsterilised** condition



a) **Experiments** sterilised **vertisol**

b) Experiment unsterilised vertisol



C. Experiment under sterilised sand culture devoid of mineral nitrogen source

Plate 2: General view of growth room experiments

Discussion

CHAPTER-V

DISCUSSION

Because of diversified use of Chickpea *(Cicer arietinum* L.) and its ability to grow better with low input under harsh edaphic and arid environment than many other crops, it is an important component of the cropping system of sustainable farming in the Indian subcontinent. Some of these attributes together with its ability to derive more than 70 per cent of its nitrogen from symbiotic nitrogen fixation makes, chickpea a promising crop for the sustainable agriculture. On the other hand many pesticides are being used for cultivation of gram crop without knowing their effect on crop beneficial microbes like *Rhizobium* . This is one of the reasons of reducing soil productivity per unit area of the country. Therefore, this study is planned in order to select suitable/ compatible doses of pesticides on the basis of their cumulative effect on Gram-*Rhizobium* symbiosis. The results of present investigation are discussed below:

There is sufficient evidence of influence of different pesticide and their doses on *Rhizobium* dynamics, nodulation, growth and N-

uptake in chickpea (Pandey and Rai 1996, Dastgheib *et al.*, 1995 Madhavi *et al,* 1994, Singh *et al,* 1994, Pahwa and Prakash, 1992, Prabhakaran and Ramasamy, 1990 and Kundu and Trimohan, 1989). Higher doses of pesticides show inhibitory effects (Trivedi *et al,* 1983), reduce seed germination and seedling development (Bangar and Puri, 1981), reduce nodulation (Clarkson *et al.* 1982) or inhibit the nitrogenase activity (Chandra and Mathur, 1986).

Keeping in view of above, the present investigation was carried out to find out the effect of different pesticides on chickpea growth, nodulation, N-uptake and *Rhizobium* population by using unsterilised/sterilised Vertisols. Sand culture experiment devoid of mineral nitrogen source was also conducted under growth room conditions to achieve the objectives of the present investigation

I Unsterilised and sterilised Vertisol

a. Growth behavior

The plant height of chickpea influenced by various doses of pesticides under both unsterilised and sterilised conditions of Vertisol. However, there was no significant effect on plant height of

chickpea at early stages of crop growth. But at later stages, height of chickpea influenced significantly due to adverse effect of pesticides oninoculated homologous *Rhizobium*. At 45 and 60 DAS the tallest plant was recorded under treatment T2 *(Rhizobium* inoculation) among other treatments containing with and without *Rhizobium* + pesticides. While the smallest plant was observed under treatment T_{11} (T_2+ Soil application with pendimethalin @ 2.25 ai ltr ha^{-1}) followed by T8 (T_2+ Soil application with chloropyrfos @ 3 ltr

T_2 + Soil application with Alachlor @ 3 ltr a.i. ha^{-1}). Plant height of chickpea decreased significantly at higher doses of pesticides as mentioned in Table 3, 7 and Fig. 1, 5. Similarly, fresh weight and dry weight also reduced significantly due to application of higher doses of pesticides (Table 4,8 and Fig. 2, 6). These results are strongly supported by Pahwa and Prakash (1992). They reported that dry weight of leaves, stem and root per plant were significantly reduced at higher concentration of herbicides. Similar findings were also reported by Kundu and Trimohan (1989), Nova *et al.* (1998) and Amrante *et al.* (1992).

Up to 60 DAS, better general performance of crop was observed under sterilised soil conditions over unsterilised conditions, it may be

because of many reasons like better nutrient availability due to autoclaving of soil including better opportunity for working of inoculated homologous root nodule bacteria of chickpea.

b. Nodulation and N-accumulation

Similar to plant height, nodulation and N-accumulation also adversely affected due to application of different doses of pesticides. *The* higher doses of pesticides significantly decreased the number of nodules, per cent N-content and its uptake in chickpea at 35 and 60 DAS (Table 5, 6, 9,10 and Fig. 3, 4, *7*, 8). The highest nodulation and N-accmulation were observed with treatment T_2 (*Rhizobium* inoculation). It was clearly indicated that the pesticides application significantly reduced effective nodulation. Number of nodules per plant under treatment T2 was 16.78 at 35 DAS and 27.11 at 60 DAS. It was concluded from these observations that the applied doses of pesticides directly or indirectly affecting adversely on chickpea-*Rhizobium* symbiosis. These observation is in line with that of Honzalez *et al.*, 1996, Silava, *et al.*, 1998, Singh, *et al.*, 1997, Miettinen and Echegoyen, 1996, Singh, *et al,* 1991, Ebrbach and Donglas, 1989, Pawha and Prakash, 1992, Glover and Schapangh, 1997 and Vaishva *et al.*, 1995. They clearly mentioned about adverse

effect of higher concentration of pesticides on working of legume root nodule bacteria.

Similarly data presented in Table 9, 10 and Fig. 7, 8 related to chickpea grown under sterilised Vertisol conditions, clearly supported findinds of unsterilised Vertisol conditions These results are in conformity with the results of Loc and Loc (1993), Madhavi *et al.*(1994) and Jan *et al.* (1994).

c. Rhizobial dynamics study

At 35 DAS and 60 DAS, data of *Rhizobium* dynamics (Table 5 and Fig. 3) revealed that *Rhizobium* population density was highest in treatment T2 *(Rhizobium* inoculation) among all the treatments under the study because of adverse effect of applied doses of pesticides on multiplication and working *Rhizobium.* Similar results were also reported by Koopman *et al.* (1993), and Zawoznik *et al.* (1995). Both unsterilised and sterilised soil conditions, population density of *Rhizobium* increased significantly from 35 to 60 DAS due to higher rhizosphere effect at 60 DAS than at 35 DAS (Subba Rao, 1988).

II Sterilised sand

Under sterilised sand culture devoid of mineral nitrogen source conditions, the observations related to growth behavior (El-Masry *et al.;*1995, Trotus and Ghizdavu; 1996 and Amarante, 1995), nodulation, accumulation of biomass and nitrogen including *Rhizobium* population density with respect to different stages of chickpea crop growth (Pandey and Rai ;1995, Zawoznik *et al.*1995and Loc and Nguyen, 1993), gave almost similar trend as obtained under unsterilised and sterilised Vertisol conditions (Table 11,12 and Fig. 9, 10).

At 60 DAS general performance of growth room grown chickpea crop DAS) was better under sterilised soil conditions over unsterilised conditions, it may be because many reasons like better nutrient availability due to autoclaving of soil including better opportunity for working of homologous inoculated root nodule bacteria in absence of other soil organisms. On the other hand the situation is reverse with sand culture experiment, it may also be due to so many reasons like lack of some growth promoting substances,

sub-optimal nitrogen nutrition etc. of chickpea plants grown under sand culture devoid of mineral nitrogen source.

Keeping in view of findings of present investigation, it can be concluded that pesticidal application should be minimized in order to improve working of root nodule bacteria of chickpea.

Summary, Conclusions & Suggestions for Future work

CHAPTER - VI

SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH WORK

The present investigation entitled "Detection of safe doses of different pesticides for chickpea *(Cicer arientinum* L.)-*Rhizobium* symbiosis" was carried out under sterilized and unsterilised Vertisol conditions. Sand culture experiment was also conducted by using Gram variety JG-11under growth room conditions. From these studies following conclusions were drawn:

1. With reference to plant height, it was concluded that higher doses of pesticides were found to suppress this character.
2. Fresh and dry weight of chickpea shoot was also adversely affected by higher doses of pesticides under all the conditions.
3. During the study, *Rhizobium* population density and nodulation decreased significantly with higher doses of pesticides.
4. Nitrogen accumulation in chickpea shoot increased with inoculation of seed with *Rhizobium* and decreased with higher concentration of different pesticides tested under the present investigation

The growth room study with unsterilised and sterilized Vertisol including sand culture devoid of mineral nitrogen indicated same conclusion that, with the pesticides application the growth behavior of chickpea, nodulation, N-uptake and *Rhizobium* population density decreases significantly.

SUGGESTIONS FOR FUTURE WORK

1. Study should be conducted to detect the safe doses of various pesticides for root nodule bacteria and other associated beneficial microbes of different legumes including chickpea in order to improve legume–Rhizobium symbiosis under field conditions especially.
2. The suitable/compatible doses of different pesticides should be tested at least for three years under rainfed regions like Chhattisgarh plains.
3. Keeping in view of very high cost pesticides, proper efforts should be made to determine economic and effective doses of pesticides.
4. Effort should also be made to detect MIC (Minimum inhibitory concentration) of different pesticides for different legume root nodule bacteria
5. Direct and indirect effects of different doses of pesticides on beneficial microbes like Rhizobium should be studied under mixed and inter cropping system of leguminous crops.

Abstract

"Detection of safe doses of different pesticides for chickpea (*Cicer arietinum* L.)-*Rhizobium* symbiosis"

By
Prashant Gupta

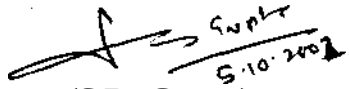
ABSTRACT

A set of experiments under sterilized and unsterilized conditions were conducted in Soil Microbiology Lab., Department of Soil Science, Indira Gandhi Agricultural University, Raipur (C.G.) during 2001-2002 to determine the suitable doses of different pesticides for effective chickpea - *Rhizobium* symbiosis. In this connection, experiments were conducted with chickpea cv. JG-11 by using unsterilized and sterilized Vertisol. Sand culture devoid of mineral N source was also conducted under growth room conditions to fulfill above mentioned objective of the present investigation.

Under both unsterilized and sterilized conditions, high concentrations of pesticides adversely affected the parameters of chickpea - *Rhizobium* symbiosis, like nodulation, nitrogen and biomass accumulation and *Rhizobium* population density. On the other hand, *Rhizobium* inoculation without pesticides was found to be beneficial,

Under all the experimental conditions medium doses of pesticides (Metasystox @ 2 ltr ha^{-1}, Chloropyrifos @ 2 ltr ha^{-1}, Alachlor @ 2 ltr a.i. ha^{-1} and Pendimethalin @ 1.5 ltr a.i. ha^{-1}) were found to be at par with the lower doses and safer (Metasystox @ 1 ltr ha^{-1}, Chloropyrifos @ 1 ltr ha^{-1}, Alachlor @ 1 ltr a.i. ha^{-1} and Pendimethalin @ 0.75 ltr a.i. ha^{-1}) in respect of chickpea - *Rhizobium* symbiosis. Herbicides (Pendimethalin and Alachlor) were found to be more harmful than insecticides (Metasystox and Chloropyrifos)

Higher concentration of Pendimethalin (@ 2.25 ltr a.i. ha^{-1}) was observed to be more toxic than others pesticides under study.



College of Agriculture, Raipur **(S.B. Gupta)**
Dat: 5-10-2002 Major Advisor & Chairman

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