

**MYCORRHIZAL EFFECT ON ROOT ROT
OF CHICKPEA CAUSED BY
*Rhizoctonia bataticola***

THESIS

**Submitted to the
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola
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**MASTER OF SCIENCE
IN
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(PLANT PATHOLOGY)**

By

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Enrolment Number - JJ-1862

2017

DECLARATION OF STUDENT

I hereby declare that the experimental work and its interpretation of the thesis entitled **“MYCORRHIZAL EFFECT ON ROOT ROT OF CHICKPEA CAUSED BY *Rhizoctonia bataticola*”** or part there of has neither been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis / publication of any University or Scientific Organization. The sources of material used and all assistance received during the course of investigation have been duly acknowledged.

Place : Akola.

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CERTIFICATE

This is to certify that the thesis entitled “**MYCORRHIZAL EFFECT ON ROOT ROT OF CHICKPEA CAUSED BY *Rhizoctonia bataticola***” submitted in partial fulfillment of the requirement for the degree of “**Master of Science in Agriculture (Plant Pathology)**” of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola is a record of bonafide research work carried out by **Uikey Darshana Wasudeo** under my guidance and supervision.

The subject of thesis has been approved by the Student’s Advisory Committee.

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THESIS APPROVED BY THE STUDENT’S ADVISORY COMMITTEE INCLUDING EXTERNAL EXAMINER (AFTER VIVA-VOCE)

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D) LIST OF ABBREVIATION

Abbreviations	Full form
%	Per cent
/	Per
@	At the rate of
°C	Degree Celsius
cm	Centimetre(s)
dd H ₂ O	Double distilled water
C.D.	Critical differences
DAS	Days after sowing
Deptt.	Department
Dr. PDKV	Dr. Panjabrao Deshmukh Krishi Vidyapeeth
e.g.	Exempli gratia (For example)
et al.	Et alia (and associates)
etc.	Et cetera
Fig.	Figure
G	Grams
ha	Hectare
i.e.	That is
J.	Journal
M.sc.	Master of Science
Sr. No.	Serial number
HCl	Hydrochloric acid
KOH	Potassium hydroxide
VAM	Vesicular Arbuscular Mycorrhiza
cfu	Colony Forming Unit
Mg	Milli gram(s)
min.	Minutes
ml	Milli litre(s)
No.	Number
U	Unit
UV	Ultra violet
SE (m) ±	Standard error of mean
viz.	Videlicet (namely)

E) **THESIS ABSTRACT**

- a) **Title of the thesis** : **“MYCORRHIZAL EFFECT ON ROOT ROT OF CHICKPEA CAUSED BY *Rhizoctonia bataticola*”**
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ABSTRACT

The experiment entitled “Mycorrhizal effect on root rot of chickpea caused by *Rhizoctonia bataticola*” was carried out during 2016-17 at Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola with the objectives to study the effect of mycorrhiza on root rot of chickpea and to know the effect of fungicides, bioagents and herbicides on mycorrhiza. The experiment was laid out in randomized block design with three replication and seven treatments.

During experimentation root nodule, root colonization by VAM, spore density of VAM, root rot incidence by *Rhizoctonia bataticola* and

population of *Rhizoctonia bataticola* were recorded at 15, 30, 45, 60 and 75 DAS.

The number of nodules/ plant was recorded as 4.4, 6.2 and 8.0 at 30, 45 and 60 DAS respectively in its treatment of VAM @ 5kg/ha. The percent root colonization of mycorrhiza in chickpea was increased in treatment of alone VAM rather than the other treatments i.e. 4.7, 8.3, 13.7, 16.3, 18.7% at 15, 30, 45, 60 and 75 DAS respectively which was significantly more compared to no. of nodules in other treatments. The maximum number of mycorrhizal spores was also observed in treatment of VAM alone @ 5kg/ha i.e. 17.3, 21.0, 24.7, 27.7, 30.7 followed by combined application of VAM + seed treatment with *Trichoderma* @ 4g/kg (12.7, 16.3, 20.0, 22.3 and 25.0) at 15, 30, 45, 60 and 75 DAS respectively. The root rot incidence was minimum due to individual application of VAM @ 5kg/ha and recorded as 0.7, 0.7, 1.3, 1.7 and 2.3% while the maximum being in control plots (4.0, 4.7, 5.3, 6.0 and 6.7%) at 15, 30, 45, 60 and 75 DAS respectively. The population of *Rhizoctonia bataticola* in individual VAM treated plots was 0.7×10^4 , 0.7×10^4 , 1.0×10^4 , 1.0×10^4 and 1.3×10^4 cfu/g of soil at 15, 30, 45, 60 and 75 DAS respectively compared to 3.0×10^4 , 3.7×10^4 , 4.7×10^4 , 5.3×10^4 and 5.7×10^4 cfu/g of soil in control plots. The application of VAM alone exhibited superiority for each component observed. But its application along with chemical and bioagents reduced the population and root colonization of VAM whereas increased the root rot incidence and population of *Rhizoctonia bataticola* as compared to its alone application. However these combinations were proved significantly superior over control treatment.

In the pot experimentation the mycorrhizal spores was recorded as 3.3, 5.3, 9.0, 13.3 and 14.0 at 15, 30, 45, 60 and 75 DAS respectively in the treatment of VAM @ 5kg/ha alone and no mycorrhizal spores was obtained in control treatment during each observation.

The fungicides Carbendazim @ 0.1% and Carboxin + Thirum @ 0.3% were efficient in completely inhibiting the growth of *Rhizoctonia bataticola* (100%) *in vitro*. The biocontrol agent *Trichoderma viride* was found antagonistic and the *in vitro* mycelia inhibition of *Rhizoctonia bataticola* was 50.9%.

CHAPTER I

INTRODUCTION

1.1 Background information

Chickpea (*Cicer arietinum* L.) is leguminous pulse crop which belongs to leguminoceae family. It is originated from south eastern Turkey. It is third important legume crop in world after bean and peas. It is first important pulse crop in India being grown in largest area in rabi season. It is grown in India for Dal making. It is good source of protein constitute in about 99% in grains on dry weight basis which is very cheap and hence referred as “Poor man’s meat”.

The largest gram producing countries are India and Pakistan. India ranks first in the world in respect of production as well as acreage followed by Pakistan. In India the production of Chana 9120 thousand tonnes during 2016-2017. The largest gram producing states in India with respect to area are Madhya Pradesh, Uttar Pradesh, Andhra Pradesh, Hariyana, Karnataka and Maharashtra. In Maharashtra it is important *rabi* crop with production of 1058 thousand tones recorded with productivity of 844 kg/ha during 2013-14. Over the years, Maharashtra surpassed Rajasthan to become the second largest Chana producing state. According to Department of Agriculture, Chana acreage in Maharashtra increased from 12.79 lakh during 2015-2016 to 15.03 lakh hectares during 2016-2017 (Shah and Murali, 2016).

The climatic variables viz. temperature, rainfall pattern and its distribution have become more erratic under the changing scenario of climate. Climate change has adverse impacts on agriculture. These changes have also affected the reproduction, spread and severity of many plant pathogens. Thus the previously categorized minor diseases have now become major threats in many crops. In chickpea drastic shift of diseases have been recorded throughout the major chickpea growing regions in India and elsewhere. Dry root rot (DRR) caused by *Rhizoctonia bataticola* (Taub.) Butler [Pycnidial stage: *Macrophomina phaseolina* (Tassi) Goid] was found as a potentially emerging constraint to chickpea production. The disease generally appears during reproductive phase of the crop. The disease may also appear

at seedling stage, however, the susceptibility of the plant increases with age. The disease generally appears when day temperature is more than 30°C.

The term mycorrhiza was first coined by a German Botanist A. B. Frank in 1885 which means “Fungal root”. Mycorrhiza is a symbiotic association between fungi and roots of higher plants, in which both members normally benefit from this association. These associations are generally divided into two main groups based primarily on morphology. The first ectomycorrhiza contains fungal mantle surrounding the host root as well as intercellular fungal growth in the cell layers of the root cortex, commonly referred as “ Harting net”. The second endomycorrhiza, contains a loose fungal network in the soil and the fungus grows intercellularly in the root cortex. The profound effect of VAM has recently been realized.

Mycorrhizal fungi are able to retard pathogen development in root system. The roots colonized by mycorrhizal fungus exhibit high chitinolytic activities. These enzymes can be effective against other fungal pathogen (Dehne, 1982)

1.2 Importance of study

Dry root rot caused by *Rhizoctonia bataticola* (*Macrophomina phaseolina*) of chickpea (*Cicer arietinum* L.) is gaining importance in the changed scenario of climate when growing crop is predisposed to high temperature and moisture stress. Being mainly a soil-inhabiting pathogen, many environmental and soil factors are responsible for the development of disease. The disease is very difficult to control after its initiation. The best management practice is to avoid the infection by physical, chemical or biological means. The ill effect of chemicals are now well known. Physical means are having number of limitations and hence biological method is best suited.

Application of new molecules in soil like herbicides and insecticides are suspected to increase the population of this pathogen in the soil. Soil microorganisms represent a spectrum from being harmful (pathogenic) to highly beneficial for plant growth and survival. A major component of these soil microorganisms is the complex of mycorrhizal fungi

that colonize plant roots and adjacent soil (rhizosphere) at the same time, thus interact directly or indirectly with pathogenic microbes and plant growth promoting rhizobacteria (PGPR) (Sood, 2003). Mycorrhizal symbiosis generally increases root exudation and influences rhizosphere microbial communities.

Both harmful *Rhizoctonia bataticola* and the beneficial mycorrhiza being soil inhabitant may interact with each other and may have suppression of probably pathogenic organism which needs to study and hence the present investigation was undertaken.

1.3 Objective of the study

Based on the evidence discussed above, we speculate that it was necessary to study “Mycorrhizal effect on root rot of chickpea caused by *Rhizoctonia bataticola*”, with following objectives

1. To study the effect of mycorrhiza on root rot of chickpea.
2. To know the effect of fungicides, bioagents and herbicides on mycorrhiza.

1.4 Scope and limitations

From the very early research on mycorrhiza, it was apparent that mycorrhizal fungi have extensive host range.

To improve production potential of Chickpea it is necessary to use proper dose of fungicides, bioagents and herbicides in combination with VAM. It also helps in economic use of resources' and maximizing profit. The biofertilizer has to improve the growth and yield of crop. It plays a very significant role in improving the soil and maximizing yield by various ways.

As root rot disease is a serious threat to chickpea cultivation, its management through VAM may be one of the added advantage of VAM application. Moreover this serious disease can be managed ecofriendly.

The selection of the most specific appropriate plant fungus association for each specific environmental and ecological situation is one of the main challenges in current research on VAM. The knowledge of the different factors influencing the diversity of VAM is essential in their use for sustainable agriculture. The desire to exploit VAM as a natural biofertilizers for the agricultural biotechnology industry was understandable, but it become clear that more knowledge was needed to commercialize the use of VAM with limited success. The influence of VAM fungi on plant root, growth may also have indirect effect on the rhizosphere through the growth and degeneration of the hyphal network.

Among the beneficial effects of VAM fungi on the host-plant physiology, the decrease of the intraradical and/or mycorrhizosphere population and/or of the disease symptoms of soilborne pathogens was shown in many biological systems but according to yet partially described but probably synergistic mechanisms. Since chemical fertilizer use is more and more restricted due to its risks to human health and the environment, implementation of sustainable agriculture has become imperative in crop industry. The understanding of the mechanisms involved in the AM-mediated biocontrol will permit the performance of an adequate management of such agroecosystems and then permit the maximization of AM benefits.

1.5 Hypothesis

Being soil borne in nature the *R. bataticola* pathogen is difficult to manage. Some systemic fungicides can check the spread of disease, but its method of application is not preferred by the cultivators. Moreover chemicals cause environmental, soil and health hazards. Under such conditions host plant resistance remains the way of avoiding disease, however with changing climatic conditions the resistance can also be break easily by the pathogen. In this contest it is felt that biological control is the most suitable, possible and cheaper way of managing this devastating pathogen.

Trichoderma is the only known potential biocontrol agent for this pathogen, but it acts as an antagonistic organism only. On the other hand besides being used as biofertilizer mycorrhiza has a potential of controlling soil borne pathogens because of their association with roots of plants which provides protective covering to the roots.

To have an alternative to the available biocontrol agent with dual purpose of having ability as biofertilizer and bioagent also, the present study was aimed.

CHAPTER II

REVIEW OF LITERATURE

An attempt has been made to present a review of related past work, on root rot of chickpea and antagonist as well as chemicals for the management of *R. bataticola* at different places by some research workers. Efforts were also made to highlight the effect of VAM on crops including chickpea.

2.1 Background information

Latha et al. (2000) conducted field trial on black gram (*Vigna mungo* (L.) Hepper) to study the effect of antagonists and chemicals on root rot disease complex caused by the root rot fungus *Macrophomina phaseolina*. Among the nine treatments tested, combination of fungicide carbendazim as seed treatment @ 2g/kg of seed with a nematicide carbofuran as soil application (3.3 kg ai/ha) was found to be the most effective in reducing the root rot incidence.

Pande et al. (2003) opined that dry root rot caused by *Rhizoctonia bataticola* is one of the most important and wider spread soil borne disease of chickpea (*Cicer arietinum*) grown between latitudes 20⁰S and 20⁰S, where the climate is relatively dry and warm. Dry root rot generally appears during late flowering and podding stages and the infected plants appear completely dried. Chemical control of dry root rot is not effective as *Rhizoctonia bataticola* has a broad host range and survives in soil for longer periods in the form of sclerotia.

Aghakhani and Dubey (2009) observed that Chickpea is prone to many diseases and among them dry root rot caused by *Rhizoctonia bataticola* (Taub) Butler [synonym-*Macrophomina phaseolina* (Tassi) Goid] is one of the major constraints in chickpea production causing 10–20% annual loss. *R. bataticola* is primarily seed and soil-borne fungal pathogen. In chickpea, infected seeds and sclerotia surviving in the soils are the major source of primary inoculum. The pathogen also has wide host range. Since 75% cultivation of chickpea in India is under rain fed, the crop faces severe moisture stress, which predisposes the crop to dry root rot development.

Akhtar and Siddique (2010) studied influence of four species of arbuscular mycorrhizal fungi, namely *Glomus intraradices*, *G. aggregatum*, *G. clarisodeum* and *Glomus sp.* when evaluated for the control of root rot fungus, *Macrophomina phaseolina* on chickpea under glasshouse conditions. Application of these AM fungi cause an increase of plant growth, pod number, nodulation, chlorophyll and N,P,K content in *Macrophomina phaseolina* inoculated plants and also reduced root rot index. Application of *G.intraradices* found to be the best for reducing root rot index and improving plant growth parameters of chickpea.

Nagamani et al. (2011) optioned that soil borne inoculum of *R. bataticola* is more important in causing infection and disease development. For soil borne pathogen, use of fungicide is not practical due to exorbitant cost and environmental hazards involved. Hence integrated management of the disease using bio-control agents and chemicals is the best alternative.

Khan et al. (2012) opined that dry root rot caused by *Rhizoctonia bataticola* (Taub.) Butler is emerging as a serious biotic constraint for chickpea production. It is the most important and widespread soil borne disease of chickpea grown between latitudes 20° N. and 20° S, where the climate is relatively dry and warm.

Mamta et al. (2012) collected fifty isolates of dry root rot pathogen *Rhizoctonia bataticola* from different agro-climatic zones in India and evaluated these isolates for the degree of subdivision in isolates level. The isolates showed variability in pathogenicity test. Genetic characteristics were analyzed based on the sequence of the rDNA-internal transcribed spacer (ITS) region. Both pathological and molecular data correlated each other and supported that the *R. bataticola* present in India were very diverse and independent to their origin.

Nene et al. (2012) stated that dry root rot (*Rhizoctonia bataticola*) of chickpea, is emerging as a potential threat to chickpea cultivation in semi-arid regions because the host plant is predisposed to infection by moisture stress and high temperatures during the flowering to pod filling stage.

Mamta et al. (2015) stated that dry root rot caused by *Rhizoctonia bataticola* of chickpea is gaining importance in the changed scenario of climate when growing crop is predisposed to high temperature and moisture stress. Being mainly a soil-inhabiting pathogen, many environmental and soil factors are responsible for the development of disease.

Rashmi and Kumar (2016) collected 120 seed samples of smooth gourd from seven areas and observed occurrence of variously discoloured seeds, brown discoloured seeds, black discoloured seeds, white discoloured seeds, small seeds, shrivelled seeds and insect damaged seeds. Eight seed samples from Bargoan (15.25%) and Mandana 38 (32.75%) carried higher frequency of *Rhizoctonia bataticola* causing root rot disease in smooth gourd. Heavily infected seeds did not germinate. Dry seed inspection, incubation tests and histopathological studies clearly showed the seed borne nature of *Rhizoctonia bataticola*.

2.2 Effect of VAM on crops

Giovannetti and Mosse (1980) stated that the assessment of infection is an essential part of many studies involving VA mycorrhiza. They calculated the standard error of four methods of assessment based on observations of stained root samples either randomly arranged in a petri dish or mounted on microscope slides.

Maria et al. (2002) concluded that many plants have the capacity to obtain phosphate via a symbiotic association with arbuscular mycorrhizal (AM) fungi. In AM associations, the fungi release phosphate from differentiated hyphae called arbuscules, that develop within the cortical cells, and the plant transports the phosphate across a symbiotic membrane, called the periarbuscular membrane, into the cortical cell.

Orlando (2003) reported that the vesicular-arbuscular mycorrhiza fungi are associated with the majority to the terrestrial plants. Mycorrhiza is the mutualistic symbiosis (non- pathogenic association) between soil borne fungi with the roots of higher plants.

Akhtar and Siddiqui (2007) observed significantly increased plant growth, pod numbers, chlorophyll, nitrogen, phosphorus and potassium contents with reduced galling, nematode multiplication and root rot index in chickpea when inoculated with *Glomus intraradices*.

Nasim et al. (2007) conducted the experiment on mung bean (*Vigna radiata* (L.) Wilczek var.M28). Seeds were sown in earthen pots and were kept in filtered air, unfiltered air and ambient air. The study documents that the species of mycorrhizal fungi sensitive to troposphere ozone failed to reproduced in ambient air and unfiltered air chambers (without dust particles).

Jalaluddin et al. (2008) found that VAM- fungi *Scutellispora auriglobosa* was consistently associated with sunflower var. Helico-250. Sunflower plants suffer from charcoal rot disease caused by *Macrophomina phaseolina*.

Wichmann et al. (2009) opioned that the there is increasing evidence for the promoting effects of arbuscular mycorrhiza fungi (AMF) on the practically important crops. The effect of treatment on colonization by AMF, rhizobial nodule number and weight, plant dry matter and soil mineral N were determined in RBD designs with five replication.

Habullah et al. (2010) showed that biomass and P uptake in cereals following legumes are higher than in cereals following cereals. This may be due to soil chemical and biological changes induced by the legumes and/or their residues. The addition of inorganic P lowered the AM colonization particularly at more than 10 mg/kg available P.

Lioussanne (2010) studied the mutualistic symbiosis of most land plants with arbuscular mycorrhizal (AM) fungi has been shown to favour mineral and water nutrition and to increase resistance to abiotic and biotic stresses. This review reports the main mechanisms involved in the control of the disease symptoms and of the intraradical proliferation of soilborne phytopathogens by root colonization with AM fungi, with a special emphasis on the role of the rhizobacteria shown to be specifically associated with the AM extraradical network and the mycorrhizosphere.

Ortas (2010) studied mycorrhiza application in horticultural production in the eastern mediterranean region of Turkey under field conditions for several years. The effects of different arbuscular mycorrhizal fungi (AMF) have been evaluated under field conditions for cucumber production. The parameters measured were seedling survival, plant growth and yield, and root colonization. The field experiment results showed that mycorrhiza inoculation significantly increased cucumber seedling survival, fruit yield, P and Zn shoot concentrations. Indigenous mycorrhiza inoculum was successful in colonizing plant roots and resulted in better plant growth and yield. The relative effectiveness of each of the inoculam tested was not consistent in the different experiments, although inoculated plants always grew better than control. The most relevant result for growers was the increased survival of seedlings.

Farzaneh et al. (2011) found that arbuscular mycorrhiza fungi (AMF) colonize roots of host plants and promote plant growth due to improved uptake of nutrients. While the effects on P uptake are well known, the relevance of AMF for the uptake of other nutrients is less investigated. A moderate level of AMF colonization (18–55% of roots), enhanced the nutrient uptake of chickpea. Soil sterilization or fertilization with N showed no significant effect on nutrient uptake and biomass production. The inoculation with AMF was successful because all inoculated plant samples were substantially colonized without sterilization, the soil obviously contained indigenous populations that were able to colonize chickpea.

Abiala et al. (2013) exhibited that mycorrhizae are symbiotic associations, formed between plants and soil fungi that play an essential role in sustainable crop production and soil fertility. Interest in Vesicular Arbuscular Mycorrhiza (VAM) fungi inocula propagation for agriculture is increasing due to their role in promotion of plant health, soil fertility, and soil aggregates stability in the developing economies. This review discussed the impact of VAM to plant growth, factors contributing to VAM utilization and associations to plant growth. The management and strategic applications of VAM to enhance growth of food crops most especially in Africa with an understanding

of exploiting VAM benefits towards sustainable agricultural development is very important.

Chitra et al. (2013) described Arbuscular Mycorrhiza (AM) fungi colonization within the roots of cultivated tea plants (*Camellia sinensis*) at four sites, that is, Goodrich, Archadia, IIP, and Vasant Vihar of Doon Valley, Dehradun, India, from April, 2008, to March, 2009. Microscopic study of sterilized and stained root segments showed presence of four species namely *Glomus fasciculatum*, *G. mosseae*, *Gigaspora margarita*, and *Acaulospora scrobiculata* belonging to three genera of mycorrhizal fungi. Study concluded that the percentage AM colonization is the function of seasonal variation in physicochemical properties of soil and presence of AM inoculums in the soil at a particular time.

Olagunju et al. (2014) observed that nutrient depletion in soil has become a serious threat to agricultural production, incorporation of factor such as AM Fungi as an alternative amendment in enhancing crop production cannot be underestimated. This paper investigates potency of Arbuscular mycorrhirza inoculation on plant growth. The experiment was designed such that 3 planting pots were seeded with *Sorghum bicolor* and AM Fungi, another 3 planting pots were seeded without AM Fungi, in all there were replicate of 4 treatments. Dieback and necrosis of the inoculated *Sorghum bicolor* were less compared to the higher value in non inoculated *Sorghum bicolor*.

Pezeshkpour et al. (2014) investigated the effects of mycorrhization, phosphate solubilizing bacteria and vermicomposting on chickpea (*Cicer arietinum* L.) yield, nutrient uptake and proteins in the Experimental Research Institute of Khorram Abad, Lorestan, Iran, in 2009-2010. Chickpea seeds were treated with inoculum (spore, hyphae and roots) of *Glomus intraradices*, (from Soil and Water Research Institute, Karaj, Iran), rock phosphate treated with *Pseudomonas striata* and vermicompost (0, 6, 12 T/ha, manure treated with *Eisenia foetida*, from Behsaman Co. Karaj, Iran). The use of soil microbes including arbuscular mycorrhizal (AM) fungi, phosphorous (P) solubilizing bacteria, and vermicomposting increases both the population of soil beneficial microbes and enhances the availability of soil

nutrients such as nitrogen (N), phosphorous (P), potassium (K) and micronutrients. This can be very useful for plant growth and crop production.

Rabinson et al. (2014) studied plant growth and physiological response of sesame in controlled environment using normal soil and indigenous Vesicular-Arbuscular Mycorrhiza (VAM) fungi treated soil. The seedlings of *Zea mays* were inoculated with *Gigaspora* species of VAM and the inoculum was multiplied with help of *Zea mays* seed bed. Sesame seeds were then inoculated into the bed and it was found that the plant height, shoots lengths, roots, biomass of shoot and roots were considerably increased in the mycorrhizal plants. The effect of VAM infection was assessed in pot experiment. In this comparative study, specific mycorrhizal fungi had consistent effects on various growth parameters such as the number of leaves, number of roots, shoot length, biomass of shoot and roots and biochemical parameters.

Sadhana (2014) used Arbuscular Mycorrhizal Fungi (AMF) as a Biofertilizer. Dual inoculation of such fungi with a *Rhizobium* and other bacterium on plant enhanced the growth and other beneficial effects viz., resistance to disease and tolerance to adverse soil and climatic conditions.

Zadehbagheri et al. (2014) found that the most common group of mycorrhizal fungi are the arbuscular mycorrhizal fungi (AMF) which colonize the roots of over 80% of land plant families Mycorrhizal fungi associated with plant roots have existed for hundred of millions of years. The use of AM fungi economizes on fertilizer use in plant production providing a sustainable and environmentally safer substitute.

2.3 Efficacy of bioagents with fungicides

Raghuchander et al. (1997) recorded that dry root rot of mungbean caused by *Marophomina phaseolina* was reduced by seed pelleting of *Trichoderma viride* isolates when talc was used as carrier. Among the isolates, *T. Viride*- III supported higher plant growth, better native *Rhizobium* nodulation and grain yield. Sclerotial number and root rot incidence were greatly reduced in seed pelleting of antagonists as compared

to row application. Rhizosphere soil had a higher number of *Trichoderma* chlamydospores in seed pelleting treatment.

Yashoda and Ammajamma (2009) described the efficacies of systemic and non-systemic fungicides against *Rhizoctonia bataticola* at different concentration. Among systemic fungicides, Carboxin (0.05 and 0.1%) and Hexaconazole, Metalaxyl and Tridemifon at 0.1 per cent completely inhibited the growth of *R. bataticola* (100%) and among the non-systemic fungicides tested, Thiram at 0.1, 0.2 and 0.3 per cent concentrations was found effective against *Rhizoctonia bataticola*.

Anita et al. (2011) used the secondary metabolites from culture filtrate and mycelial mass of potential isolates of *Trichoderma viride*, *T. virens* (IARI P3) and *T. harzianum* which was extracted by solvent extraction and soxhlet water bath distillation methods and evaluated at different concentrations against *Rhizoctonia bataticola* (dry root rot of chickpea) and *Fusarium oxysporum* f. sp. *ciceris* (wilt of chickpea). Among the secondary metabolites present in culture filtrate and that in mycelia mass, the metabolites of *T. harzianum* caused maximum growth inhibition followed by *T. viride* and *T. virens* against *R. bataticola*.

Rashmi et al. (2012) studied antifungal potential of different strains of *Trichoderma* species against growth and development of *Macrophomina phaseolina*. In dual culture all strains of *Trichoderma* showed inhibitory effects on *M. phaseolina* and they were ranked according to the degree of inhibition. Different concentration of culture filtrates of *Trichoderma* on colony growth of *M. phaseolina* were also studied. It was found that *T. harzianum*-1 showed maximum inhibition of *M. phaseolina* at 40% concentration of culture filtrates followed by other screened culture filtrates of *Trichoderma* strains.

Rekha (2012) reported root rot as an important disease of mungbean caused by *Macrophomina phaseolina* from farmers field in Rajasthan. For the integrated management of the disease, biocontrol agents, fungicides, herbal oils, plant extracts and organic manure as well as their combinations was used. Integrated management showed that vermicompost and (Carbendazim) in combination was more effective in reducing the root rot incidence in pot condition.

Amrutha et al. (2014a) isolated ten *Trichoderma* spp. from chickpea rhizosphere and root endophytic region by using serial dilution technique and purified by single hyp hal tip method. *Trichoderma* isolate-7 showed highest compatibility with validamycin (72.22%) followed by copper oxychloride (66.66%).

Reetha et al. (2014) recorded that *Trichoderma* sp. was the most antagonistic microorganism to charcoal rot disease pathogen i.e. *Macrophomina phaseolina*. All the antagonists reduced the colony growth of *Macrophomina phaseolina* significantly compared to the control.

Malleswari et al. (2015) revealed probable influence of plant growth promotion and induced systemic resistance (ISR) in enhancing the disease resistance against root rot disease by PGPR bioformulations. The bio control agents not only controlled dry root rot, but also promoted plant growth and this gives them an advantage over the use of chemical fungicides against root rot in disease management.

Mohamedy et al. (2015) tested the efficiency of *Trichoderma harzianum* (bio control agent) in combination with sorbic and benzoic acid (chemical resistance inducers) in management of root rot diseases on green bean plants caused by *Rhizoctonia bataticola*. In green house, combination between *T. harzianum* and sorbic and/or benzoic acid treatments were more effective in control line *Rhizoctonia* and *Fusarium* root rots on bean plants, than each of them separately. The use of biological seed treatment combined with chemical plant resistance inducers was considered as safe, cheap and easily applied method for controlling such soil-borne plant pathogens considering the avoidance of environmental pollution and the side effect of pesticide application.

Nandini et al. (2015) found that seed treatment with fungicides does not protect the crop for very long against *Macrophomina phaseolina*. Chemical control measures like soil drenching with fungicides is uneconomical. Hence biological control method can be a promising approach for the management of root-rot caused by *M. phaseolina* in safflower.

Nevadita et al. (2015) observed that *Trichoderma viride* can thrive in diverse environmental conditions as aggressive colonizers of soil and the roots of plants and act as natural bioagent to protect plants from infection by soil-borne fungal pathogens. Laboratory experiments were conducted to test the possibility of combining fungicides with *Trichoderma viride* to work out their compatibility to devise a suitable integrated management of soil borne plant diseases. Six fungicides Blitox, Thiophenate methyl, Roxiltabucanazole, Ridomil, Bavistin and Captan were evaluated at different concentration. Present investigation suggests that compatible fungicides can be used with *Trichoderma* in an IDM package to control soil borne plant pathogens.

Viswanathan et al. (2015) promoted biological control as a component of an integrated management approach under arid ecological conditions, for management of dry root rot of black gram by using *Trichoderma spp.* Among the *Trichoderma spp.* tested, *Trichoderma viride* exhibited strong inhibition of the growth (77.77%) against *M. phaseolina*. Their culture filtrates were also found to be effective in inhibiting the *in vitro* growth of pathogen.

Sangappa and Mallesh (2016) found that a new disease of blackgram *i.e.* aerial blight and dry root rot caused by *Rhizoctonia bataticola* is primarily a soil inhabitant. An attempt was made to manage the disease with biocontrol agents and fungicides. Among the biocontrol agents tested against *Rhizoctonia bataticola*, *Pseudomonas fluorescens* (DAPG+ve)-RP46 was found more effective as compared to other bio-control agents and inhibited maximum mycelia growth (68.95%) of *R. bataticola* followed by *T. harzianum* (Th-R) (61.85%) and *T. viride* (Tv-R) (61.11%) under *in vitro* condition.

2.4 Virulence of the pathogen

Anilkumar and Sastry (1980) observed variation among eight isolates of *Rhizoctonia bataticola* from different hosts with regard to growth rate, effect of media, sclerotial initiation, sclerotial number and size, effect of incubation temperature, fungicides, and virulence. The isolates varied with regard to above characters. Isolates from brinjal and beans were highly virulent and less sensitive to fungicides. The study indicates the existence of different strains within *R. bataticola*.

Sharma et al. (2003) studied Charcoal root rot and wilt, two economically important diseases of many crop plants in North and South America, Asia and Africa and some parts of Europe and found genetic variation in 43 isolates of *Macrophomina phaseolina* and 22 isolates of *Fusarium* species, collected from geographically distinct regions over a range of hosts.

Kanchan and Biswas (2009) recorded that potency of infection of isolates of *Rhizoctonia bataticola* varied on four different cultivars of pigeon pea viz. Prabhat, Bahar, T-7 and T-21. The isolates, T-4 and T-5 showed maximum disease severity with 48.3% each on Bahar T-1 isolate on T-21 and Prabhat with 54.4 and 53.3 % respectively. The maximum disease severity was recorded in the plant which had attained the age of 47 days followed by 57 and 67 days old plant. The early age group of plant showed low disease severity as compared to middle and above middle age group.

Pancheshwar and Verma (2013) conducted study on 22 isolates of *Rhizoctonia bataticola* collected from various soybeans growing area of Madhya Pradesh for their reactions against soybean cultivars. On the basis of their Pathogenicity two isolates I₂ and I₁₀ were identified as a highly virulent isolates. Both isolates of *Rhizoctonia bataticola* were tested against seventy seven cultivars of soybean.

Amrutha et al. (2014b) identified the pathogen based on its mycelial and sclerotial characters and pathogenicity test was proved by soil inoculation method. The fungicides copper oxychloride, captan, hexaconazole and tebuconazole were found to be highly effective (100%) in inhibiting the mycelia growth of the highly virulent pathogen at all the concentrations tested.

CHAPTER III

MATERIAL AND METHODS

The present investigation was carried out during 2016-17 at Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. The details of the material used and methods followed are described in this chapter.

3.1 Materials required

Following material were used for experimentation.

3.1.1 Seeds

Seeds of CSV 27 of sorghum from sorghum research unit and Digvijay (Phule G-9425-5) of chickpea from Pulses Research Unit were obtained from Dr. PDKV, Akola for experimentation.

3.1.2 Glass wares and Plastic wares

During the course of investigation following glass wares and plastic wares were used: Glass Petri plates (Borosil and Schott Duran, Germany), test tubes (Borosil, Germany), conical flasks of 250ml, 500ml and 1000ml (Schott Duran, Germany), funnel (Schott Duran, Germany), beaker (Schott Duran, Germany), glass pipette (Schott Duran, Germany), measuring cylinder (Schott Duran, Germany), slides, cover slip, etc.

3.1.3 Equipments

Standard laboratory equipments used for different experiments were Autoclave (Equitron, India), BOD incubator (Sanco, India), laminar airflow (Klenzaid, India), student microscope (Olympus, India), stereoscopic binocular (Nikon, India), refrigerator (LG, India), hot air oven (Bio-techniques, India), digital weighing balance (Anamed, India), Bunsen burner, digital camera (Kodak, India), double distillation unit (JSGW, India), soil sterilization tank (locally made), etc.

3.1.4 Chemicals

Agar agar, dextrose for preparation of media, Streptomycin Sulphate, Mercuric chloride, Spirit etc. were obtained from Department of

Plant Pathology, Post Graduate Institute Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

3.1.5 Preparation of Reagent

Chemicals like Potassium hydroxide (KOH) and Hydrochloric acid (HCL) were used for root clearing and staining. Ten g of Potassium hydroxide (Pellets)(KOH) was dissolved in 100 ml water for preparation of 10% KOH solution. For preparation of 0.1N HCL solution, 8.3 ml HCL was dissolved in 992 ml water.

3.1.6 Bioagent used

Bioagent used in the studies *Trichoderma viride* was obtained from Department of Plant Pathology Dr. PDKV, Akola.

3.1.7 Other materials

Blotter paper, non-absorbent cotton, muslin cloth, polyethylene bags, cork borer (5 mm), inoculation needle, micropipette, dissection needle, forceps, paper bags, butter paper bags, pencil, permanent marker, cello tapes, Whatman filter paper (4 mm), tags, polyethylene sheets, test tube stand, tray, hand sprayer, wash bottle, thread, wooden sticks, potato, rubber band, scissors, etc were used during study.

3.2 Methodology

3.2.1 Field experiment

Commercially available VAM culture was procured from local market. To establish the population of VAM spores in experimental plot, the culture was applied to field @ 5 kg/ha during *kharif* season and the sorghum variety CSV 27 was grown in that field. The sorghum was allowed to grow and after harvest of the crop the root debris containing VAM spores were mixed in the soil. On the same piece of land the *rabi* experiment as per treatment detail was conducted. The control treatment was without application of VAM in both the season.

3.2.2 Experimental details

The study was conducted by using seeds of Digvijay (Phule G-9425-5) of chickpea under field condition. The experimental details are given below.

1. Year of experiment : *Kharif and Rabi* 2016-2017.
2. Design of experiment : Randomized Block Design (RBD).
3. No. of Replications : Three.
4. Crop : Chickpea.
5. Variety : Digvijay of Chickpea
6. Spacing : 30 cm x 10 cm
7. Plot size (Gross) : 4m x 3m
8. No. of treatments : 7

3.2.3 Treatment details (*Rabi* season)

T₁ : Soil application of Mycorrhiza @ 5kg/ha.

T₂ : T₁ + Seed treatment with Carbendazim 50 WP (0.1%)

T₃ : T₁ + Seed treatment with Carboxin + Thirum @ 2.0 g/kg

T₄ : T₁ + Seed treatment with *Trichoderma* @ 4g/kg

T₅ : T₁ + Soil application of Pendamethelin @ 1.5 kg/ha

T₆ : T₁ + Soil application of *Trichoderma* @ 2.5 kg/ha

T₇ : Control.

3.2.4 Root samples

The root samples from experimental field were collected up to the base of main stem from randomly selected 5 plants of each plot.

3.2.5 Maintenance and preservation of roots

After collection of plants root samples were thoroughly washed in tap water to remove soil particles. Selected and cleaned roots were fixed in formaldehyde/ acetic acid solution (Johansen, 1940). About 5 root samples from each plants were observed. The arbuscular mycorrhiza fungi do not

cause morphological changes to the roots; however, they produce arbuscules and in many cases vesicles in roots. To observe AM fungus structures within the roots it is necessary to clear cortical cells of cytoplasm and phenolic compounds which usually hide them and then to differentially stain the fungus tissue. Clearing procedures, which use chemical agents to remove cell contents and cell wall pigments, are routinely used to view internal features in plant tissues. (Gardner 1975).

3.2.6 Staining of Vesicular Arbuscular Mycorrhiza for root colonization

1. Roots were collected from field and washed with tap water to remove soil particles. Then roots were cut into 1cm length by sterilized blade.
2. Root pieces were placed in a small beaker with enough 10% KOH solution.
3. The beaker with root pieces in 10% KOH solution was autoclaved at 1.04 kg/cm².
4. The KOH solution was decanted from the beaker with leaving roots behind.
5. Roots were rinsed with about 20 ml distilled water .
6. Twenty ml of 0.1N HCl was added in the beaker, swirl and left it for a minute.
7. HCl solution was decanted.
8. Sufficient amount of cotton blue or tryphan blue solution was added to cover the roots generously.
9. Then root bits were observed under microscope.

Percentage of root colonization =

$$\frac{\text{Number of root pieces contains vesicles}}{\text{Total number of root pieces observed}} \times 100$$

3.2.7 Counting VAM spores from soil by wet sieving and decanting method

1. First 10 g soil sample was taken and dissolved in 100 ml distilled water in conical flask.
2. Then conical flask was shaken for 30 min.
3. After that the conical flask was kept in undisturbed condition for 30 min.
4. The heavier particles were allowed to settle down.
5. Suspension was decanted through a 710 μm sieve to remove organic matter and roots.
6. The obtained suspension was decanted through 250 μm , 75 μm and 45 μm sieves consequently.
7. The entire residue was collected on 45 μm sieve.
8. After settlement residue was dissolved in distilled water and filtered through filter paper.
9. This paper was spread in Petri dish and a residue present on filter paper was taken and mounted on a slide and was examined under microscope.

3.2.8 Estimation of *R. bataticola* population by serial dilution technique

Serial dilution and pour plate method was followed for counting Population of *Rhizoctonia bataticola* fungus from soil. .

1. The 1 g soil sample was weighed and added to 9 ml sterile water in a test tube. Shaken thoroughly for 1 minute. This has given soil dilution of 10^{-1} .
2. Then 1 ml suspension from 10^{-1} dilution was transferred to next 9 ml sterile water blank and mixed thoroughly by shaking for one minute. Thus 10^{-2} dilution of original soil sample was prepared.
3. The above steps were repeated and dilution of 10^{-3} and 10^{-4} of the soil sample were obtained in test tubes containing sterile water.
4. The 10^{-4} dilutions were used for estimation of population. One ml aliquot of the desired dilution was transferred to sterile Petri plate containing PDA at 45°C agar medium.

5. The soil dilution with medium was mixed by gently rotating the plates for uniform mixing and allowed to solidify it.
6. The Petri plates were placed in BOD incubator at $28\pm 2^{\circ}\text{C}$ for 48 hrs and observations regarding growth were recorded.

3.3 *In vitro* study

3.3.1 Sterilization of glassware and media

During entire course of investigation glasswares viz., petriplates, pipettes, flasks, etc were sterilized in hot air oven at 180°C for 1 hr. before its use, whereas distilled water and media were sterilized in autoclave at 15 lbs for 15 minutes.

3.3.2 Preparation of potato dextrose agar medium (PDA)

The medium was prepared by following ingredients

Potato Dextrose Agar (PDA)

1) Potato (peeled)	200 g
2) Dextrose	20 g
3) Agar agar	20 g
4) Distilled water	1000 ml

- Peeled potatoes were sliced into pieces and boiled in 600 ml water till properly cooked.
- The starch extract was strained through muslin cloth and measured. In remaining water, dextrose and agar were added.
- Potato starch extract was added to it and volume was made up to one litre.
- The medium was distributed and poured in conical flask then plugged with non-absorbent cotton and autoclaved at 15 lbs. pressure for 15 minutes.
- This media was used for purification of fungi and for maintenance of culture.

3.3.3 Precautions to eliminate contamination

All work and inoculation of microbial culture was carried out in laminar air flow. The laminar flow was sterilized by glowing ultra violet light for ½ hr prior to commencement of work. The working surface of laminar flow and side glasses were surface sterilized with denatured spirit. Moreover, other such necessary care was taken to maintain and carryout work under aseptic condition.

3.3.4 Isolation of *R. bataticola*

R. bataticola was isolated from root rot infected chickpea plants on PDA by tissue isolation method.

3.3.5 Purification and maintenance of pure culture

Fungal culture was purified by picking the colonies and transferring it on PDA by adopting subsequent sub culturing at regular intervals and the plates were kept at room temperature. Seven days old culture was used for further studies (Islam et al. 2008).

3.3.6 Mass multiplication of *R. bataticola*

The sorghum grain soaked partially for one hour in warm water (40⁰ to 45⁰C) and then spread on clean blotting paper for air drying. About 300 g moistened grains were filled in each 1000 ml flask with 10 ml water and autoclaved for 30 min. at 15 lbs pressure. The mycelial bits of pure culture of *Rhizoctonia bataticola* were inoculated under aseptic condition in those flask containing grains and incubated at 28+2⁰ C for 10 days. Mean while flask were shaken to avoid clumping of grains and to facillitate early growth of test fungus. The grains turn blackish due to growth of test fungus.

3.3.7 Preparation of sick soil for pathogenicity test

Soil was put in gunny bag and sterilized in autoclave at 1.05 kg/cm² pressure for 1 hour consequently for 3 days. The mass multiplied inoculum was added in 1:10 proportion to soil and thoroughly mixed. Thus soil was made sick. The pots were taken and surface sterilised with 0.1% formalin. The sick soil was filled in sterilized pot 1/4th of its capacity. The pots were watered lightly and incubated for 4 days. Chickpea seeds of Digvijay

were sown (12 seeds per pot) and the pots were monitored for seedling mortality.

3.3.8 Dual culture Technique

Dual culture technique as described by Vincent (1927) was adopted for estimating the ability of antagonistic organisms as an antagonist against *Rhizoctonia bataticola*.

- The pathogen *R. bataticola* and antagonistic *Trichoderma* was grown separately on PDA up to 7 days.
- The discs of 5 mm from 7 days old culture of *R. bataticola* were placed on one side of the petri-plates containing PDA. Similarly, 5 mm disc of *T. viride* were cut from 7 days old culture and placed on opposite sides of the pathogen. Each treatment was replicated three times.
- The inoculated plates were incubated at $28 \pm 2^{\circ}\text{C}$ for 5 days. On seventh day the growth of *Rhizoctonia bataticola* was measured.

The per cent growth inhibition was calculated using following formula.

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

Where,

I = Per cent inhibition

C = Growth of fungus in control (mm)

T = Growth of fungus in treatment (mm)

3.3.9 Efficacy of fungicides against *Rhizoctonia bataticola* (Poison food technique)

Poison food technique was employed to test the efficacy of commonly used fungicides i.e. Carbendazim @ 0.1% and Carboxin + Thirum (combined products) @ 0.3%. against *Rhizoctonia bataticola*

- Prepared PDA in flask (used separate stock for each fungicide) and sterilized.

- Measured quantity of chemicals were added to make required final concentration and mixed well.
- Poured in sterilized petri plates and allowed to solidify.
- The small discs (0.5 cm dia) of 7 days old fungal culture were cut and placed on the petri plates in the centre with proper control (PDA without chemicals). Three replications were maintained for each chemical and control Plates were incubated for 7 days at $28 \pm 2^{\circ}\text{C}$.
- The colony diameter was measured and per cent growth inhibition was calculated by following formula.

$$\text{PI} = \frac{\text{C} - \text{T}}{\text{C}} \times 100$$

Where,

PI - Inhibition percentage

C - Growth in control

T - Growth in treatment

3.3.10 Statistical analysis

Statistical analysis was done by using method of analysis of variance Means were tested for significance and critical difference was used for comparing the differences between the treatments (Panse and Sukhatme,1967).

CHAPTER IV

RESULT AND DISCUSSION

The research work on “Mycorrhizal effect on root rot of chickpea caused by *Rhizoctonia bataticola*” was carried out during 2016-17 in Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

The observations recorded and the data generated through different experimentation is interpreted and presented in this chapter. Microbial pesticides are emerging as major driving force to the chemicals, as indiscrimination and excessive use of chemicals disturb the eco-system. In this context a field experiment in Randomized Block Design with seven treatments were replicated thrice to evaluate treatment effect of VAM on root rot of chickpea.

In present investigation, VAM culture containing *Glomus species* was applied to sorghum crop during *kharif* for the establishment of VAM population in soil. After harvest of the crop the same field was used for present investigation during *rabi* on chickpea crop. The treatments were applied as per detail and the results obtained are discussed briefly in this chapter so as to reach the proper conclusion. Rhizospheric soil were used for observing the number of mycorrhizal spores and the spores were identified based on their morphology. During the investigation, association of mycorrhiza with characteristic feature was observed. Only one type of spores could be seen in rhizosphere which was found singly and in cluster. The shape was globuse, smooth or shiny roughened from adherent debries. Some spores were light yellow brown or bright yellow and transparent to translucent when young and became black brown to black at maturity. Some spore was two layered, outer wall thicker than inner filled with granular partical. Spore was also found with one straight to recovered funnel shape subtending hyphae. The colour of hyphae was observed as yellow to brown. The hyphal growth and penetration of hyphae in roots could be seen under microscope.

4.1 Effect of different treatments on number of nodules per plant

Data presented in Table and fig 1 indicated that there was progressive increase in plant nodules from 30 DAS to 60 DAS. Treatments were found to be significant at all the stages of chickpea crop.

Table 1. Effect of different treatments on number of nodules / Plant at different interval

Tr. No.	Treatments	Number of nodules/plant		
		30 DAS	45 DAS	60 DAS
T ₁	VAM @ 5kg/ha.	4.4	6.2	8.0
T ₂	T ₁ + Seed treatment with Carbendazim 50 WP @ 0.1%	2.6	4.7	5.2
T ₃	T ₁ + Seed treatment with Carboxin + Thirum @ 2.0 g/kg	3.1	4.2	5.4
T ₄	T ₁ + Seed treatment with <i>Trichoderma</i> @ 4g/kg	3.8	4.5	6.4
T ₅	T ₁ + Soil application of Pendamethelin @ 1.5 kg/ha	2.7	3.4	4.4
T ₆	T ₁ + Soil application of <i>Trichoderma</i> @ 2.5 kg/ha	3.4	5.1	6.2
T ₇	Control	4.2	5.3	5.7
	F test	Sig	Sig	Sig
	SE(m)	0.21	0.39	0.38
	CD (0.05)	0.67	1.22	1.18

At 30 DAS treatment T₁ i.e. soil application of VAM @ 5kg/ha recorded significantly more number of nodules (4.4) than all other treatments followed by the treatment T₄ (3.8) where *Trichoderma* was applied as seed treatment along with soil application of VAM. Application of chemicals either fungicides or herbicide along with VAM reduced the no. of nodules/plant

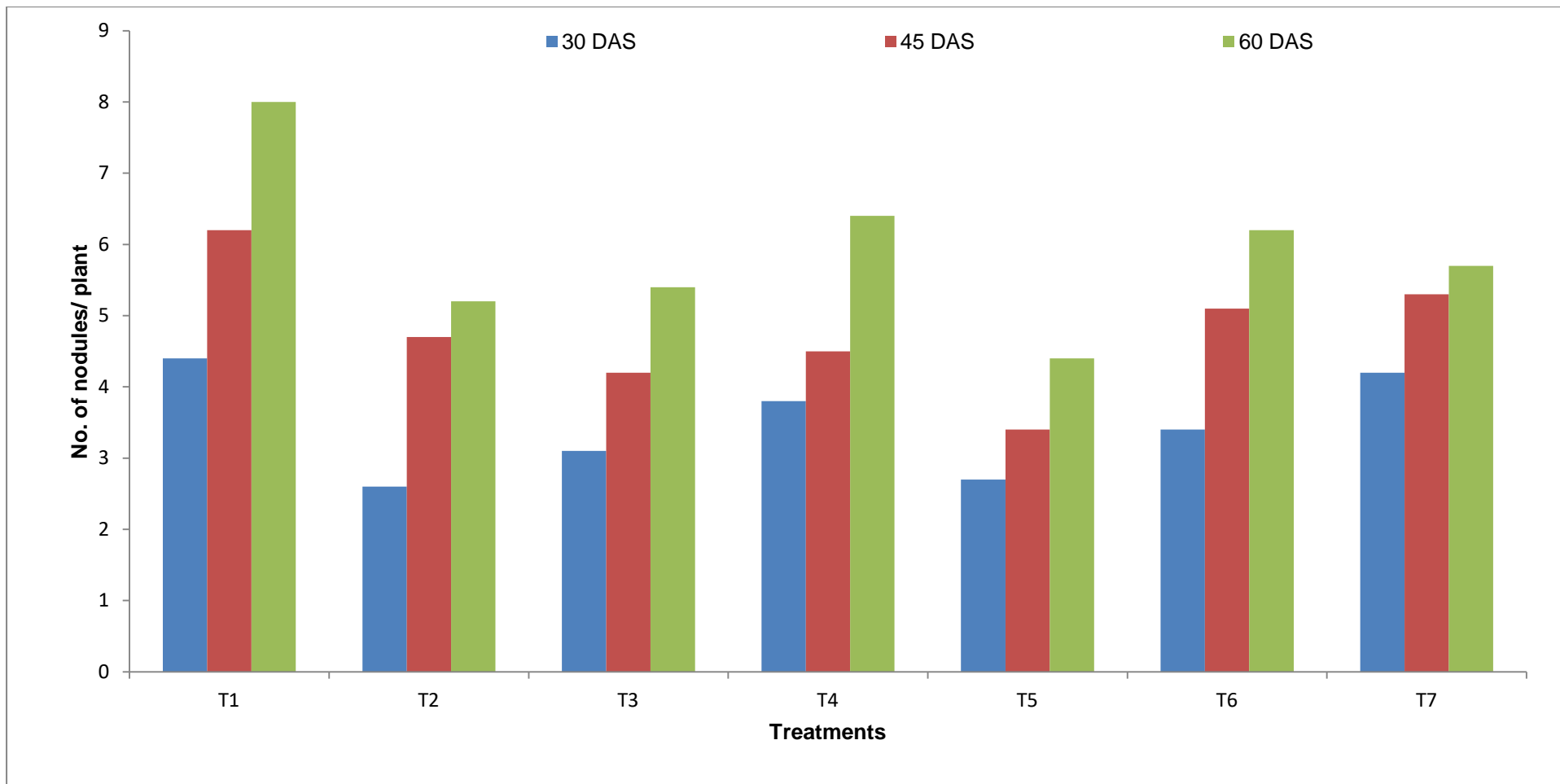


Fig. 1. Effect of different treatments on number of nodules / Plant

compared to control exhibiting adverse effect of these chemicals on soil inhabiting beneficial microbes particularly Rhizobium.

Similar trend was observed in no. of nodules at 45 and 60 DAS. The maximum no. of nodules were recorded in treatment of VAM followed by its combination with *Trichoderma* either as seed treatment or soil application (Plate 1).

Beneficial effect of VAM on crops by enhancing the efficiency of useful microbes have been recorded by number of workers including Maria et al. (2002), Habullah et al. (2010) and Pezeshkpour et al. (2014). Wichman et al. (2009) opined increasing evidence for promoting effects of AMF on rhizobial nodule number and weight, plant dry matter and soil mineral N. Sadhana (2014) could find enhanced growth and other beneficial effect viz. resistance to disease and tolerance to adverse soil and climatic conditions by co-inoculating AMF with Rhizobium and other bacterium. The findings of present study confirms the earlier reports of having enhanced number of nodules with application of VAM.

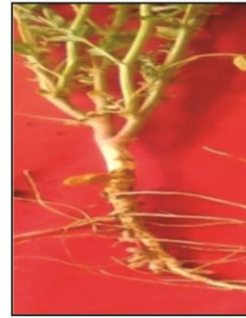
4.1.2 Effect of different treatments on percent root colonization of VAM

Table, fig and plate 2 showed the effect of treatments on root colonization of VAM in chickpea crop.

The percent root colonization of VAM increased from 15 DAS to 75 DAS in each treatment. At 15 DAS the colonization was maximum in the treatment of soil application of VAM alone (T_1) where was recorded as 4.7% compared to 0% in control. Ortas (2010) found that indigenous mycorrhiza inoculam was successful in colonizing plant roots in cucumber. All other treatments where VAM was applied in combination with chemicals and bioagent exhibited adverse effect on VAM root colonization as the percent colonization was decreased compared to alone application of VAM in these treatments and exhibited statistically at par colonization with control. Similar trend was recorded at 30 DAS with maximum root colonization in treatment of VAM (8.3%) but during this period of observation it was at par with T_4 (T_1 + seed treatment with *Trichoderma*) i.e. 8.0% compared to 4.8% in control. At 45, 60 and 75 DAS the root colonization recorded due to application of VAM



T1 VAM



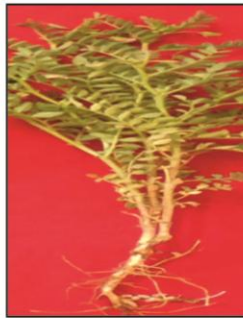
T2: T1+ Carbendazim



T3: T1+ Carboxin +Thirum



T4: T1+*Trichoderma* (ST)



T5: T1+ Pendamethalin



T6: T1+ *Trichoderma* (SA)



T7 Control

Plate 1: Effect of different treatments on number of nodules at 60 DAS

was 13.7, 16.3 and 18.7% respectively followed by 10.3, 11.0 and 14.0% due to combined application of VAM and *Trichoderma* as seed treatment at 45, 60 and 75 DAS respectively. Although Farzaneh et al. (2011) found that the inoculation with AMF was successful in chickpea because all inoculated plant samples were substantially colonized, the finding of present study exhibiting adverse effect of chemicals and bioagents on root colonization by VAM could not be supported due to lack of literature. Chita et al. (2013) concluded that the percentage AM colonization is the function of seasonal variation in physiochemical properties of the soil. The applied chemicals/ bioagent might be playing role for this variation which needs confirmation.

Table 2. Effect of different treatments on percent root colonization of VAM

Tr. No.	Treatments	Root colonization of VAM (%)				
		15 DAS	30 DAS	45 DAS	60 DAS	75 DAS
T ₁	VAM @ 5kg/ha.	4.7	8.3	13.7	16.3	18.7
T ₂	T ₁ + Seed treatment with Carbendazim 50 WP @ 0.1%	0.0	4.0	6.0	7.7	8.7
T ₃	T ₁ + Seed treatment with Carboxin + Thirum @ 2.0 g/kg	0.7	4.7	7.0	7.3	9.0
T ₄	T ₁ + Seed treatment with <i>Trichoderma</i> @ 4g/kg	1.0	8.0	10.3	11.0	14.0
T ₅	T ₁ + Soil application of Pendamethelin @ 1.5 kg/ha	0.0	2.0	5.0	7.3	8.0
T ₆	T ₁ + Soil application of <i>Trichoderma</i> @ 2.5 kg/ha	1.7	7.0	8.7	10.0	12.3
T ₇	Control	0.0	0.0	0.0	1.0	0.7
	F test	Sig	Sig	Sig	Sig	Sig
	SE(m)	0.62	0.48	0.53	0.92	0.69
	CD (0.05)	1.92	1.49	1.63	2.86	2.15

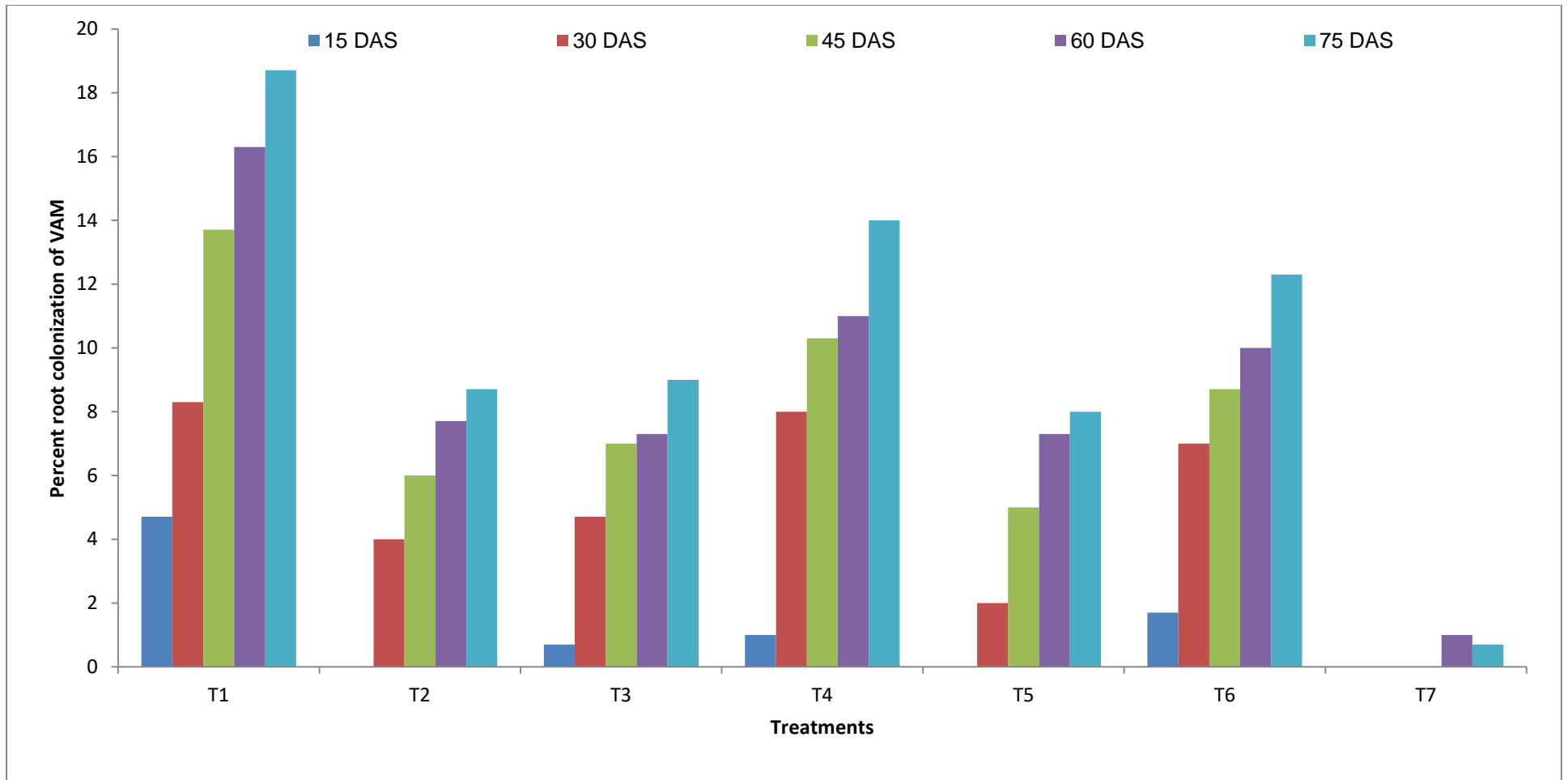
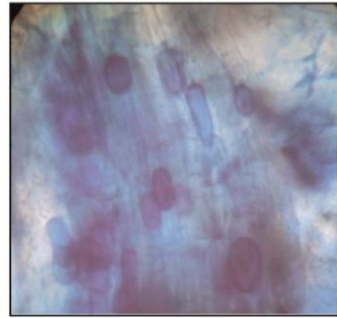


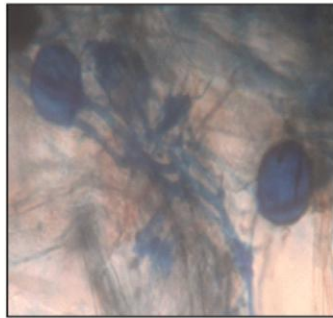
Fig. 2. Effect of different treatments on percent root colonization of VAM



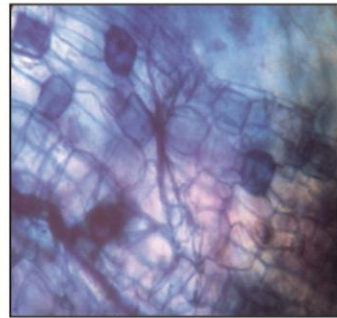
T1: VAM



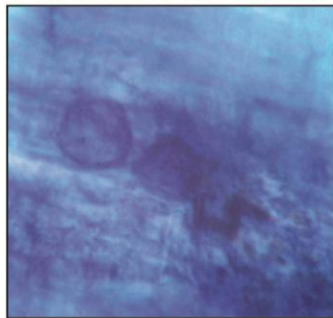
T2: T1+ Carbendazim



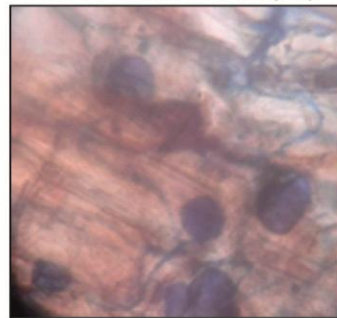
T3: T1+ Carboxin + Thiram



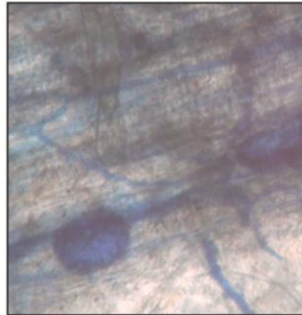
T4:T1+ *Trichoderma* (ST)



T5: T1+ Pendamethalin



T6: T1+ *Trichoderma* (SA)



T7 Control

Plate 2: Effect of different treatments on root colonization of VAM at 75 DAS

4.1.3 Effect of different treatments on mycorrhizal Spores

The mycorrhizal spores were counted in plots of different treatments and the data recorded is presented in Table and fig 3.

Table 3. Effect of different treatments on mycorrhizal spores in soil

Tr. No.	Treatments	Mycorrhizal Spores				
		15 DAS	30 DAS	45 DAS	60 DAS	75 DAS
T ₁	VAM @ 5kg/ha.	17.3	21.0	24.7	27.7	30.7
T ₂	T ₁ + Seed treatment with Carbendazim 50 WP @ 0.1%	9.7	11.3	14.3	17.0	19.3
T ₃	T ₁ + Seed treatment with Carboxin + Thirum @ 2.0 g/kg	10.3	12.7	15.3	16.7	18.7
T ₄	T ₁ + Seed treatment with <i>Trichoderma</i> @ 4g/kg	12.7	16.3	20.0	22.3	25.0
T ₅	T ₁ + Soil application of Pendamethelin @ 1.5 kg/ha	8.3	11.7	15.0	17.3	18.3
T ₆	T ₁ VAM + Soil application of <i>Trichoderma</i> @ 2.5 kg/ha	11.3	16.3	18.7	21.3	23.7
T ₇	Control	5.7	8.3	9.3	10.3	13.7
	F test	Sig	Sig	Sig	Sig	Sig
	SE(m)	0.45	0.42	0.35	0.45	0.60
	CD (0.05)	1.39	1.32	1.09	1.41	1.86

Maximum number of spores were observed in the treatment of soil application of VAM alone (T₁) which was 17.3, 21.00, 24.7, 27.7 and 30.7 at 15, 30, 45, 60 and 75 DAS respectively (plate 3 and 4). The observations of Orlando (2003) and Jalaluddin et al. (2008) supports this findings. The application of VAM along with *Trichoderma* as seed and soil application proved next best treatment, however the VAM spores decreased to 12.7, 16.3, 20.0, 22.3 and 25.0 in treatment T₄ i.e. VAM + seed treatment with *Trichoderma* at 15, 30, 45, 60 and 75 DAS respectively. In all the treatments

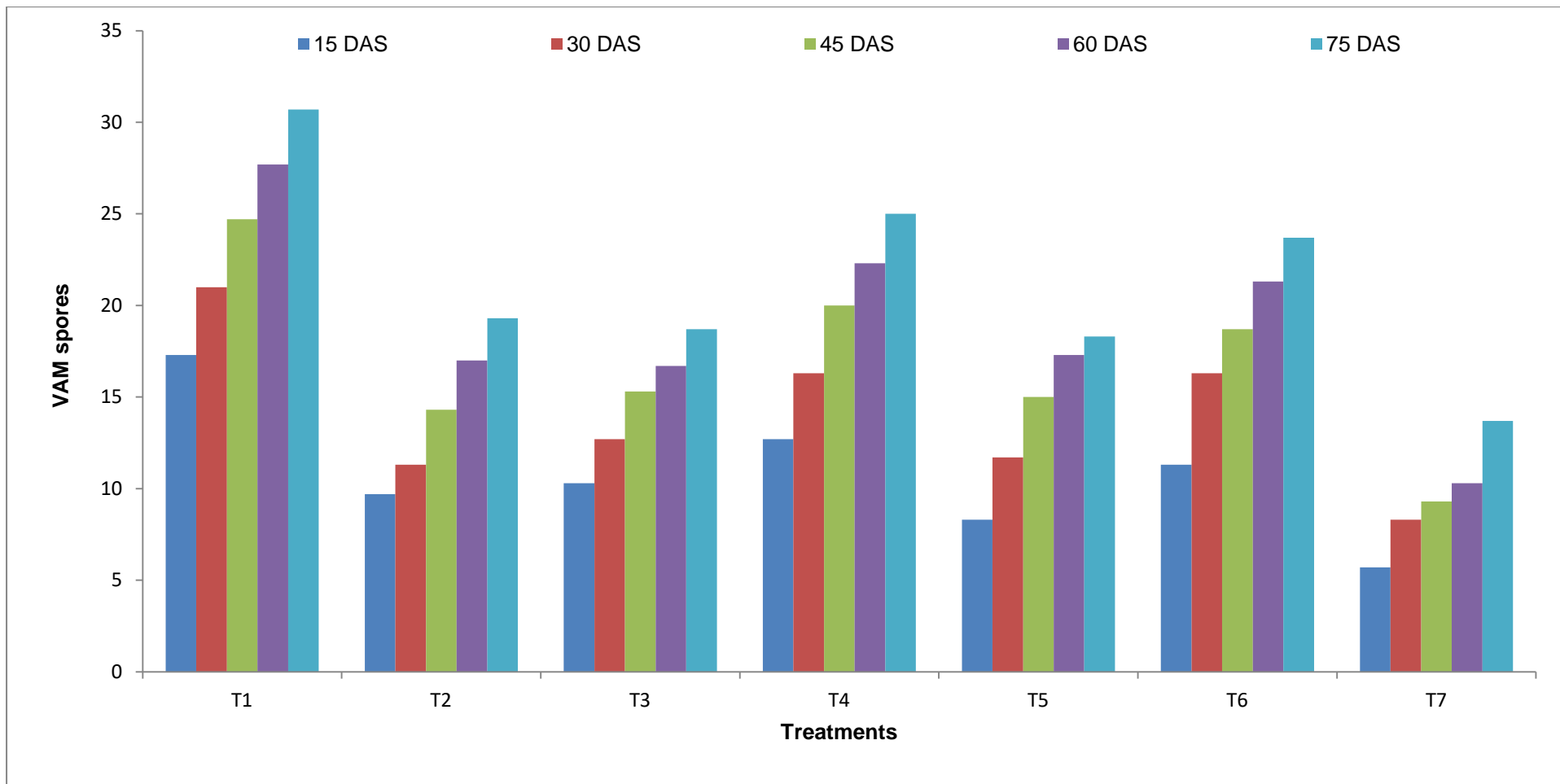
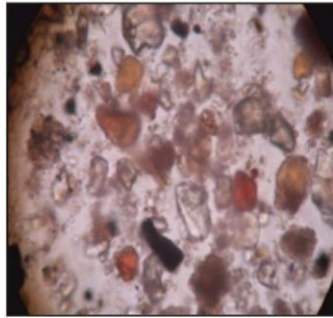
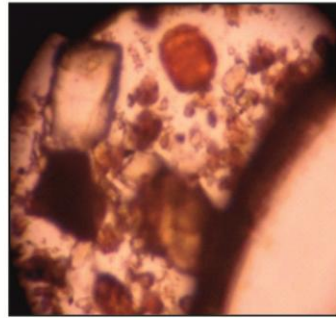


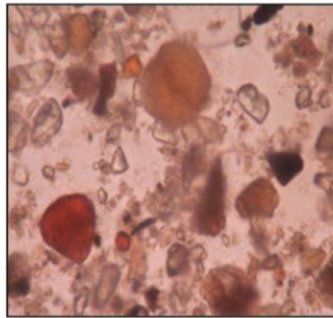
Fig. 3. Effect of different treatments on mycorrhizal spores in soil



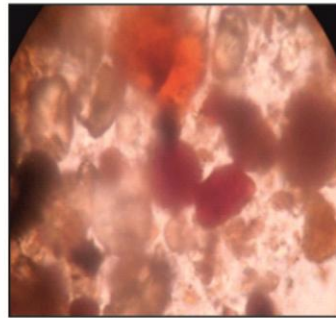
T1: VAM



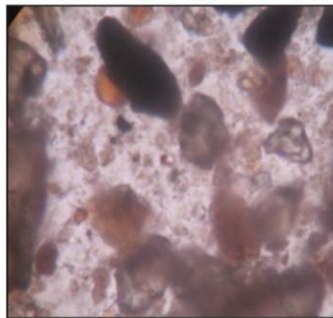
T2: T1+ Carbendazim



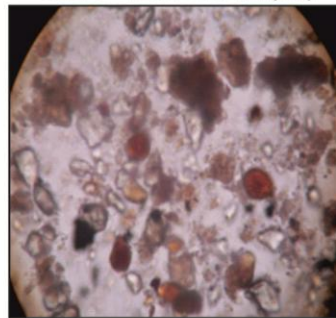
T3: T1+ Carboxin + Thirum



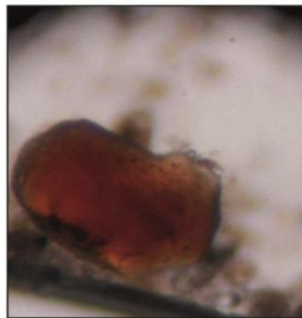
T4: T1+ *Trichoderma* (ST)



T5: T1+ Pendamethalin

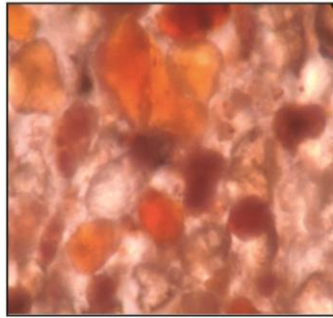


T6: T1+ *Trichoderma* (SA)

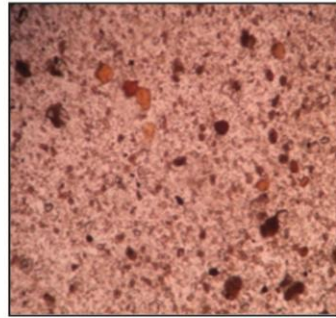


T7 Control

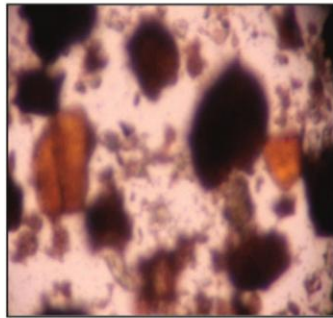
Plate 3: Effect of different treatments on mycorrhizal spores at 15 DAS



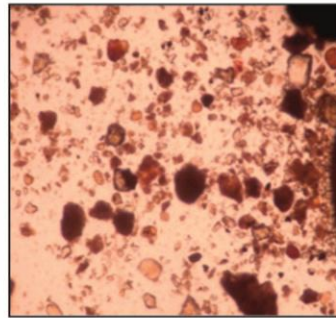
T1: VAM



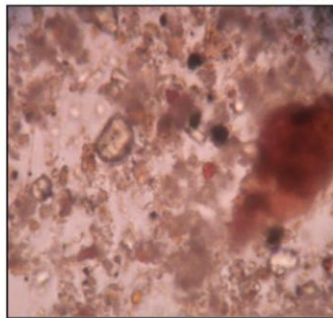
T2: T1+ Carbendazim



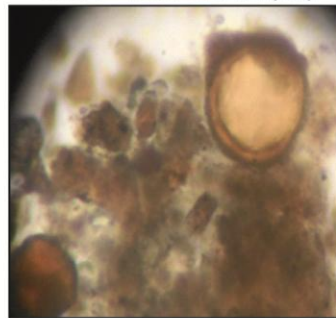
T3: T1+ Carboxin + Thirum



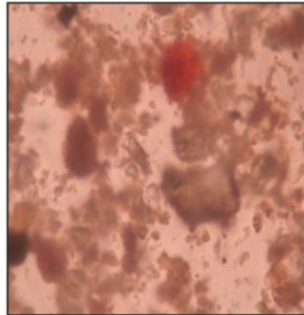
T4:T1+ *Trichoderma* (ST)



T5: T1+ Pendamethalin



T6: T1+ *Trichoderma* (SA)



T7 Control

Plate 4: Effect of different treatments on mycorrhizal spores at 75 DAS

mycorrhizal spores were more compared to control treatment from 15 to 75 DAS. The reduced number of mycorrhizal spores due to combined application of VAM with chemicals and bioagent compared to VAM application alone exhibited adverse effect of chemicals and bioagent on population of VAM which needs to be studied further, as earlier workers evaluated the effect of biofertilizers on performance of VAM but the effect of bioagents and chemicals on VAM could not be find in available literature.

4.1.4 Effect of different treatments on root rot incidence

The incidence of root rot disease was recorded at 15, 30, 45, 60 and 75 DAS and the data obtained in presented in Table and fig 4.

Table 4. Effect of different treatments on root rot incidence

Tr. No.	Treatments	Root Rot Incidence				
		15 DAS	30 DAS	45 DAS	60 DAS	75 DAS
T ₁	VAM @ 5kg/ha.	0.7	0.7	1.3	1.7	2.3
T ₂	T ₁ + Seed treatment with Carbendazim 50 WP@ 0.1%	0.0	1.3	1.3	2.7	3.0
T ₃	T ₁ + Seed treatment with Carboxin + Thirum@ 2.0 g/kg	0.0	1.0	1.7	2.7	3.0
T ₄	T ₁ + Seed treatment with <i>Trichoderma</i> @ 4g/kg	0.7	1.0	1.3	2.0	2.3
T ₅	T ₁ + Soil application of Pendamethelin @ 1.5 kg/ha	2.3	2.7	3.3	4.7	5.0
T ₆	T ₁ + Soil application of <i>Trichoderma</i> @ 2.5 kg/ha	0.0	0.7	1.3	2.3	2.7
T ₇	Control	4.0	4.7	5.3	6.0	6.7
	F test	sig	sig	sig	sig	Sig
	SE(m)	0.42	0.63	0.89	0.69	0.64
	CD (0.05)	1.29	1.98	2.78	2.16	2.01

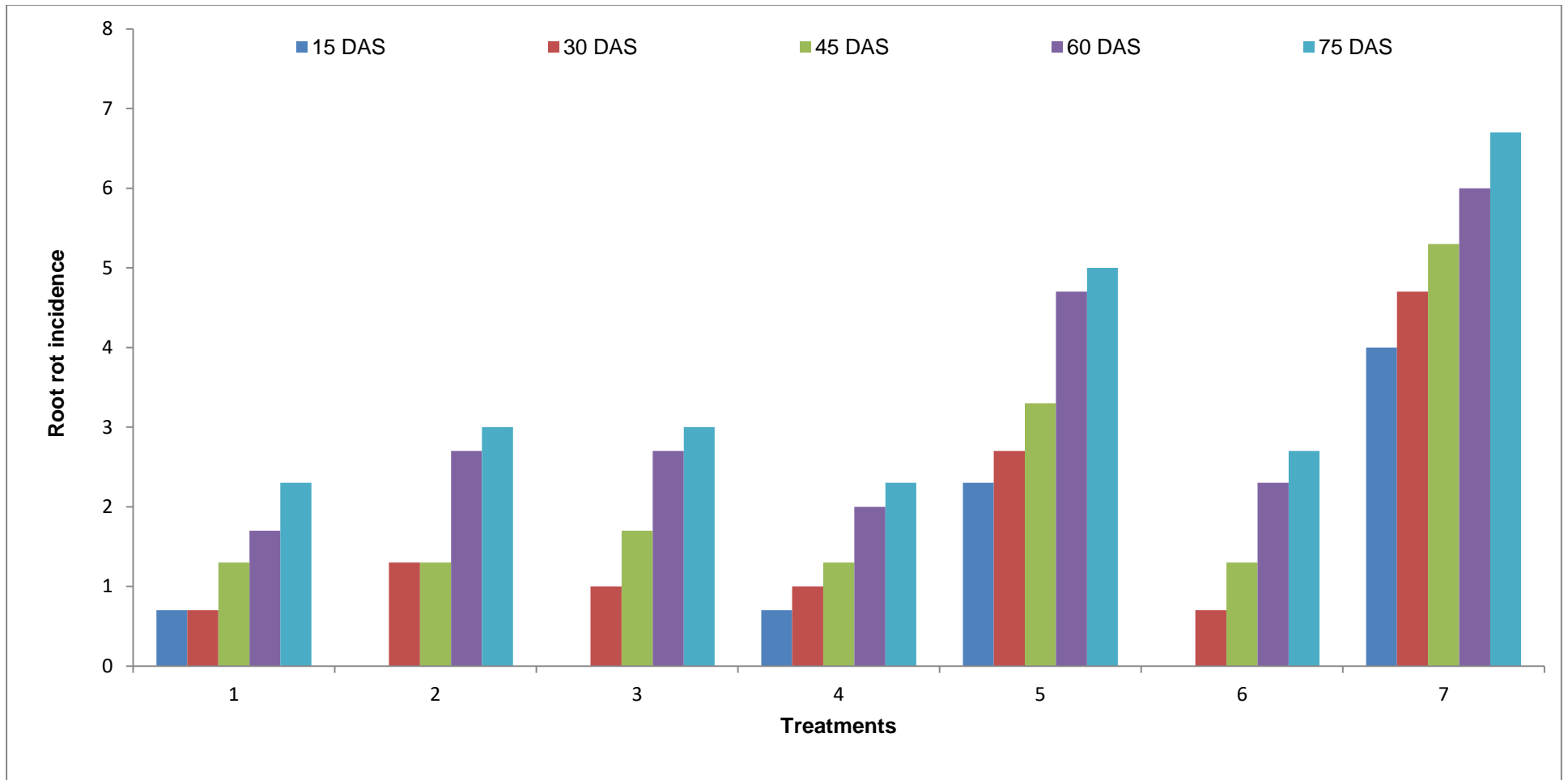


Fig. 4. Effect of different treatments on root rot incidence caused by *R. bataticola*

Progressive increase in root rot incidence was recorded during each observation which ranged from 4.0% to 6.7% in control treatment from 15 to 75 DAS respectively. The application of chemical fungicides i.e. carbendazim and Carboxin + Thirum protected the crop from infection by root rot pathogen upto 15 DAS. The herbicide Pendamethalin reduced the incidence (2.3%) as compared to control (4.0%) but failed to eliminate the incidence compared to chemical fungicides and bioagent. Although the application of VAM alone exhibited less control of root rot incidence compared to fungicides and bioagent initially, it checked the further spread of root rot incidence progressively compared to control which was 0.7, 0.7, 1.3, 1.7 and 2.3% compared to 4.0, 4.7, 5.3, 6.0 and 6.7% in control at 15, 30, 45, 60 and 75 DAS respectively. Akhtar and Siddique (2010) studied influence of four species of arbuscular mycorrhizal fungi namely *Glomus intraradices*, *G. aggregatum*, *G. clariodeum* and *Glomus sp.* for the control of root rot fungus *Macrophomina phaseolina* on chickpea under glasshouse conditions. Application of these AM fungi cause an increase of plant growth, pod number, nodulation, chlorophyll and N,P,K content in *Macrophomina phaseolina* inoculated plants and also reduced root rot index as observed by Akhtar and Siddique (2007). The findings of Liossanne (2010) also flowed in same direction. Olagunju et al. (2014) obtained similar result in sorghum against Diaback and Necrosis. The result of present study is in line of agreement of these findings.

4.1.5 Effect of different treatments on population of *Rhizoctonia bataticola*

Population of *Rhizoctonia bataticola* as estimated from plots of different treatments is depicted in Table and fig. 5.

Table 5 indicated statistically significant differences in population of *R. bataticola* due to different treatments. Treatment T₁ i.e. soil application of VAM exhibited minimum population of *Rhizoctonia bataticola* which was 0.7 x 10⁴, 0.7 x 10⁴, 1.0 x 10⁴, 1.0 x 10⁴ and 1.3 x 10⁴ cfu/g of soil at 15, 30, 45, 60 and 75 DAS as against to 3.0 x 10⁴, 3.7 x 10⁴, 4.7 x 10⁴, 5.3 x 10⁴ and 5.7 x 10⁴ cfu/g of soil respectively in control. VAM application along with chemical fungicides, herbicide and biocontrol agent *Trichoderma* resulted in increased

population of *R. bataticola* compared to its alone application however all these combinations yielded less number of cfu of *R. bataticola* compared to control. The increase in population of *R. bataticola* from 15 to 75 DAS was minimum in VAM application whereas the increase in population at different interval was more in other treatments and maximum in control.

Table 5. Effect of different treatments on population of *Rhizoctonia bataticola* at different interval

Tr. No.	Treatments	Population of <i>Rhizoctonia bataticola</i> (cfu X 10 ⁴ /g)				
		15 DAS	30 DAS	45 DAS	60 DAS	75 DAS
T ₁	VAM @ 5kg/ha.	0.7	0.7	1.0	1.0	1.3
T ₂	T ₁ + Seed treatment with Carbendazim 50 WP @ 0.1%	1.3	1.7	2.0	2.3	2.7
T ₃	T ₁ + Seed treatment with Carboxin + Thirum @ 2.0 g/kg	1.3	1.3	1.7	2.0	2.3
T ₄	T ₁ + Seed treatment with <i>Trichoderma</i> @ 4g/kg	1.0	1.0	1.3	1.7	2.0
T ₅	T ₁ + Soil application of Pendamethelin @ 1.5 kg/ha	1.7	1.7	2.3	2.7	3.0
T ₆	T ₁ + Soil application of <i>Trichoderma</i> @ 2.5 kg/ha	1.3	1.3	1.7	2.0	2.3
T ₇	Control	3.0	3.7	4.7	5.3	5.7
	F test	Sig	Sig	Sig	Sig	Sig
	SE(m)	0.38	0.40	0.39	0.57	0.65
	CD (0.05)	1.18	1.23	1.22	1.78	2.03

The data of present study showed positive effect of VAM application in reducing population of *R. bataticola*. However, its decreased effectivity in combination with fungicides, herbicide and also with *Trichoderma* might be due to the adverse effect of these chemicals/ bioagent on

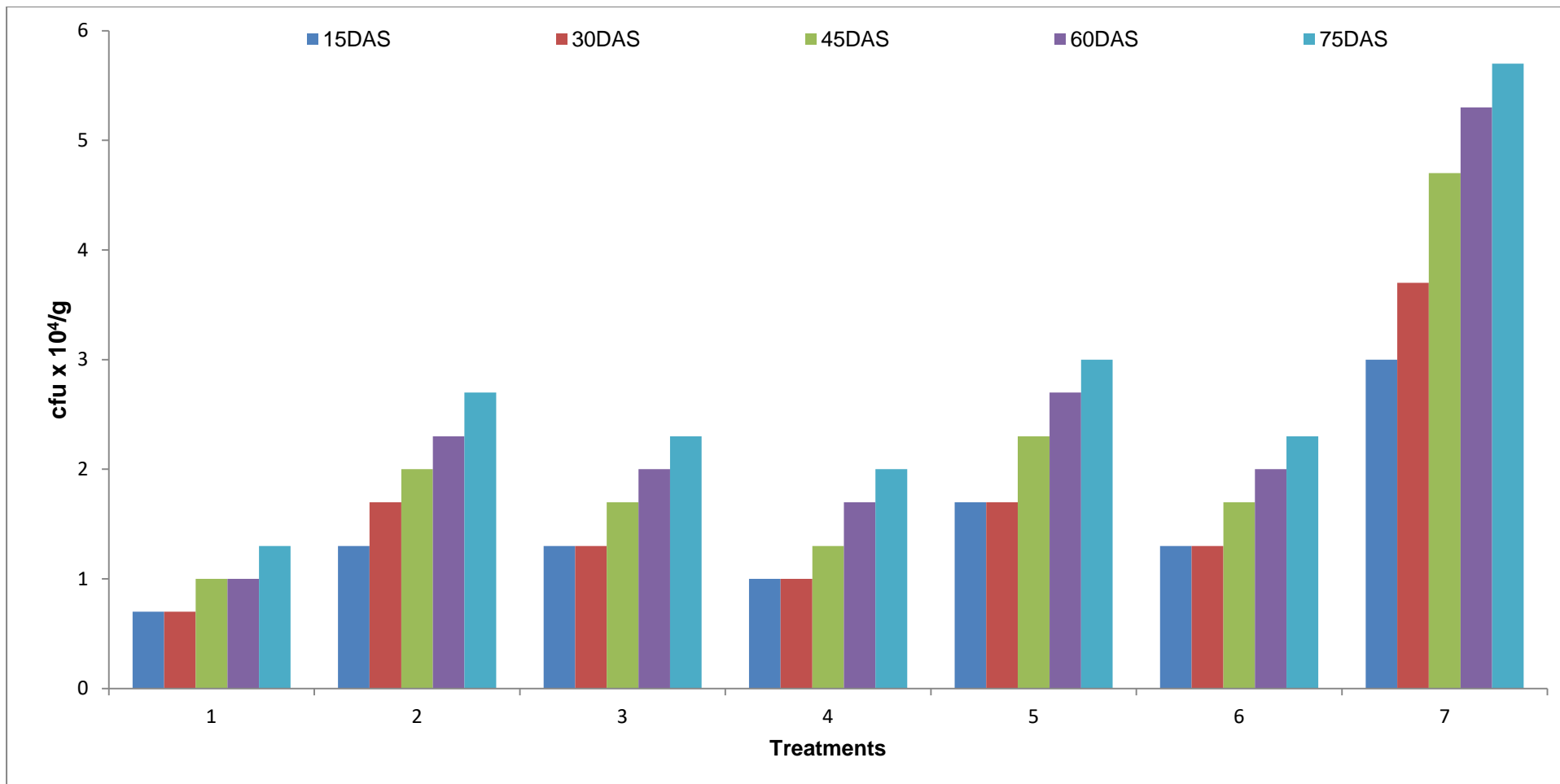


Fig. 5. Effect of different treatments on population of *Rhizoctonia bataticola*

performance and survival of VAM which was also noticed and observed as presented in Table 2 and 3 of this chapter.

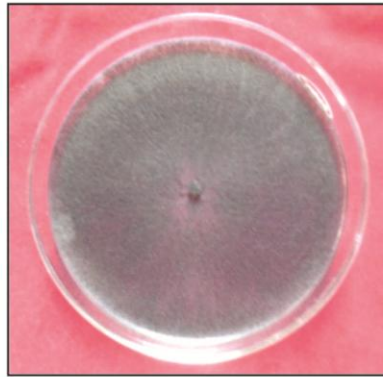
4.2 Pathogenicity Test of *Rhizoctonia bataticola*

The infected leaves of chickpea showing typical symptoms of root rot were used for isolation of the pathogen. The isolated pathogen was inoculated on healthy plants by soil inoculum method and then re-isolated from plants showing symptoms of root rot to confirm that the *R. bataticola* was pathogenic to chickpea (plate 5). The fungus grew rapidly on potato dextrose agar (PDA) and produced brown to grey coloured mycelium that became darker with age. The hypha was thin, hyaline, aseptate and dichotomously branched and later produced typical black sclerotia. After proving pathogenicity of *R. bataticola* the pot culture experiment was conducted in *R. bataticola* sick soil. Amrutha et al. (2014b) also proved the pathogenicity of *R. bataticola* in chickpea by soil inoculation method.

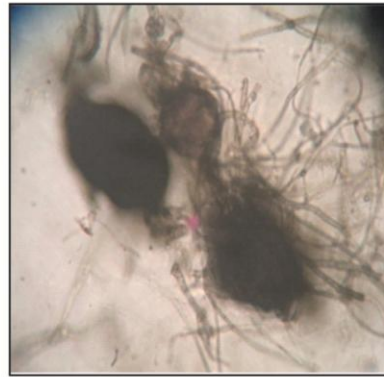
4.2.1 Effect of different treatments of mycorrhizal spores in pot culture

To confirm the trend of data observed in field condition the same experiment was also conducted in pot culture and the data obtained is depicted in Table and fig 6.

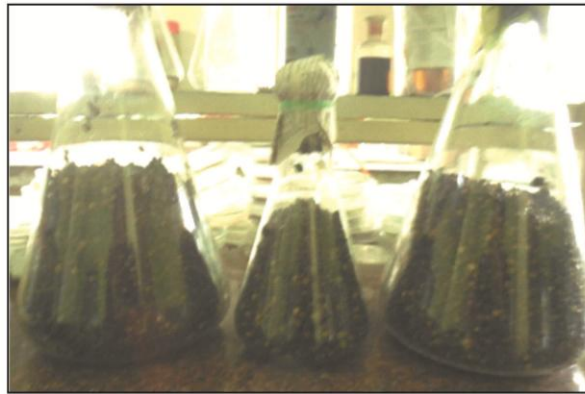
Table 6 revealed that maximum number of mycorrhizal spores was recorded in the treatment of VAM application alone i.e. 3.3, 5.3, 9.0, 13.3 and 14.0 at 15, 30, 45, 60 and 75 DAS. The trend of the results was same as obtained in field experiment. However under field condition, control treatment also exhibited some VAM spores might be due to migration of spores from one plot to another which is restricted in pot culture. Obviously no mycorrhizal spores was obtained in control treatment during each observation as it was completely devoid of VAM application. In pot culture experiment also the fungicides and herbicide exhibited adverse effect on VAM spores as their number get reduced in combination treatments compared to alone treatment of VAM. Although during the observations application of VAM with *Trichoderma* either as seed treatment or soil application yielded statistically at par mycorrhizal spores with alone application of mycorrhiza, the mycorrhizal spores get reduced with *Trichoderma* also at 75 DAS.



Rhizoctonia bataticola



Sclerotia



Mass culture of *Rhizoctonia bataticola*



Healthy plant



Infected plant

Plate 5: Pathogenicity test of *Rhizoctonia bataticola* *in-vitro*

Table 6. Effect of different treatment on mycorrhizal spores in pot culture

Tr. No.	Treatment	Mycorrhizal spores in pot culture				
		15 DAS	30 DAS	45 DAS	60 DAS	75 DAS
T ₁	VAM @ 5kg/ha.	3.3	5.3	9.0	13.3	14.0
T ₂	T ₁ + Seed treatment with Carbendazim 50 WP @ 0.1%	2.0	3.3	6.0	7.0	7.3
T ₃	T ₁ + Seed treatment with Carboxin + Thirum @ 2.0 g/kg	2.3	4.0	7.0	7.0	9.7
T ₄	T ₁ + Seed treatment with <i>Trichoderma</i> @ 4g/kg	3.3	5.3	12.0	13.0	9.3
T ₅	T ₁ + Soil application of Pendamethelin @ 1.5 kg/ha	2.3	3.7	6.3	5.3	6.3
T ₆	T ₁ + Soil application of <i>Trichoderma</i> @ 2.5 kg/ha	3.3	5.0	7.7	11.0	11.7
T ₇	Control	0.0	0.0	0.0	0.0	0.0
	F test	Sig	Sig	Sig	Sig	Sig
	SE(m)	0.34	0.54	0.44	0.85	1.56
	CD (0.05)	1.05	1.67	1.38	2.65	4.87

From the studies of field and pot culture experiment it is evident that VAM alone performed better in suppressing the root rot incidence and population of *R. bataticola* and its application with chemicals and bioagent had adverse effect on root colonization and population of VAM in chickpea.

4.2.2 Effect of bioagent and fungicides on growth of *Rhizoctonia bataticola* in vitro

Rhizosphere mycoflora has an ability to inhibit the growth of disease causing pathogen. Therefore antagonistic effects of *Trichoderma* were assessed against the pathogenic fungi *R. bataticola* by dual culture method (plate 6a) and the effect of fungicide viz., Carboxin + Thirum (0.3%)

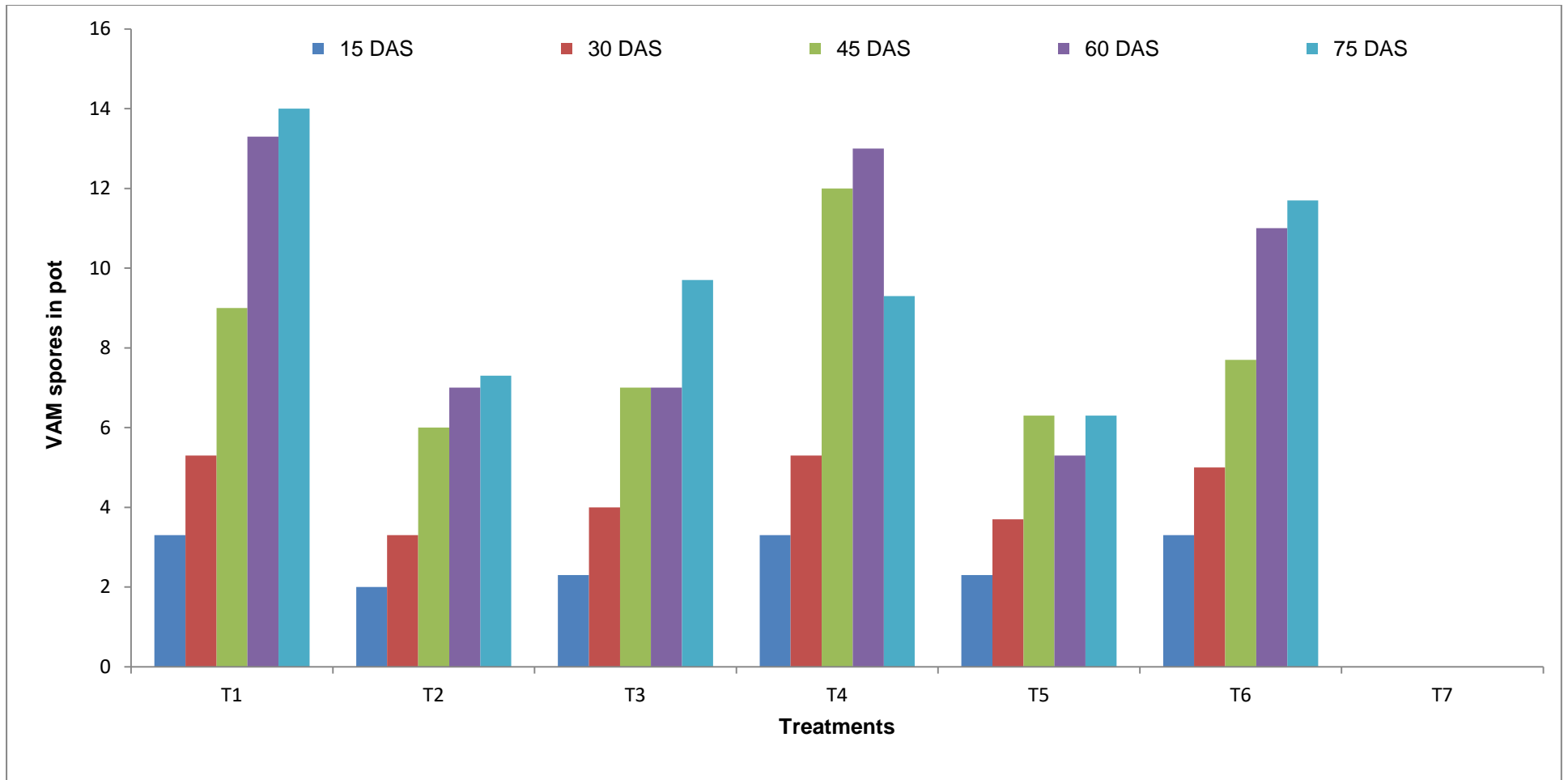


Fig. 6. Effect of different treatment on mycorrhizal spores in pot culture

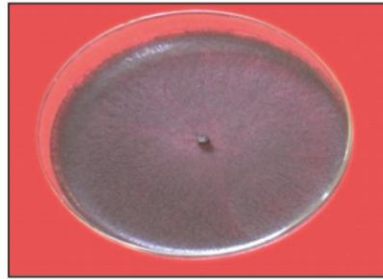
and Carbendazim (0.1%) were evaluated against this pathogen by poison food technique (plate 6b). The growth inhibition of *R. bataticola* due to *Trichoderma* and chemical fungicides is calculated and presented in Table 7.

Table 7. Efficacy of bioagents and fungicides against *Rhizoctonia bataticola* by dual culture and poison food method

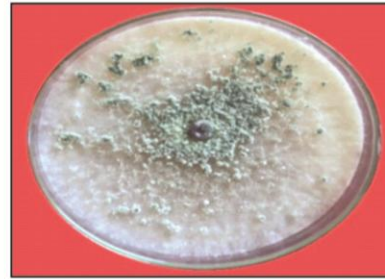
Particulars	Mean radial mycelial growth (mm) of <i>R. bataticola</i>	Growth inhibition (%)
(Bioagent)		
1. <i>Trichoderma</i>	44.16	50.9
2. Control	90	-
(Fungicides)		
1. Carboxin + Thirum (0.3%)	0	100
2. Carbendazim (0.1%)	0	100
3. Control	90	-

Radial mycelial growth of *R. bataticola* in presence of *Trichoderma* was recorded as 44.16 mm with inhibition of *R. bataticola* by 50.9%. Reetha et al. (2014) reported that *Trichoderma sp.* was the most antagonists microorganism to charcoal rot disease pathogen i.e. *Macrophomina phaseolina* and reduced the colony growth of *Macrophomina phaseolina* significantly compared to the control. Viswanathan et al. (2015) and Rasmi (2012) also demonstrated strong inhibition of *R. bataticola/ M. phaseolina* by *Trichoderma* in vitro.

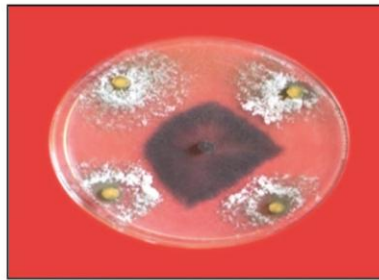
Both the tested fungicides i.e. Carboxin + Thirum (0.3%) and Carbendazim (0.1%) were found effective for arresting 100% myelial growth of *Rhizoctonia bataticola*. Khan et al. (2012) also reported 100% mycelial inhibition of *Rhizoctonia bataticola* in dry root rot of chickpea with Carbendazim (0.1%).



Rhizoctonia bataticola

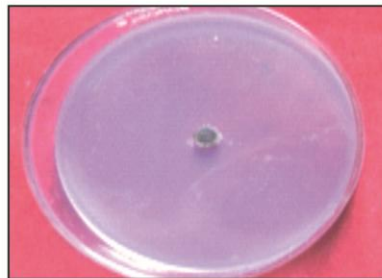


Trichoderma viride



Rhizoctonia bataticola + *Trichoderma viride*

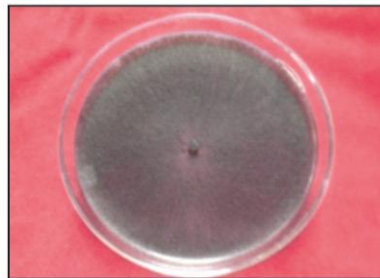
Plate 6a: *In vitro* efficacy of *Trichoderma viride* against *Rhizoctonia bataticola*



Carboxin + *Thirum*



Carbendazim



Control

Plate 6b: *In vitro* efficacy of fungicides against *Rhizoctonia bataticola*

CHAPTER V

SUMMARY AND CONCLUSION

Chickpea is third important legume crop in world after bean and peas. It is first important pulse crop in India being grown in largest area in *rabi* season. Among number of biotic and abiotic constrains in chickpea root rot caused by *Rhizoctonia bataticola* is becoming severe threat in its cultivation with the changing disease scenario under changing climatic condition which causes heavy losses in grain yield.

The experiment entitled “Mycorrhizal effect on root rot of chickpea caused by *Rhizoctonia bataticola*” was carried out during 2016-17 at Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola with the objectives to study the effect of mycorrhiza on root rot of chickpea and to know the effect of fungicides, bioagents and herbicides on mycorrhiza.

The observations regarding morphology of spore exhibited globuse structure of vesicles formed intracellularly and golbuse, smooth or shiny roughened spore. The spore colour was light yellow to bright yellow, transparent and matured spore were brown black to dark black. There was hypha attachment with few spore but some spore showed straight to recoverd funnel shape suspending hyphae which was yellow to brown. This morphological features were identical with the genus *Glomus* and on that basis spores were confirmed as *Glomus*.

The experiment was laid out in Randomized Block Design in which treatments were replicated thrice. Mycorrhiza was applied to soil during *kharif* and sorghum was sown to multiply mycorrhiza in the field. After harvest of sorghum crop the root debris was mixed in the soil and the experiment was conducted on same piece of land on chickpea during *rabi*. The treatments were T₁ (Soil application of Mycorrhiza @ 5kg/ha), T₂ (T₁ + Seed treatment with Carbendazim 50 WP @ 0.1%), T₃ (T₁ + Seed treatment with Carboxin + Thirum @ 2.0 g/kg),T₄ (T₁ + Seed treatment with *Trichoderma* @ 4g/kg),T₅ (T₁ + Soil application of Pendamethelin @ 1.5 kg/ha),T₆ (T₁ + Soil application

of *Trichoderma* @ 2.5 kg/ha) and T₇ (Control). Chickpea variety Digvijay was used for experiment.

The diseased samples of root rot infected plants were collected from field of Department of Plant Pathology and fungal pathogen *Rhizoctonia bataticola* was isolated on Potato Dextrose Agar medium. *R. bataticola* produced a white septate mycelia growth, latter turned dark grey, produced profuse, dark brown, irregular round shaped sclerotia on PDA medium.

The number of root nodules/ plant was increased considerably in all treatments compared to control. The maximum root nodules were found in treatment T₁ i.e. VAM @ 5kg/ha which was 4.4, 6.2 and 8.0 at 30, 45 and 60 DAS respectively.

The percent root colonization of mychorrhiza in chickpea was increased from 15 to 75 DAS. The root colonization was maximum in T₁ (application of VAM @ 5kg/ha) i.e. 4.7, 8.3, 13.7, 16.3, 18.7% followed by combined application of VAM + seed treatment with *Trichoderma* @ 4g/kg and VAM + soil application of *Trichoderma* @ 2.5 kg/ha. The VAM when used alone exhibited more colonization in roots than the treatments having combination of VAM with other chemical/ bioagent.

The vesicular arbuscular mychorrhizal spore density was estimated by following wet sieving and decanting method. The inoculation of VAM alone @ 5kg/ha recorded maximum VAM spores as compared to other treatments i.e. 17.3, 21.0, 24.7, 27.7 and 30.7 at 15, 30, 45, 60, 75 DAS respectively followed by combined application of T₄ VAM + seed treatment with *Trichoderma* @ 4g/kg which was 12.7, 16.3, 20.0, 22.3 and 25.0 at 15, 30, 45, 60 and 75 DAS respectively. The control treatment recorded least VAM spores than all other treatments having 5.7, 8.3, 9.3, 10.3 and 13.7 spores at 15, 30, 45, 60 and 75 DAS respectively. All treatments were significantly superior over control.

The minimum root rot incidence was recorded in T₁ (VAM @ 5kg/ha) i.e. 0.7, 0.7, 1.3, 1.7 and 2.3 and the maximum was observed in control plots (4.0, 4.7, 5.3, 6.0 and 6.7%) at 15, 30, 45, 60 and 75 DAS respectively. The population of *Rhizoctonia bataticola* was minimum in alone

applied VAM plots i.e. 0.7×10^4 , 0.7×10^4 , 1.0×10^4 , 1.0×10^4 and 1.3×10^4 cfu/g of soil at 15, 30, 45, 60 and 75 DAS compared to 3.0×10^4 , 3.7×10^4 , 4.7×10^4 , 5.3×10^4 and 5.7×10^4 cfu/g of soil in control plots.

The pathogenicity of *R. bataticola* was proved and observed the mortality to the extent of infection caused by *R. bataticola* to chickpea seedling which showed reddish brown discolouration at the emerging portion of hypocotyls. Seedling of ten to twelve days appeared sick and collapse and uprooted easily and roots were rotted. The uprooted seedlings showed greyish black discolouration of the roots.

In the pot experimentation the mycorrhizal spores was recorded as 3.3, 5.3, 9.0, 13.3 and 14.0 in the alone treatment of VAM @ 5kg/ha at 15, 30, 45, 60 and 75 DAS respectively and no mycorrhizal spores was obtained in control treatment during each observation.

The poisoned food technique was employed to test the efficacy of commonly used fungicides against *Rhizoctonia bataticola*. Carbendazim (0.1%) and Carboxin + Thirum (0.3%) were efficient in completely inhibiting the growth of *R. bataticola* (100%) *in vitro*. The biocontrol agent *Trichoderma* was found antagonistic and the *in vitro* mycelial inhibition of *Rhizoctonia bataticola* was 50.9% due to this bioagent.

Conclusion

Following conclusions were drawn from the present study.

- There was considerable increase in nodules due to application of VAM at 30, 45 and 60 DAS.
- The percent root colonization of VAM and VAM spore density was more in inoculation of VAM alone @ 5kg/ha than the combined treatments of VAM + other chemicals/ bioagent.
- The root rot incidence was minimum in due to alone application VAM followed by combined application of VAM + *Trichoderma* (ST).
- The population of *Rhizoctonia bataticola* was reduced in VAM applied plots than control and the minimum was due to alone application of VAM.

- Fungicides Carbendazim @ 0.1% and Carboxin + Thirum @ 0.3 % completely inhibited the growth of *Rhizoctonia bataticola* *in vitro*.
- The biocontrol agent *Trichoderma viride* was found antagonistic to *Rhizoctonia bataticola* and inhibited the growth of *R. bataticola* by 50.9% *in vitro*.

CHAPTER VI

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