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Darshana Uikey
Department of Plant Pathology, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidhyapeeth, Akola, Maharashtra, India

VR Gupta
Department of Plant Pathology, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidhyapeeth, Akola, Maharashtra, India

KW Uikey
Department of Plant Pathology, Mahatma Phule Agriculture University, Rahuri, Maharashtra, India

Correspondence
Darshana Uikey
Department of Plant Pathology, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidhyapeeth, Akola, Maharashtra, India

Mycorrhizal effect on root rot of chickpea caused by *Rhizoctonia bataticola*

Darshana Uikey, VR Gupta and KW Uikey

Abstract
The present investigation was carried out to study the effect of mycorrhiza on root rot of chickpea. 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 maximum number of mycorrhizal spores was 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.

Keyword: mycorrhizal, root rot, chickpea caused, *Rhizoctonia bataticola*

Introduction
Chickpea (*Cicer arietinum* L.) is leguminous pulse crop which belongs to Leguminosae 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). 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 30^0C.

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.

Material and Methods
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 fiel0d was allowed to

The sorghum 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.

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

Treatment details (*Rabi* season)

T_1: Soil application of Mycorrhiza @ 5kg/ha.
T_2: T_1 + Seed treatment with Carbendazim 50 WP (0.1%)
T_3: T_1 + Seed treatment with Carboxin + Thirum @ 2.0 g/kg
T_4: T_1 + Seed treatment with *Trichoderma* @ 4g/kg
T_5: T_1 + Soil application of Pendamethelin @ 1.5 kg/ha
T_6: T_1 + Soil application of *Trichoderma* @ 2.5 kg/ha
T_7: Control.

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.

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 produres, which use chemical agents to remove cell contents and cell wall pigments, are routinely used to view internal features in plant tissues. (Gardner 1975) [4].

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 in 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/cm^2.
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.

$$\text{Percentage of root colonization} = \frac{\text{Number of root pieces contains vesicles}}{\text{Total number of root pieces observed}} X\ 100$$

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, 75µm and 45µm sieves consequently.
7. The entire residue was collected on 45µm 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.

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 45^0C 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 ± 2^0C for 48 hrs and observations regarding growth were recorded.

Results and Discussion

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.

Effect of different treatments on number of nodules per plant

Data presented in Table 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	45DAS	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 *Trichoderma* @ 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 *Trichoderma* @ 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 T_1 i.e. soil application of VAM @ 5kg/ha recorded significantly more number of nodules (4.4) than all other treatments followed by the treatment T_4 (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 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).

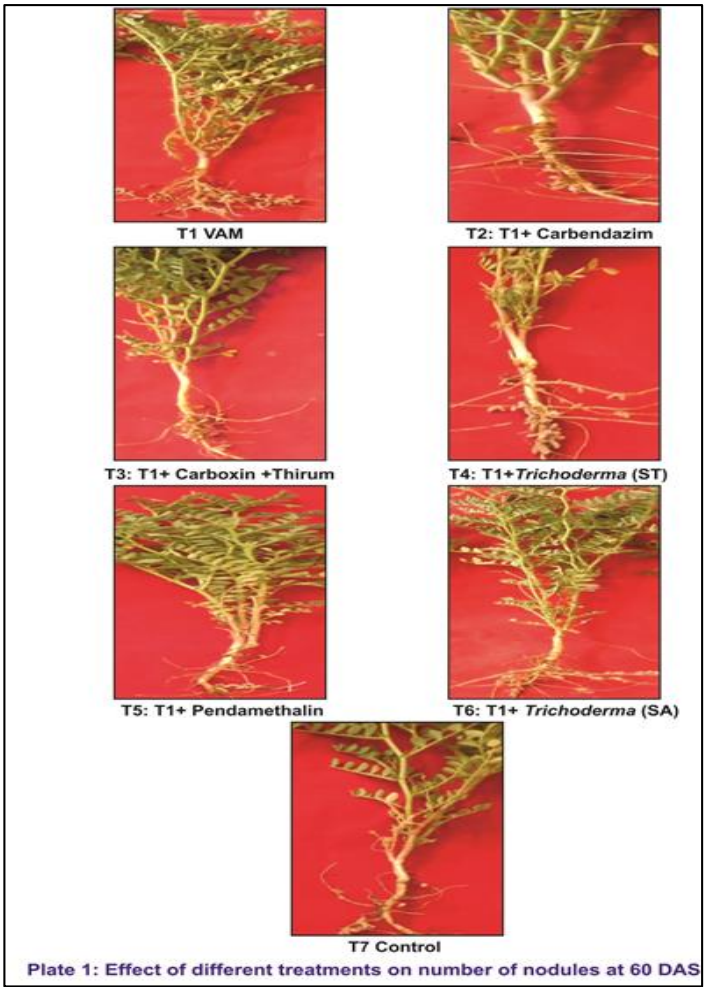

Plate 1: Effect of different treatments on number of nodules at 60 DAS

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) [5] and Pezeshkpour *et al*. (2014). Wichman *et al*. (2009) [14] opioned increasing evidence for promoting effects of AMF on rhizobial nodule number and weight, plant dry matter and soil mineral N. Sadhana (2014) [12] 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.

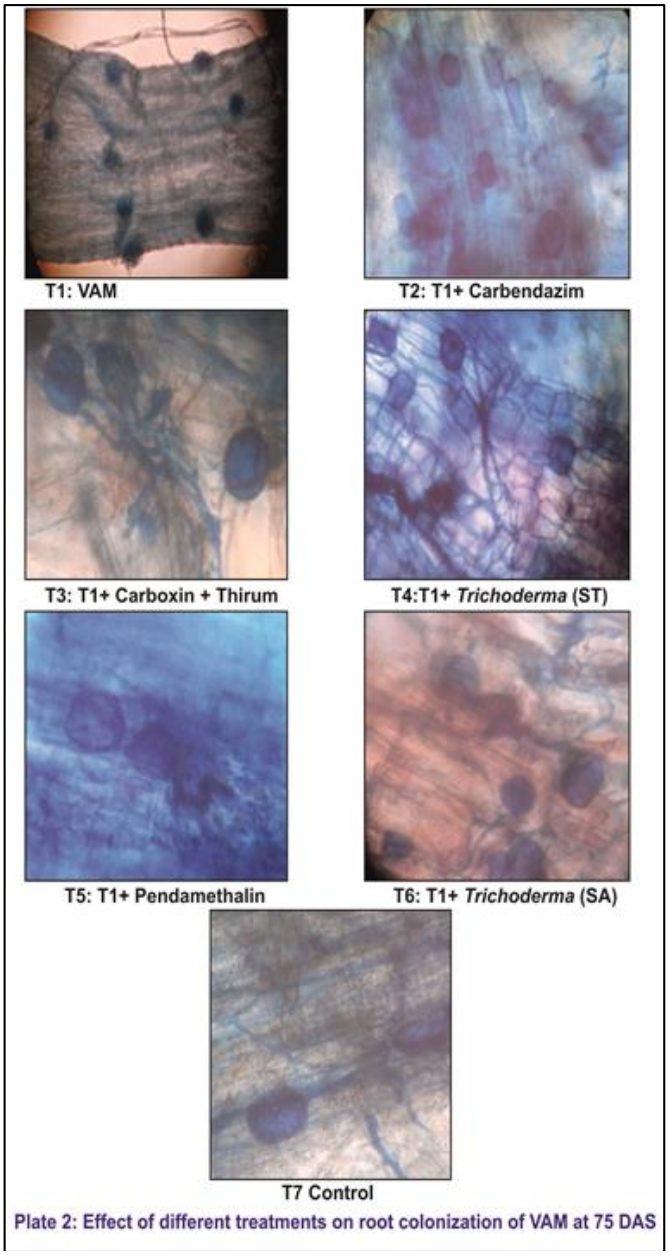

Plate 2: Effect of different treatments on root colonization of VAM at 75 DAS

Effect of different treatments on percent root colonization of VAM

Table 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 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 *Trichoderma* @ 4g/kgs	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 *Trichoderma* @ 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

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 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 *Trichoderma* @ 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_1VAM + Soil application of *Trichoderma*@ 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 (T_1) 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) [10] and Jalaluddin *et al*. (2008) [6] 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 T_4 i.e. VAM + seed treatment with *Trichoderma* at 15, 30, 45, 60 and 75 DAS respectively. In all the treatments 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 bio agent compared to VAM application alone exhibited adverse effect of chemicals and bio agent 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.

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 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 *Trichoderma* @ 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 *Trichoderma* @ 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

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) [1] 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) [7] also flowed in same direction. Olagunju *et al*. (2014) [9] obtained similar result in sorghum against Diaback and Necrosis. The result of present study is in line of agreement of these findings.

Effect of different treatments on population of *Rhizoctonia bataticola*

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

Table 5 indicated statistically significant differences in population of *R. bataticola* due to different treatments. Treatment T_1 i.e. soil application of VAM exhibited minimum population of *Rhizoctonia bataticola* which was 0.7 x 10^4, 0.7 x 10^4, 1.0 x 10^4, 1.0 x 10^4 and 1.3 x 10^4 cfu/g of soil at 15, 30, 45, 60 and 75 DAS as against to 3.0 x 10^4, 3.7 x 10^4, 4.7 x 10^4, 5.3 x 10^4 and 5.7 x 10^4 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 *Rhizoctonia bataticola* (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 *Trichoderma* @ 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 *Trichoderma* @ 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 performance and survival of VAM which was also noticed and observed as presented in Table 2 and 3.

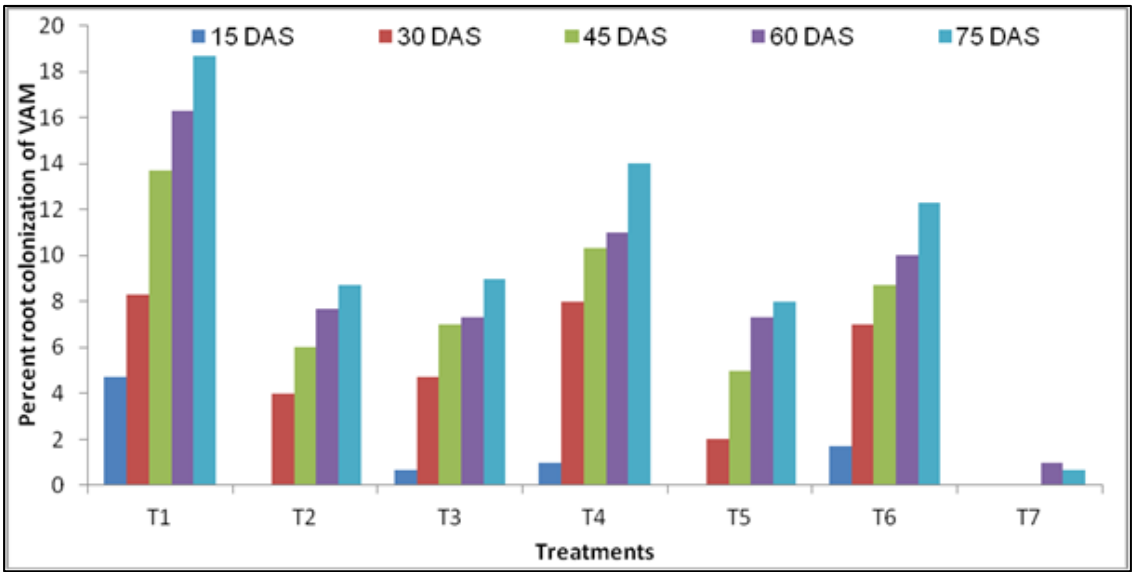

Fig 1: Effect of different treatments on percent root colonization of VAM

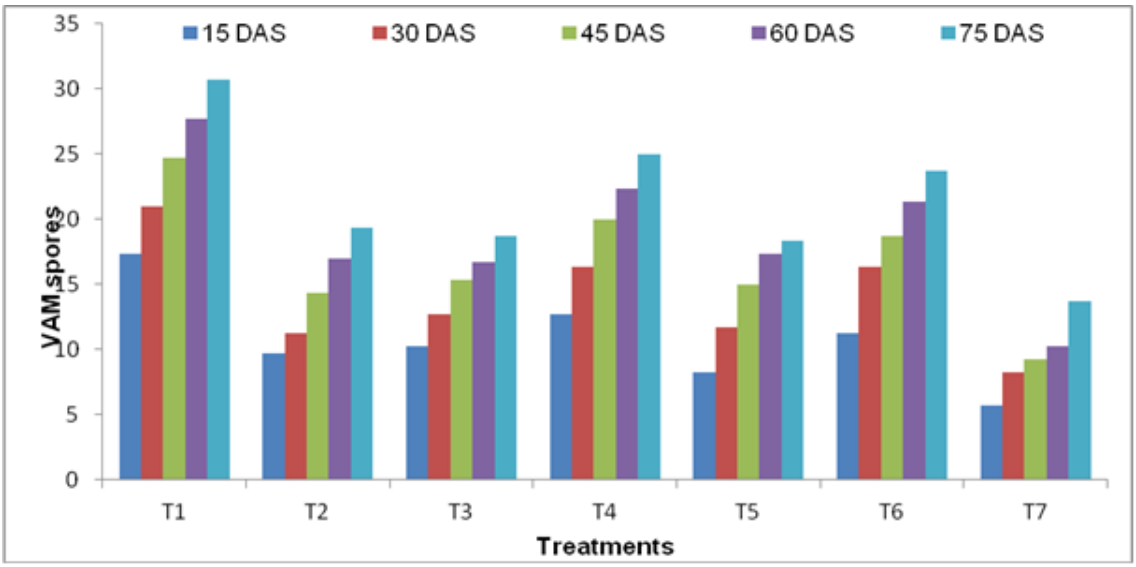

Fig 2: Effect of different treatments on mycorrhizal spores in soil

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