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Research article

INTEGRATED DISEASE MANAGEMENT OF DRY ROOT ROT OF CHICKPEA

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ABSTRACT: The effect of different organic amendments like FYM, vermicompost and neem cake individually and in combination with potential fungicidal tolerant fungal antagonist *Trichoderma* isolate-7 (CT7), bacterial antagonist CREB-16 screened from dual culture studies, fungicide copper oxychloride were tested against *Rhizoctonia bataticola* in pot culture under green house conditions. The results revealed that treatment T_8 i.e seed treatment with fungicide (Copper oxychloride) + soil application of potential fungal (*Trichoderma isolate-7)* and bacterial biocontrol agent (CREB-16) was found to be superior as it recorded the highest germination percentage (100 %), highest initial (10.00) and final population of chickpea (9.66), least PDI (16.00 %), maximum plant height (25.61 cm), root length (13.40 cm) and maximum shoot (0.49 g) and root dry weights (0.11 g). The population dynamics of both antagonists and pathogen were estimated at two different intervals initially at 7 DAS and then on 45 DAS in pot culture experiment. With the increase in antagonists population, the population dynamics of pathogen were significantly reduced from 7 DAS to 45 DAS over control in all the treatments with the maximum reduction in T_8 treatment.

Key words: Chickpea, *Rhizoctonia bataticola*, *Trichoderma*, Endophytic bacteria, copper oychloride, organic amendments

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INTRODUCTION

Chickpea is the third important legume grown in about 50 countries around the world. India is the leading producer of chickpea contributing to about 70 per cent of the world's chickpea production. Among the major pulse crops, chickpea contributes nearly 42 to 47 per cent of total pulse production. In Andhra Pradesh, it is grown in an area of 5.84 lakh ha with an annual production and productivity of 7.19 lakh tonnes and 1233 kg ha^{-1} respectively (Annual Report, 2010-2011). Chickpea suffers from several soil borne fungal diseases among these, dry root rot (*Rhizoctonia bataticola* (Taub.) Butler) cause considerable yield losses in chickpea which may be as high as 50 to 71 per cent (Ahmed and Mohammad, 1986). Presently there are no resistant varieties to dry root rot disease in chickpea. Biological control using microbial antagonists is considered as good alternative of management of root diseases in many crops. Integrated disease management is gaining importance which involves blending of compatible systems of control measures for effective management of disease from profitability to food and environmental safety (Jacksen and Backman, 1993).

MATERIALS AND METHODS

Isolation of pathogen

The pathogen was isolated from dry root rot infected chickpea plants by using tissue segment method (Rangaswamy and Mahadevan, 1999). The pathogen culture was purified by single hyphal tip method and maintained on PDA by periodical transfer throughout the present investigation.

Mass multiplication of *Rhizoctonia bataticola*

The test pathogen *Rhizoctonia bataticola* was mass multiplied on sterilized sorghum seeds for pot culture studies. For this, 100g of sorghum seeds were washed thoroughly in tap water and soaked in water overnight in 250 ml conical flask with addition of 20 ml of 4 per cent dextrose. After removing the water, the flasks were autoclaved for 20 min at 15 p.s.i and inoculated with 2-3 discs of 4 days old culture of test pathogen. After seven days the inoculum was mixed with sterilized soil in pots @100g kg^{-1}.

Isolation of *Trichoderma* from Chickpea rhizosphere

Composite soil sample was collected from rhizosphere of healthy chickpea plants. *Trichoderma* were isolated by following serial dilution technique (Johnson and Curl, 1977) using *Trichoderma* selective medium. Three days old colonies of *Trichoderma* were picked up and purified by single spore/hyphal tip method. Among 10 *Trichoderma* isolates, CT-7 showed highest inhibition (83.33%) in dual culture studies against *Rhizoctonia bataticola* and compatible with Copper oxychloride.

Preparation of talc based formulation of potential fungal isolate

Two to three discs of seven day old culture of potential *Trichoderma* spp. were added separately into conical flasks containing potato dextrose broth and incubated for seven days at room temperature (28 ± 2°C). One kg of talc powder (Montmorillonite) was taken in a metal tray under aseptic conditions and pH was adjusted to 7.0 by adding $CaCo_3$ at the rate of 15 g kg^{-1}. Ten g of carboxy methyl cellulose (CMC) was added to 1 kg of talc powder, mixed well and the mixture was autoclaved for 30 min. at 121°C for 2 successive days. The mycelial mat along with broth was homogenized, mixed with talc powder @ 1:2 v/w and shade dried until it attains 20 per cent moisture content under aseptic conditions. After mixing, the clumps were broken and homogenized uniformly, then the mixture was packed in polypropylene bags and sealed.

The initial population of the fungal biocontrol agent was evaluated by serial dilution method and used in integrated disease management.

$$\text{Number of cfu/g} = \frac{\text{Number of colonies}}{\text{Amount of sample plated} \times \text{dilution}}$$

Isolation of native antagonistic bacteria from Chickpea root endophytes

For isolation of endophytes, five g of root was surface sterilized for 5 min with 70 per cent ethanol and homogenized in 20 ml of sterilized phosphate buffer (0.2M Na_2HPO_4 + 0.2M NaH_2PO_4) pH 7.0 using mortar and pestle. Appropriate dilutions (10^{-6} for bacteria) of these suspensions were plated on NA for the isolation of bacteria. The plates were incubated for 72 h at 28 ± 2°C (Kishore *et al.*, 2005). One day old colonies of bacteria were picked up and purified by streak plate method. Among the 20 endophytic bacteria isolated, CREB-16 showed highest inhibition (95.55%) against *Rhizoctonia bataticola* and compatible with fungicide copper oxychloride

Preparation of talc based formulation of potential bacterial isolate

Talc based formulation of potential bacterial biocontrol agent CREB-16 was prepared by following the method as described by Vidhyasekaran and Muthamilan (1995).

A loopful of potential antagonistic bacteria was inoculated into Nutrient broth and incubated in a rotary shaker at 150 rpm min^{-1} for 48 hours at room temperature (28 ± 2°C). One kg of talc powder (Montmorillonite) was taken in a metal tray under aseptic conditions and pH was adjusted to 7.0 by adding $CaCo_3$ at the rate of 15 g kg^{-1}. Ten g of carboxymethyl cellulose (CMC) was added to 1 kg of talc powder, mixed well and the mixture was autoclaved for 30 min. at 121°C for 2 successive days. 400 ml of the bacterial suspension containing 1 x 10^8cfu/ml was mixed with carrier CMC mixture under aseptic conditions. After drying to 35 per cent moisture content overnight under aseptic conditions, the mixture was packed in polypropylene bags and sealed.

The initial population of the bacterial biocontrol agent was evaluated by serial dilution method and used in integrated disease management.

Seed treatment

Chickpea seeds were treated with talc based formulation of potential fungal biocontrol agent *Trichoderma* isolate-7 and bacterial biocontrol agent CREB-16 @ 10 g kg^{-1} of seed and the seeds were used for sowing. For treatment with fungicide, the chickpea seeds were treated with compatible and effective fungicide copper oxychloride @ 2.5 g kg^{-1} of seeds and sown in the pathogen infested soil in the pots.

The effect of seed treatment with fungal and bacterial bio-control agents and fungicide on seed germination was evaluated by blotter technique under *in vitro*. Total number of seeds plated per each plate was five.

Soil application

The population was estimated in talc based formulation after preparation and before use. It was 2.0×10^5 cfu/g in the case of *Trichoderma* isolate-7 and it was $1.0x10^8$ cfu/g in the case of CREB-16. The talc based formulation of potential fungal and bacterial biocontrol agents were applied @ 100 g pot^{-1} before sowing.

Pot culture studies

This experiment was conducted under greenhouse conditions. In this experiment, the potential fungicidal compatible antagonists along with effective fungicides and different organic amendments was evaluated.

Estimation of population levels of *Rhizoctonia bataticola, Trichoderma* (CT7) and bacterial antagonist (CREB16)

The population levels of test pathogen, *Rhizoctonia bataticola*, fungal antagonist, *Trichoderma* and bacterial antagonist from different treatments in pot culture experiment were enumerated at 7 DAS and final population at 45 DAS following serial dilution plate count technique (Johnson and Curl, 1977).

RESULTS AND DISCUSSION

In *vitro* evaluation of efficacy of different seed treatments on seed germination by blotter technique

The effect of different seed treatments on per cent seed germination was calculated by blotter technique under *in vitro* conditions and the results are presented in Table 1.

The results revealed that seed treatment with fungicide, biocontrol agents alone and in combination, has given 100 per cent seed germination under *in vitro* conditions. There was no significant difference between the treatments, all the treatments has given good germination percentage. The seeds started germinating at 24 hours after incubation and the time taken for 100 per cent seed germination in all the treatments was 78 hours. As antagonistic bacteria CREB-16 has shown more inhibition (93.33%) compared to *Trichoderma* isolate-7 (83.33%) under *in vitro* conditions in dual culture against the pathogen, hence it was selected as potential antagonist seed treatment in combination with organic amendments.

Similarly Rangeshwaran *et al*. (2001) reported that seed treatment with bioagents and fungicide gave 100 per cent germination of chickpea seedlings under *in vitro* conditions in the absence of pathogen. Nautiyal (1997) reported that seed bacterization with *Pseudomonas fluorescens* NBRI1303 increased the germination of chickpea by 25%.

OBSERVATIONS

The data on per cent germination, initial and final population of chickpea, per cent incidence of dry root rot and plant growth parameters *viz.*, plant height, root length, shoot dry weight and root dry weight of chickpea in each of the treatment were recorded and presented in Table 2 and Table 3.

Per cent germination

Maximum per cent germination (100 per cent) was recorded in treatment T_8 (seed treatment with fungicide and soil application of potential fungal and bacterial biocontrol agent) and T_7 (soil application of potential fungal and bacterial biocontrol agent). There was no significant difference between the treatments T_6, T_5, T_{14} and T_{13}. It is evident from table 2 that least germination percentage (60.00) was recorded in inoculated control (T_{15}).

Seed treatment with talc based formulations of biocontrol agents proved better and thus may serve as ecofriendly alternative to copper oxychloride. Our results showed that the antagonists have some stimulatory effect on seed germination. This might be due to the production of some hormones. It is interesting to identify those compounds involved in stimulation of seed germination.

Bagri *et al*. (2004) reported that seed treatment with captan gave 86 per cent germination and minimum pre and post emergence mortality (11 and 5%) respectively against *Macrophomina phaseolina* causing root rot disease in chickpea.

Initial and Final population

From the data (Table 2) it is evident that initial population was highest in the treatments T_8 (10.00) and T_7 (10.00) and the lowest population was recorded in the treatment T_{15} (6.00). Similarly regarding final population also, highest number was recorded in the treatments T_8 (9.66) and T_7 (9.66) and the lowest population was recorded in the treatment T_{15} (3.00). The final population were in accordance with the per cent disease incidence in each treatment.

Table: 1 *In vitro* evaluation of efficacy of different seed treatments on seed germination by blotter technique

S.No	Treatment	Number of seeds germinated*	Per cent germination
1	Copper oxychloride @ 2.5 g/kg	5.00	100.00(90.00)
2	Trichoderma isolate-7 (CT7) @ 10 g/kg	5.00	100.00(90.00)
3	Bacterial isolate CREB-16 @ 10 g/kg	5.00	100.00(90.00)
4	*Trichoderma* isolate-7 (CT7) @ 10 g/kg + Bacterial isolate CREB-16 @ 10 g/kg	5.00	100.00(90.00)
5	Control	5.00	100.00 (90.00)
	S.Em ±		-
	CD (0.05)		NS

* Mean of three replications

Figures in parenthesis are angular transformed values

Table: 2 Effect of antagonists, fungicide and organic amendments on per cent germination, initial population, final population and per cent disease incidence of chickpea cv. JG-11 in Rhizoctonia bataticola infested soil in pot culture

Treatment	*Per cent germination	*Initial population	*Final population	*Per cent disease incidence
T1 : Seed treatment with potential fungal bio-control agent @ 10 g/kg	83.33 (66.14)	8.33 (16.77)	7.33 (15.65)	37.80 (37.93)
T2 : Seed treatment with potential bacterial bio-control agent @10g/kg	83.33 (70.07)	8.33 (16.73)	7.33 (15.70)	36.40 (37.10)
T3 : Seed treatment with potential fungal and bacterial bio-control agents @10g/kg	86.66 (68.85)	8.66 (17.11)	7.66 (16.06)	32.00 (34.44)
T4 : Seed treatment with fungicide	86.66 (72.28)	8.66 (17.09)	7.66 (16.02)	34.50 (35.96)
T5 : Soil application of potential fungal bio-control agent	93.33 (77.71)	9.33 (17.78)	8.66 (17.09)	21.40 (27.55)
T6 : Soil application of potential bacterial bio-control agent	96.66 (83.85)	9.66 (18.10)	9.00 (17.44)	20.50 (26.91)
T7 : Soil application of potential fungal and bacterial bio-control agents @10g/kg	100.00 (90.00)	10.00 (18.43)	9.66 (18.10)	18.20 (25.25)
T8 : $T_4+T_5+T_6$	**100.00(90.00)**	**10.00(18.43)**	**9.66 (18.10)**	**16.00(23.55)**
T9 : Application of Neem cake @ 12.5 q/ha	76.66(61.21)	7.66(16.06)	6.00(14.09)	52.00(46.14)
T10 : Application of FYM @ 5 t/ha	80.00(63.92)	8.00(16.40)	6.66(14.95)	47.50(43.56)
T11 : Application of Vermicompost @5 q/ha	80.00(63.43)	8.00(16.42)	6.66(14.92)	49.80(44.88)
T12 : Seed treatment with potential bacterial bio-control agent and soil application of Neem cake	90.00 (71.56)	9.00 (17.45)	8.33 (16.77)	27.80 (31.81)
T13 : Seed treatment with potential bacterial bio-control agent and soil application of FYM	90.00 (75.00)	9.00 (17.44)	8.33 (16.73)	22.50 (28.31)
T14 : Seed treatment with potential bacterial bio-control agent and soil application of Vermicompost	90.00 (78.90)	9.00 (17.40)	8.33 (16.69)	25.60 (30.38)
T15 : Control	60.00(51.14)	6.00(14.09)	3.00(9.88)	83.00(65.66)
S Em ±	5.79	0.56	0.78	0.52
CD (0.05)	16.80	1.63	2.27	1.52

Table: 3 Effect of fungicide, biocontrol agents and organic amendments on growth parameters of chickpea cv. JG-11 in *Rhizoctonia bataticola* infested soil in pot culture

Treatment	*Plant height (cm)	*Root length (cm)	*Dry weight of shoot (g)	*Dry weight of root (g)
T1 : Seed treatment with potential fungal bio-control agent @ 10 g/kg	19.84 (26.43)	10.20 (18.61)	0.24 (2.80)	0.06 (1.42)
T2 : Seed treatment with potential bacterial bio-control agent @10g/kg	20.57 (26.96)	10.63 (19.02)	0.25 (2.86)	0.06 (1.44)
T3 : Seed treatment with potential fungal and bacterial bio-control agents @10g/kg	21.40 (27.55)	11.25 (19.59)	0.29 (3.08)	0.07 (1.51)
T4 : Seed treatment with fungicide	21.09 (27.33)	11.00 (19.34)	0.26 (2.91)	0.06 (1.48)
T5 : Soil application of potential fungal bio-control agent	24.13 (29.42)	12.20 (20.43)	0.40 (3.61)	0.09 (1.71)
T6 : Soil application of potential bacterial bio-control agent	24.64 (29.76)	12.78 (20.94)	0.42 (3.71)	0.09 (1.77)
T7 : Soil application of potential fungal and bacterial bio-control agents @10g/kg	24.88 (29.92)	12.90 (21.04)	0.46 (3.88)	0.10 (1.81)
T8 : $T_4+T_5+T_6$	**25.61 (30.40)**	**13.40 (21.46)**	**0.49 (4.01)**	**0.11 (1.89)**
T9 : Application of Neem cake @ 12.5 q/ha	16.86 (24.24)	8.80 (17.25)	0.17 (2.35)	0.05 (1.33)
T10 : Application of FYM @ 5 t/ha	19.43 (26.15)	9.82 (18.25)	0.22 (2.68)	0.05 (1.36)
T11 : Application of Vermicompost @ 5 q/ha	19.14 (25.94)	9.30 (17.75)	0.20 (2.54)	0.05 (1.31)
T12 : Seed treatment with potential bacterial bio-control agent and soil application of Neem cake	21.65 (27.72)	11.50 (19.82)	0.32 (3.24)	0.07 (1.57)
T13 : Seed treatment with potential bacterial bio-control agent and soil application of FYM	23.49 (28.99)	12.13 (20.38)	0.37 (3.47)	0.08 (1.68)
T14 : Seed treatment with potential bacterial bio-control agent and soil application of Vermicompost	22.61 (28.39)	11.90 (20.17)	0.34 (3.33)	0.08 (1.62)
T15 : Control	14.43 (22.32)	7.40 (15.76)	0.14 (2.12)	0.05 (1.28)
S Em ±	0.342	0.36	0.14	0.03
CD (0.05)	0.99	1.04	0.42	0.11

Per cent disease incidence

From the data (Table 2) it is evident that all the treatments were significantly superior over control in reducing the per cent disease incidence. Maximum reduction was observed in treatment T_8 (seed treatment with fungicide and soil application of potential fungal and bacterial biocontrol agent) in which PDI of 16.00 per cent was recorded followed by T_7 (18.20%), T_6 (20.50%) and T_5 (21.40%) in comparison to 83.00 % in inoculated control.

The reasons for a good control of root rot by fungal and bacterial antagonists in combination with copper oxychloride in our study may be due to the different or complementary modes of action of antagonists or their abilities to colonize root microsites or lesser sensitivity of antagonists to copper oxychloride or due to the sensitivity of *R. bataticola* as evident from poisoned food technique.

The results of per cent disease incidence in treatments including seed treatment with bacterial biocontrol agent and soil application of organic amendments revealed that lowest PDI was observed in T_{13} (22.50%) i.e seed treatment with bacterial biocontrol agent and soil amendment of FYM, followed by T_{14} (25.60%) i.e seed treatment with bacterial biocontrol agent and soil amendment of vermicompost, T_{12} (27.80%) i.e seed treatment with bacterial biocontrol agent and soil amendment of neem cake.

Increase in disease control in combined treatments i.e seed treatment with bacterial biocontrol agent and soil amendment of FYM or neem cake or vermicompost observed may be due to enhanced population of antagonists or due to suppression of *Rhizoctonia bataticola* or due to inhibition of growth of *Rhizoctonia bataticola* by toxic and volatile products released during breakdown of organic amendment in soil.

Seed treatment with both fungal and bacterial biocontrol agents was found to be significantly superior in reducing the disease incidence when compared to individual treatments.

The per cent disease incidence was low when antagonists were applied in soil compared to seed treatment alone or in combination of seed treatment and soil application of organic amendments. This might be due to the application of more number of antagonistic propagules which have multiplied at faster rate and inhibited the growth of pathogen.

Similar results have been reported by several workers in the management of several soil borne diseases by integration of biocontrol agents with chemicals.

Gaur *et al*. (2005) reported that dry root rot of chickpea (16%) can be effectively managed by the soil application of 10-15 days pre-incubated *Trichoderma harzianum* @10 kg ha^{-1} in 200 kg of FYM when compared to inoculated control (79.5%). Seed treatment with *Pseudomonas fluorescens* plus soil amendment with mustard cake provided best control of dry root rot caused by *Macrophomina phaseolina* in chickpea (Khan and Gangopadhyay, 2008).

Table: 4 Population dynamics of Potential fungal isolate *Trichoderma* isolate-7 (CT7)

Treatment	*Population of *Trichoderma* isolate-7 cfu/g soil (× 10^5)	
	7 DAS	45 DAS
T1 : Seed treatment with potential fungal bio-control agent @ 10 g/kg	4.60(12.38)	10.50(18.91)
T2 : Seed treatment with potential bacterial bio-control agent @10g/kg	-	-
T3 : Seed treatment with potential fungal and bacterial bio-control agents @10g/kg	4.00(11.54)	9.60(18.05)
T4 : Seed treatment with fungicide	-	-
T5 : Soil application of potential fungal bio-control agent	**7.80(16.22)**	**15.20(22.95)**
T6 : Soil application of potential bacterial bio-control agent	-	-
T7 : Soil application of potential fungal and bacterial bio-control agents	7.00(15.34)	13.80 (21.81)
T8 : $T_4+T_5+T_6$	6.80 (15.11)	13.60 (21.64)
T9 : Application of Neem cake @ 12.5 q/ha	-	-
T10 : Application of FYM @ 5 t/ha	-	-
T11 : Application of Vermicompost @ 5 q/ha	-	-
T12 : Seed treatment with potential bacterial bio-control agent and soil application of Neem cake	-	-
T13 : Seed treatment with potential bacterial bio-control agent and soil application of FYM	-	-
T14 : Seed treatment with potential bacterial bio-control agent and soil application of Vermicompost	-	-
T15 : Control	-	-
S Em ±	0.21	0.15
CD (0.05)	0.69	0.51

* Mean of three replications

Figures in parenthesis are angular transformed values

EFFECT OF DIFFERENT TREATMENTS ON PLANT GROWTH PARAMETERS

In the present investigation, an attempt was made to observe whether the treatments imposed have any stimulatory (or) inhibitory effect on mean plant height, root length and shoot and root dry weight of chickpea plants

a) Plant height

Maximum plant height (25.61cm) was recorded in treatment T_8 (seed treatment with fungicide and soil application of potential fungal and bacterial biocontrol agent) followed by treatment T_7 (24.88 cm), T_6 (24.64 cm) and T_5 (24.13 cm). It is evident from Table 3 that least plant height (14.43cm) was recorded in inoculated control (T_{15}).

It is evident that treatment T_8 stimulated the plant growth and development (25.61cm) when compared to inoculated control (14.43cm). It is attributed that copper oxychloride could arrest the pathogen and antagonists could parasitize the pathogen and promote growth by secreting growth promoting metabolites.

The results were in agreement with Vinod Kumar *et al*. (2007) who reported that *Pseudomonas fluorescens* isolate pf 4-99 reduced the incidence of charcoal rot of chickpea and enhanced the plant height by 29.40 per cent against the control.

b) Root length

Maximum root length was recorded in treatment T_8 (13.40 cm) that was on par with treatment T_7 (12.90 cm) and T_6 (12.78 cm). It is evident from Table 3 that least root length (7.4 cm) was recorded in inoculated control (T_{15}).

Similarly Bharati *et al*. (2004) reported that soil application of *Trichoderma harzianum* and *B. subtilis* reduced the incidence of damping-off in tomato caused by *Pythium aphanidermatum* and also increased shoot and root length and biomass production. Rangeshwaran *et al*. (2008) reported that maximum root length (6.7 cm) and shoot length (5.9 cm) was recorded in seed treatment with *Bacillus* sp. (UASEBCH2) after 14 days of germination against *Rhizoctonia solani* in chickpea.

c) Dry weight of shoot and root

The maximum shoot weight was recorded in treatment T_8 (0.49 g) that was on par with T_7 (0.46 g), T_6 (0.42 g) and T_5 (0.40 g). It is evident from Table 3 that least shoot weight (0.14 g) was recorded in inoculated control (T_{15}).

Maximum root weight (0.11g) was recorded in treatment T_8 that was on par with T_7 (0.10 g), T_6 (0.09 g) and T_5 (0.09 g) with the least (0.05 g) recorded in inoculated control (T_{15}).

Rajan *et al*. (2000) reported that the endophytic bacteria enhanced tillering, overall growth of the plants and suppressed the pathogens and disease incidence in ginger.

Thus overall, the efficacy of treatment T_8 (seed treatment with fungicide and soil application of potential fungal and bacterial biocontrol agent) was found to be superior which recorded highest germination per cent, least PDI, highest initial and final population of chickpea, maximum plant height, root length, shoot and root dry weight when compared to other treatments.

Estimation of Population levels of Pathogen, fungal and Bacterial Antagonists

Population dynamics of potential fungal isolate *Trichoderma* isolate-7 (CT7)

Population dynamics of potential fungal isolate *Trichoderma* isolate-7 (CT7) was estimated at two different intervals, initially 7 DAS and then on 45 DAS and the results are presented in the Table 4.

It is evident that maximum population was obtained in the treatment T_5 (7.80×10^5), followed by T_7 (7.00×10^5) and T_8 (6.80×10^5) which were on par with each other. The least population was obtained from the treatment T_3 (4.00×10^5) at 7 DAS. Similarly at 45 DAS, maximum population was obtained in the treatment T_5 (15.20×10^5), followed by T_7 (13.80×10^5) and T_8 (13.60×10^5) which were on par with each other. The least population was obtained from the treatment T_3 (9.60×10^5).

In the present investigation, a rapid increase in the antagonist population of the rhizosphere soil was observed from 7 DAS to 45 DAS. The initial increase in population might be due to germination of different spore forms and their subsequent proliferation with or without food base. Odunfa and Oso (1979) also found that initial increase of antagonist population in rhizosphere could be due to the abundance of sugar and amino acids exuded from roots while the subsequent decrease could be ascribed to a reduction or change in composition of root exudates as the plants age.

The above results showed that the populations levels of potential fungal isolate *Trichoderma* isolate-7 (CT7) was more when applied as soil treatment compared to seed treatment. Further, fungal population was more when applied singly compared to combination treatment with bacteria and fungicide. This might be due to lack of complete compatibility of bacteria and fungicide with *Trichoderma* isolate-7 (CT7) as evident from *in vitro* results or due to competition in the zone of rhizosphere.

Application of suitable biocontrol agents capable of competing with other microbes in the soil can result in great success of biological control of crop diseases.

Choudhary *et al*. (2010) reported that the mean population densities of *Trichoderma harzianum* (10.20×10^6) recorded minimum dry root rot incidence (16.67%) on mungbean when compared to control (66.67%).

Population dynamics of potential bacterial isolate CREB-16

Population dynamics of potential bacterial isolate CREB-16 was estimated at two different intervals, initially 7 DAS and then at 45 DAS and the results are presented in the Table 5.

It is evident that maximum population was obtained in the treatment T_6 (3.90×10^8), followed by T_7 (3.37×10^8) and T_8 (3.20×10^8) which were on par with each other. The least population was obtained from the treatment T_3 (1.30×10^8) at 7 DAS. Similarly at 45 DAS, maximum population was obtained in the treatment T_6 (4.97×10^8), followed by T_7 (4.60×10^8) and T_8 (4.50×10^8) which were on par with each other. The least population was obtained from the treatment T_3 (2.40×10^8).

The results of the treatments including organic amendments along with seed treatment with bacterial isolate CREB-16, showed that the population levels of bacteria were more with FYM both at 7 DAS (2.80×10^8) and at 45 DAS (3.67×10^8) compared to vermicompost and neem cake.

The above results showed that the population levels of potential bacterial isolate CREB-16 was more when applied as soil treatment compared to seed treatment. FYM was found to be the best suitable substrate for multiplication of the bacteria.

Khan and Gangopadhyay (2008) reported that the population of *Pseudomonas fluorescens* in FYM amended soil were increased from 17.3 to 28.7×10^{10} cfu/g soil with the increase in FYM dose from 5 g to 20 g/kg soil.

Table: 5 Population dynamics of Potential bacterial isolate CREB-16

Treatment	*Population of CREB-16 cfu/g soil ($\times 10^8$)	
	7 DAS	**45 DAS**
T1 : Seed treatment with potential fungal bio-control agent @ 10 g/kg	-	-
T2 : Seed treatment with potential bacterial bio-control agent @10g/kg	1.67(7.41)	2.80(9.63)
T3 : Seed treatment with potential fungal and bacterial bio-control agents @10g/kg	1.30(6.54)	2.40(8.90)
T4 : Seed treatment with fungicide	-	-
T5 : Soil application of potential fungal bio-control agent	-	-
T6 : Soil application of potential bacterial bio-control agent	**3.90(11.39)**	**4.97(12.88)**
T7 : Soil application of potential fungal and bacterial bio-control agents	3.37(10.57)	4.60(12.38)
T8 : $T_4+T_5+T_6$	3.20(10.30)	4.50(12.24)
T9 : Application of Neem cake @ 12.5 q/ha	-	-
T10 : Application of FYM @ 5 t/ha	-	-
T11 : Application of Vermicompost @ 5 q/ha	-	-
T12 : Seed treatment with potential bacterial bio-control agent and soil application of Neem cake	2.23 (8.59)	3.10 (10.14)
T13 : Seed treatment with potential bacterial bio-control agent and soil application of FYM	2.80 (9.63)	3.67 (11.04)
T14 : Seed treatment with potential bacterial bio-control agent and soil application of Vermicompost	2.43 (8.95)	3.33 (10.52)
T15 : Control	-	-
S Em ±	0.22	0.14
CD (0.05)	0.66	0.43

* Mean of three replications

Figures in parenthesis are angular transformed values

Effect of fungicide, biocontrol agents and organic amendments on population levels of *Rhizoctonia bataticola* at different intervals associated with chickpea cv. JG-11

Population dynamics of *Rhizoctonia bataticola* was estimated at two different intervals, initially 7 DAS and then at 45 DAS and the results are presented in the Table 6.

It is evident that there was no significant difference between the treatments regarding population levels of the pathogen at 7 DAS. Maximum reduction in population levels of *Rhizoctonia bataticola* was observed in the treatment T_8 (3.00×10^4) followed by T_7 (4.33×10^4) and the least reduction was obtained in the treatment T_9 (15.33×10^4) at 45 DAS.

The above results indicated that the pathogen population were found to be decreased from 7 DAS to 45 DAS in all the treatments imposed. Maximum reduction in pathogen population was observed in treatment T_8 (seed treatment with fungicide and soil application of potential fungal and bacterial biocontrol agent).

The decrease in pathogen population may be due to increased levels of antagonists and fungitoxicity of copper oxychloride. Whereas the pathogen population was very high in control, which may be due to lack of competition with any other microbes and fungi toxicants in soil.

Choudhary *et al*. (2010) reported that high levels of suppression of *Macrophomina phaseolina* (1.10×10^3) were observed in combination treatment of *Trichoderma harzianum* and *Bacillus firmus* at the mean population densisties of 10.14×10^6 and 23.10×10^8 respectively, when compared to control (1.60×10^3).

Table: 6. Effect of antagonists, fungicide and organic amendments on population levels of *Rhizoctonia bataticola*

	***Population of *Rhizoctonia bataticola* cfu/g soil ($\times 10^4$)**	
Treatment	**7 DAS**	**45 DAS**
T1 : Seed treatment with potential fungal bio-control agent @ 10 g/kg	34.00 (35.66)	11.33(19.61)
T2 : Seed treatment with potential bacterial bio-control agent @10g/kg	33.33(35.26)	11.00(19.35)
T3 : Seed treatment with potential fungal and bacterial bio-control agents @10g/kg	33.00(35.05)	9.66(18.09)
T4 : Seed treatment with fungicide	33.00(35.06)	10.00(18.42)
T5 : Soil application of potential fungal bio-control agent	31.00(33.82)	6.00(14.14)
T6 : Soil application of potential bacterial bio-control agent	31.00(33.80)	5.33(13.34)
T7 : Soil application of potential fungal and bacterial bio-control agents	30.33(33.41)	4.33(11.99)
T8 : $T_4+T_5+T_6$	**30.00(33.20)**	**3.00(9.88)**
T9 : Application of Neem cake @ 12.5 q/ha	35.00(36.26)	15.33(23.03)
T10 : Application of FYM @ 5 t/ha	34.33(35.86)	13.00(21.09)
T11 : Application of Vermicompost @ 5 q/ha	35.00(36.26)	14.00(21.94)
T12 : Seed treatment with potential bacterial bio-control agent and soil application of Neem cake	31.66(34.23)	8.66(17.11)
T13 : Seed treatment with potential bacterial bio-control agent and soil application of FYM	32.00(34.42)	7.33(15.70)
T14 : Seed treatment with potential bacterial bio-control agent and soil application of Vermicompost	31.66(34.23)	8.00(16.40)
T15 : Control	36.00(36.86)	65.00(53.73)
S Em ±	0.812	0.693
CD (0.05)	NS	2.01

* Mean of three replications

Figures in parenthesis are angular transformed values

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