

Original Research Article

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Evaluation of Formulations through Testing the Bioefficacy of Selected *Trichoderma* Isolate against *S. rolfsii*

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ABSTRACT

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The collar rot caused by *Sclerotium rolfsii* is an important fungal pathogen in chickpea root disease complex causing serious losses. In the present study, efforts were made to prepare and test the bioefficacy of eight solid formulations and three liquid formulations against chickpea collar rot (*In vitro* and *In vivo*). The results indicated that, among all the eight solid substrates used, talc powder formulation was found to be the best carrier material to retain and support maximum propagules of *Trichoderma* compared to the remaining carriers. The mean cfu was highest in talc powder (540×10^8 cfu/g) followed by pesta granules (450×10^8 cfu/g); In liquid formulations, mean cfu revealed that mineral oil formulation was found to yield higher cfu (450×10^{11} cfu/ml). Among the eight solid formulations and three liquid formulations of *Trichoderma* assessed using soil application and seed treatment methods, talc formulation (51.0% and 65.9%, respectively), pesta granules (47.7% and 66.4% respectively in solid) mineral oil formulation (50.0% and 54.0%, respectively in liquid) gave superior disease control against chickpea collar rot.

Introduction

Chickpea (*Cicer arietinum* L.) is one of the major pulse crops widely cultivated in tropical, sub-tropical and temperate regions of the world. Among various factors attributed to the low productivity of chickpea, susceptibility to diseases is the most important factor. More than 50 pathogens have been reported to infect chickpea in different parts of the world (Nene *et al.*, 1989). The collar rot caused by *Sclerotium rolfsii* is an important fungal pathogen in

chickpea root disease complex causing serious losses. Among the biotic factors contributing towards low production of chickpea, the collar rot caused by *S. rolfsii* is a major disease reported to cause 55–95% mortality of chickpea seedlings. Chemical control of the disease is not effective as the soil borne pathogens has a wide host range and survives in soil for a longer period through resting structures. Biological control is proved to be a promising disease management technology against soil borne plant pathogens when applied either alone or

in combination with other management practices (Papavizas, 1985 and Mukhopadhyay, 1987). Among the successful bioagents, *Trichoderma* spp. is being extensively used and is available as commercial formulations to manage several soil borne plant pathogens. *Trichoderma* has compatibility with other management practices in controlling many diseases (Papavizas, 1985).

Trichoderma spp. were the most widely used biocontrol agents since they have antifungal activities (Zaidi and Singh, 2004). Though several species of *Trichoderma* are found to be effective in managing plant diseases, isolate variation existed in their antagonistic efficacy (Upadhyay and Mukhopadhyay, 1986 and Patibanda *et al.*, 2002). The success of a biocontrol agent depends on its ability to produce inoculum in excess, grow and proliferate well on the plant parts whenever applied. The population of bioagent in the formulation is an important factor deciding the quantity of product necessary to apply in the field. Failure of antagonist to survive due to shorter shelf life is a major hindrance to consistent field performance. The efficacy can also be increased through strain improvement.

Materials and Methods

The present investigation was carried out in the Department of Plant Pathology, Agricultural College, Bapatla. The *T. harzianum* isolate, Th4 was used to prepare formulations using different carriers. Solid and liquid formulations of *Trichoderma* were prepared in the present study to test the viability and bioefficacy of biocontrol agent.

Formulation Development

Potato dextrose broth (PDB 50 ml) medium in sterilized 250 ml Erlenmeyer flasks was inoculated with four day old mycelial discs of the isolate Th4 and incubated at $29 \pm 1^{\circ}\text{C}$ for

ten days. The mycelial mat with conidial sporulation was separated from the broth and the mat was macerated using pestle and mortar to make it as biomass. The biomass product was then mixed with the carrier material such as talc powder, farm yard manure (FYM), neem cake powder, vermicompost, gypsum, lignite powder, pesta granules and conidial formulation @ 1:4 ratio (w/w basis) and the mixture was shade dried properly to reduce the moisture content to 8%. The formulations thus prepared were estimated for cfu on TSM. Liquid substrates or carriers such as mineral oil, neem oil and palm oil were used to prepare oil based liquid formulations.

Pesta granules (Connick *et al.*, 1991): Biomass was added to wheat flour and mixed to form cohesive dough by adding required quantity of sterile water. The dough was pressed flat and folded by hand several times. Then one mm thick sheets (pesta) were prepared and air dried to make them crisp. After drying, dough sheet was ground and passed through an 18 mesh sieve and granules were collected.

Conidial formulation: Conidial formulation was prepared by harvesting conidia in sterile water from PDA plates and the spore mass was centrifuged at 6000 rpm for 15 min. The pellet formed at the bottom of the centrifuge tube was harvested and dried in shade to obtain conidial powder. For delivery, the conidial powder was suspended in sterile water to get the desired concentration (10^8 spores/ml) (Pan and Das, 2009).

Oil based formulation: Oil based formulation was prepared by harvesting conidia in sterile water from PDA plates and mixed with paraffin oil and Tween-20 (surfactant). The spore mass was then centrifuged twice at 6000 rpm for 15 min. The pellet formed at the bottom of the centrifuge tube was suspended in sterile water to form a

stable emulsion (Ramanujam *et al.*, 2010).

Bioefficacy of Th4 formulation against *S. rolfii* in vivo

Twenty day old sorghum grain culture of *S. rolfii* was inoculated in the top two inches soil of the 6 kg soil pot @ 6 g/pot and watered sufficiently. Seed treatment and soil application methodologies were used to evaluate the bioefficacy of biocontrol agent against collar rot of chickpea.

Seed treatment with Th4 formulation

Chickpea seeds of cv. Annegiri were coated with dry powder formulation of Th4 just before sowing @ 4 g of formulation per kg seed (Mukhopadhyay *et al.*, 1992) and each pot was sown with ten chickpea seeds.

Soil application with Th4 formulation

Two kg Th4 formulation was mixed in 90 kg FYM and eight kg neem cake powder and covered for seven days with polythene sheet. The mixture was sprinkled with water intermittently and turned once in every 3-4 days. When the growth of *Trichoderma* was observed all through the heap, then the mixture (10 g/pot) was applied to the pots by mixing in top 2 inches soil.

Seeds were sown in pots @ 10 seeds/pot twenty four hours after pathogen inoculation. Pathogen inoculated and uninoculated pots were maintained stand and disease incidence.

The following formulae were used to assess treatment effects in pot culture.

$$\text{Plant stand (\%)} = \frac{\text{Number of plants in treatment}}{\text{Plant stand in absolute check}} \times 100$$

$$\text{Disease Control (\%)} = \frac{\text{Mortality \% in pathogen check} - \text{Mortality \% in treatment}}{\text{Mortality \% in pathogen check}} \times 100$$

Results and Discussion

Evaluation of Formulations of *Trichoderma* in vitro

The results indicated that, among all the eight solid substrates used, talc powder formulation was found to be the best carrier material to retain and support maximum propagules of *Trichoderma* compared to the remaining carriers. The mean cfu was highest in talc powder (540×10^8 cfu/g) followed by pesta granules (450×10^8 cfu/g), conidial formulation (390×10^8 cfu/g), FYM (370×10^8 cfu/g), neem cake powder (320×10^8 cfu/g), vermicompost (300×10^8 cfu/g), lignite (290×10^8 cfu/g) and gypsum (200×10^8 cfu/g). On the other hand, in liquid formulations, mean cfu revealed that mineral oil formulation was found to yield higher cfu (450×10^{11} cfu/ml) followed by neem oil (130×10^{11} cfu/ml) and palm oil (100×10^{11} cfu/ml).

In comparison to solid formulations prepared on w/w basis, cfu in liquid formulations prepared on w/v basis were higher. Further, among solid formulations, talc powder offered better result with maximum cfu (540×10^8 cfu/g), while in liquid formulations, mineral oil was found to be the best with 450×10^{11} cfu/ml. Further assessment of different formulations for the bioefficacy against chickpea collar rot was done in pot culture by normalizing the cfu in every formulation to $1-9 \times 10^8$ cfu/g or ml.

The population density of different formulations is variable and it depends upon nature and type of carrier material used. Deshmukh *et al.*, 1999 reported that the viability of propagules in different formulations may be less because of its low level of nitrogen. FYM was proved to be the most suitable carrier material resulted in high cfu count (7.2×10^8 cfu/g) followed by talc

powder having cfu count of 5.3×10^8 cfu/g at the time of preparation (Sarode *et al.*, 1998). However, they also reported that neem seed powder has a deleterious effect on *Trichoderma*, which resulted in gradual decline in population.

Sathiyaseelan *et al.*, (2009) evaluated five different carrier materials *viz.*, paraffin oil + glycerol, paraffin oil + soybean oil, soybean oil + glycerol, paraffin oil and soybean oil for preparation of liquid formulation of *T. viride* and found that *T. viride* in paraffin oil was better than other formulations with 5×10^9 cfu/ml at the time of preparation. Similar result was also observed by Al-Taweil *et al.*, (2010).

Bioefficacy of formulations of *Trichoderma* in vivo using soil application method

In pathogen inoculated control, germination per cent was 43.3% and plant stand was only 10% equivalent to 90% plant mortality compared to pathogen uninoculated check (100% germination and 100% plant stand) (Table 1a).

When per cent plant stand was estimated at 30 DAS, among all the eight formulations applied, talc formulation was proved superior with 56.6% plant stand though all the remaining formulations such as pesta granules (53.0%), neem cake (46.6%), vermicompost (43.3%), gypsum (43.3%), conidial formulations (43.3%), FYM (36.7%) and lignite (36.6%) were on par with talc formulation.

Per cent reduction in disease over control was also estimated based on per cent plant stand. Highest per cent disease control was noticed in talc formulation with 51% and was significantly on a par with pesta granules

(47.7%), neem cake (40.6%), conidial formulation (36.9%), vermicompost (36.9%) and gypsum (36.5%). The lowest disease control was observed in FYM (29.4%) and Lignite (29.1%) which were significantly lower in comparison with the best formulation *i.e.*, talc powder.

When liquid formulations of *Trichoderma* such as mineral oil, neem oil and palm oil were assessed for their bioefficacy against *S. rolfsii*, germination per cent in pathogen inoculated control (36.0%) was significantly lower compared to all the *Trichoderma* applied treatments and plant stand was only 12.0% equivalent to 88% plant mortality (Table 1b).

When per cent plant stand was observed, the highest plant stand was noticed in mineral oil formulation (56.0%) which was significantly on par with neem oil formulation (44.0%). Palm oil formulation has 36.0% plant stand which was significantly lower than that in mineral oil formulation but on par with neem oil formulation. Per cent disease reduction over control was estimated and mineral oil formulation (50.0%) was significantly higher compared to neem oil (35.8%) and palm oil formulations (26.6%).

From the above data, it may be interpreted that when *Trichoderma* formulations were applied in soil, talc powder formulation among solid formulations and mineral oil formulation among liquid formulations were proved better in controlling chickpea collar rot caused by *S. rolfsii*. Several workers reported on bioefficacy of different *Trichoderma* formulations through soil application to manage disease caused by soil borne plant pathogenic fungi (Jegathambigai *et al.*, 2010; Dubey *et al.*, 2011, Yang *et al.*, 2011 and Mishra *et al.*, 2012).

Table.1 Bioefficacy of different solid and liquid formulations of *Trichoderma* Th4 using soil application method against chickpea collar rot

Table.1a Solid formulations

S. No.	Treatments	Plant stand (%)	Disease reduction over control(%)
1	Talc	56.6 (48.8) ^b	51.0 (45.6) ^a
2	Neem cake	46.6 (43.1) ^b	40.6 (39.6) ^{abc}
3	FYM	36.7 (37.2) ^b	29.4 (32.8) ^{bc}
4	Vermicompost	43.3 (41.1) ^b	36.9 (37.4) ^{abc}
5	Gypsum	43.3 (41.1) ^b	36.5 (37.0) ^{abc}
6	Lignite	36.6 (37.2) ^b	29.1 (32.4) ^c
7	Pesta granules	53.0 (46.9) ^b	47.7 (43.6) ^{ab}
8	Conidial formulation	43.3 (41.1) ^b	36.9 (37.4) ^{abc}
9	Pathogen control	10 (15.0) ^c	
10	Uninoculated control	100 (90.0) ^a	
SEm±		3.0	2.7
CD (P=0.01)		12.0	10.9
CV (%)		11.9	12.0

Values in parentheses are arc sine transformed values
Each pot was sown with 10 chickpea seeds of cv. Annegiri

Table.1b Liquid formulations

S. No.	Treatment	Plant stand (%)	Disease reduction over control (%)
1	Mineral oil	56.0 (48.4) ^b	50.0 (48.2) ^a
2	Neem oil	44.0 (41.5) ^{bc}	35.8 (35.3) ^b
3	Palm oil	36.0 (36.8) ^c	26.6 (35.3) ^b
4	Pathogen control	12.0 (18.0) ^d	
5	Uninoculated control	100 (90.0) ^a	
SEm±		2.4	2.4
CD (P=0.01)		9.6	10.1
CV (%)		11.6	14.2

Table.2 Bioefficacy of different solid and liquid formulations of *Trichoderma* Th4 using seed treatment method against chickpea collar rot

Table.2a Solid formulations

S. No.	Treatment	Plant stand (%)	Disease reduction over control (%)
1	Talc	70.0 (57.0) ^b	65.9 (54.5) ^a
2	Neem cake	43.3 (41.1) ^c	36.5 (37.0) ^a
3	FYM	50.0 (45.0) ^{bc}	44.0 (41.5) ^a
4	Vermicompost	53.0 (46.9) ^{bc}	47.2 (43.2) ^a
5	Gypsum	56.7 (48.9) ^{bc}	51.4 (45.8) ^a
6	Lignite	60.0 (50.8) ^{bc}	54.8 (47.8) ^a
7	Pesta granules	70.0 (56.8) ^b	66.4 (54.6) ^a
8	Conidial formulation	56.6 (48.8) ^{bc}	51.5 (45.8) ^a
9	Pathogen control	10 (15.0) ^d	
10	Uninoculated control	100 (90.0) ^a	
SEm±		3.7	4.3
CD (P=0.01)		14.6	17.6
CV (%)		12.8	16.1

Values in parentheses are arc sine transformed values

Each pot was sown with 10 chickpea seeds of cv. Annegiri

Table.2b Liquid formulations

S. No.	Treatment	Plant stand (%)	Disease reduction over control (%)
1	Mineral oil	60.0 (50.8) ^b	54.0 (47.3) ^a
2	Neem oil	48.0 (43.8) ^b	40.3 (39.3) ^a
3	Palm oil	50.0 (45.0) ^b	42.8 (40.7) ^a
4	Pathogen control	12.0 (18.0) ^c	
5	Uninoculated control	100 (90.0) ^a	
SEm±		2.7	3.0
CD (P=0.01)		10.5	12.5
CV (%)		12.0	15.6

Bioefficacy of formulations of *Trichoderma vivo* using seed treatment method

In pathogen inoculated control, germination per cent was 43.3% and plant stand was only 10% equivalent to 90% plant mortality (Table 2a).

Plant stand at 30 DAS was highest in talc powder formulation (70.0%) and in pesta granules (70.0%) which were on par with each other. Least plant stand was recorded with neem cake powder (43.3%) which was significantly lower than the best treatment, *i.e.*, talc powder formulation. Seed treatment with lignite (60.0%), gypsum (56.7%), conidial formulation (56.6%), vermicompost (53.3%) and FYM (50.0%) were superior to neem cake formulation but inferior to talc powder formulation. Highest per cent disease reduction over control was noticed in pesta granules and talc powder formulation with 66.4% and 65.9%, respectively.

Further, liquid formulations of *Trichoderma* such as mineral oil, neem oil and palm oil were used to assess their seed treatment efficacy against *S. rolfsii*. In pathogen inoculated control, germination per cent was 36.0% and plant stand was only 12.0% equivalent to 88% plant mortality (Table 2b). When data on plant stand data was recorded at 30 DAS the highest plant stand was noticed in mineral oil formulation (60.0%) which was significantly on par with neem oil and palm oil formulation (48.0% and 50.0%).

All the three formulations *i.e.*, mineral oil, neem oil and palm oil formulation were significantly on par among them with 54.0%, 40.3% and 42.8% disease control, respectively. Among all these three, mineral oil formulation was proved better with maximum disease control over others.

Finally from the above seed treatment experiment using solid and liquid formulations, it is clear that talc powder and pesta granule formulation in solid substrates and mineral oil formulation in liquid substrates were proved better in improving germination and disease control in comparison to the other treatments when bioefficacy of formulations were tested *in vivo* against collar rot pathogen, *S. rolfsii*.

Dubey *et al.*, (2011) and Mishra *et al.*, (2012) used different formulations and carrier material to improve bioefficacy of *Trichoderma*.

Among different solid formulations tested for the cfu just after preparation and bioefficacy against chickpea collar rot, talc powder formulation proved better with 540×10^8 cfu/g of formulation and 65.9% and 51.0% disease control when applied through seed and soil application methods, respectively. Among different liquid formulations tested for the cfu just after preparation and bioefficacy against chickpea collar rot, mineral oil formulation proved better with 450×10^{11} cfu/ml of formulation and 54.0% and 50.0% disease control when applied through seed treatment and soil application methods, respectively.

Based on the results obtained in the present investigation, seed treatment method appeared to offer better disease control compared to soil application as talc powder seed treatment (65.9%) out yielded talc powder as soil application (51.0%) and mineral oil seed treatment (54.0%) out yielded mineral oil as soil application (50.0%) in terms of disease control. *Trichoderma* when applied as seed treatment grows readily and multiplies along with the developing root system by improving the biological soil suppressiveness. The effectiveness of *Trichoderma* as seed treatments is probably determined not only by their biocontrol qualities but also by their

abilities to multiply in the rhizosphere when applied to seed.

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