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Control of cowpea (*Vigna sinensis*) root and collar rot (*Rhizoctonia solani*) with some organic formulations of *Trichoderma harzianum* under field condition

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A B S T R A C T

Cowpea (*Vigna sinensis*) has been considered as an important legume fodder and green manure crop during pre-*kharif* and *kharif* season. It has been considered even better in performance than maize. In some parts of India it is also used as green vegetable and pulse. The crop is known to suffer from a variety of diseases. Among those root rot and collar rot are important and *R. solani* has been an important factor behind these two main diseases. A field trial was conducted during pre-*kharif* season of 2011 to control root and collar rot of cowpea caused by *Rhizoctonia solani* with two organic formulations of *Trichoderma harzianum* along with other treatment combinations. It was observed that seed priming with mycelia preparation of the antagonist *Trichoderma harzianum* at 4g/kg of seed and organic formulation of the antagonist in vermicompost along with 20% neem cake (w/w) gave best disease control along with yield of cowpeas over leaf mold +20% neem cake formulation of the antagonist, talc base formulation and seed treatment with carbendazim at 2g/kg of seed. Seed priming with mycelial mat of the antagonist improved seed germination vigour of the cowpea plants, basal girth, number of branches & pod number, pod vigour of the bio primed seeds over other treatment combinations. Soil application of *Trichoderma* in vermicompost +20% neem cakes gave better disease control over the others.

***Keywords*:** Cowpea, root and collar rot, *Rhizoctania solani*, organic formulation, vermicompost, leaf mold, neem cake, *Trichoderma harzianum*, seed priming

Introduction

Soil borne plant pathogens limit the production of several crops in tropical, subtropical and temperate regions of the world (Punja 1988). *Trichoderma* spp. is well known as effective biological control agents of plant diseases caused by soil-borne fungi (Whipps & Lumsden 2001; Pan & Bhagat 2007). They are also capable to colonise roots and help to increase the population with the abundance of healthy roots in the ecosystem (Harman 2000). Formulation of *Trichoderma* spp. to reduce the incidence of the diseases caused by soilborne pathogens in the field is of significance in biocontrol of such diseases. The present work was aimed to determine the efficacy of application of some organic formulations of *Trichoderma* spp. used as soil treatment against root and collar rot of cowpea under field conditions.

Materials and Methods

Isolation of Trichoderma spp.

Trichoderma spp. was isolated from the rhizosphere soils on *Trichoderma* specific medium (Saha & Pan 1997) using dilution plate technique and the culture was purified by repeated subculturing (Pan & Jash 2009). Antagonistic potentiality of the biocontrol agent was assessed against many soil borne plant pathogens (Bose *et al*. 2005;Pan 2009). The isolates of *Trichoderma* spp. were maintained on potato dextrose agar (PDA) at 4°C for subsequent use.

Study of disease management under green house condition

The pathogen (*R. solani*) was cultured in sand maize meal medium (sand: maize meal: distilled water-980:20:250 ml). The inoculum of the

pathogen was then used in the plots in field as per treatment schedule.

T. harzianum was mass multiplied on organic substrates (vermicompost and leaf mold) amended with required quantity (20% of neem cake) and used as organic based formulation of the antagonist.

For seed treatment seeds were primed with homogenized mycelial mat of isolates of *Trichoderma* spp. at 4g/kg of seed grown in potato dextrose broth at 4g/kg of seed. A pinch sodium salt of carboxy methyl cellulose (CMC) was added with the mycelial mat as adhesive during priming so that mycelial mat forms a persistent coating on the seed surface. The seeds along with the mycelial mat were shaken gently in a wrist action shaker for 5 min to get a uniform coating on the seeds.

Field experiment was conducted to determine the efficacy of *Trichoderma* against root and collar rot of cowpea during *pre kharif* season of 2011 at Jaguli Instructional Farm, Bidhan Chandra Krishi Viswavidyalaya (BCKV). The experiment was laid out in Randomized Block Design (RBD) with a total of seven treatments and each treatment replicated thrice. Seeds were treated with antagonist and fungicide separately as per treatment schedule before sowing. Plants were raised following the normal agronomic practices. The observations on seed germination percentage, mortality percentage, per cent disease reduction and survival percentage of cowpea plants were recorded starting from seedling emergence up to 45 DAS at 15 days interval. Growth, vigour index and biomass of seedling was recorded up to 45 days after sowing and yield parameters were recorded at harvesting. The treatments are:

T_0 - Control (seeds without treatment, soil without inoculated with pathogen)

T_1 - Control (seeds without treatment, soil inoculated with pathogen)

T_2 - Seeds treated with antagonist + soil inoculated with pathogen

T_3 - Seeds treated with carbendazim + soil inoculated with pathogen

T_4 - Seed without treatment + soil application of Trichoderma bioformulation (VC + 20% NC) + soil inoculated with pathogen

T_5 - Seed without treatment + soil application of Trichoderma bioformulation (LM + 20% NC) + soil inoculated with pathogen

T_6 - Seed without treatment + soil application of talc based formulation of Trichoderma + soil inoculated with pathogen

Results and Discussion

The treatment effect under field condition was found significant with respect to seed germination, percent mortality, plant growth and yield in cowpea. Seedling emergence due to inoculation of the pathogen before sowing in the field was reduced marginally. Inoculation with *R. solani* grown in sand-maize-meal medium in field after emergence of seedling caused significant seedling mortality in all cases.

In case of cowpea plant results (Table-1) revealed that all the treatments significantly (P=0.05) enhanced the field emergence and reduce the disease incidence. Seed germination of cowpea under field condition was recorded in the range of 72-87%. Maximum seed germination was recorded with carbendazim treated seed (87.3%) followed by *T. harzianum* formulation in vermicompost+20% neem cake treatment (85%).

The per cent mortality of cowpea plants due to root rot and collar rot was reached at 64.2% at 45 DAS where only pathogen was added (Table-1)

but where no pathogen inoculam was applied, percent mortality due to natural infection was nil. After 45 DAS per cent mortality of cowpea reduced significantly while either *Trichoderma* formulation or fungicide application as seed dressing or soil application method before seed sowing was done. Maximum survival of cowpea was obtained where no inoculum either pathogen or antagonist was applied. Whereas only 35.8% cowpea plant could survive escaping the pathogen applied. Situation improved significantly (P=0.05) under either *T. harzianum* application in any form or synthetic fungicide application. Maximum survival (85.3%) in dual inoculation system was obtained when *T. harzianum* was applied in vermicompost+20% neem cake formulation followed by leafmold+20% neem cake formulation (83.1%). The mortality of percentage of cowpea plant due to *R. solani* infection, increased with days after sowing which was very drastic where only pathogen inoculum was applied (64.2%) at 45 DAS. The effect of seed priming (19.9%) and talc based formulation treatments (19.8%) were at par. In case of fungicides treated seed the percent mortality of cowpea plant due to pathogen infection was reached at 22.5% at 45 DAS.

The treatment effect on growth, vigour and biomass of cowpea plant was also evaluated at 45 DAS (Table-2). *T. harzianum* for seed biopriming or soil application as organic base formulation or by talc formulation promoted plant growth and biomass significantly compared to control (without *Trichoderma* and *R. solani*; only *R. solani* inoculation or only carbendazim as seed treatment). The performance of cowpea due to treatment with *T. harzianum* with respect to plant height, basal girth, number of pods/plant, pod dry weight, branches/plant and overall total plant biomass production 45 DAS. Maximum plant height (112.4cm) was obtained with organic formulation of *T. harzianum* in vermicompost

Table 1.
Seedling emergence, mortality percentage of cowpea at different days after sowing

Treatment	Seedling emergence*	Percent mortality*			Percent disease reduction (%)	Percent survival (%)
		15DAS	30DAS	45DAS		
T_0	77.3 (61.9)	0.0 (4.05)	0.0 (4.05)	0.0 (4.05)	-	100
T_1	72.7 (58.8)	26.1 (31.1)	44.6 (42.2)	64.2 (53.5)	-	35.8
T_2	84.0 (66.8)	11.2 (20.0)	15.1 (23.3)	19.9 (26.9)	69	80.1
T_3	87.3 (69.6)	12.0 (20.7)	16.2 (24.1)	22.5 (28.7)	64	77.5
T_4	85.0 (67.6)	9.8 (18.7)	12.5 (21.1)	14.7 (22.9)	77.1	85.3
T_5	84.0 (66.8)	10.2 (19.1)	13.4 (21.8)	16.9 (24.7)	73.7	83.1
T_6	84.7 (67.4)	11.8 (20.5)	15.5 (23.6)	19.8 (26.8)	69.15	80.2
SEm (±)	0.157	0.283	0.275	0.312	-	-
CD (5%)	0.338	0.577	0.589	0.646	-	-

*Mean of 5 replications

+20% neem cake followed by leaf mold+ 20% neem cake (94.6cm). Plant height in cases of *T. harzianum* primed seeds (71.2cm) and carbendazim treated seeds (59.9cm) was better than talc based formulation of *T. harzianum* (48.4cm). Maximum basal girth of plant was obtained with leaf mold formulation of *T. harzianum* (4.3 cm). Similarly highest pod/plant (11no), pod length (20.34cm), dry weight of pods (4.224g) was obtained with vermicompost +20% neem cake based formulation of *T. harzianum*. Effect of *T. harzianum* application in any form on plant biomass was non-significant except talc based formulation (Table 2).

Yield and yield parameters of cowpea plant at 45 and 60 DAS were also influenced by the treatments significantly at 5% level (Table- 3). Highest yield of pod (142.05g) along with highest number of pod was obtained with vermicompost+20% neem cake formulation of *T. harzianum* (210 nos) followed by leaf mold+20% neem cake formulation after second harvesting. Even pod yield (11.31g) was found higher with *T. harzianum* primed seed than talc based formulation (93.8g).

Biocontrol fungi of the genus *Trichoderma* have got the ability to interact both parasitically and symbiotically, with different substrates and living organisms, including plants and microbes (Harman & Kubicek 1998). In the present investigation the *Trichoderma* was used as seed bioprimer, soil application through organic based and talc based formulations against root and collar rot of cowpea (*R. solani*) under field condition of artificial inoculation by the respective pathogen inocula. Among the six treatments soil application of *T. harzianum* through organic based formulations (vermicompost+20% neem cake or leafmold+20% neem cake) was found superior over other treatments in reducing the disease incidence as well as improving seedling emergence. Seedling stand was improved when the antagonist was applied as different preparation of agro-substrates in soil. Use of *Trichoderma* as a biocontrol agent has many constraints because *Trichoderma* colonize the spermosphere effectively but do not survive well in rhizosphere and are active only in some soil and seasons (Deacons 1994). Many isolates of *Trichoderma* do not readily proliferate in the soil and confined to seed coat/seed surface in case of seed treatment.

Table 2.

Growth, vigour, biomass of cowpea at different stages of growth in field

Treatment	Plant height (cm)*	Basal girth (cm)*	Branches /pl*	Pod /pl*	Pod length (cm)*	Dry weight of pod (g)*	Plant biomass (g)*
T_0	31.8	3.1	3	6	13.94	1.105	5.95
T_1	38.7	3.2	3	4	14.86	1.122	5.7
T_2	71.2	4.2	7	8	19.82	4.215	13.9
T_3	59.9	3.5	5	7	16.34	2.868	10.6
T_4	112.4	4.1	7	11	20.34	4.224	13.76
T_5	94.6	4.3	6	8	18.38	3.892	13.7
T_6	48.4	3.9	5	8	17.86	3.118	10.9
SEm (±)	1.621	0.023	0.022	0.773	0.511	-	-
CD (5%)	4.081	0.204	0.191	1.032	0.822	-	-

*Means of 10 replications

Table 3.
Yield and yield parameters of cowpea at different days of sowing in field

Treatment	No of Pod (1^{st} pluck)*	No of Pod (2^{nd} pluck)*	Total no of Pod	Pod yield (1^{st} pluck)*	Pod yield (2^{nd} pluck)*	Total yield (g)
T_0	57	68	125	31.12	40.12	71.24
T_1	44	50	94	23.41	25.60	49.01
T_2	74	89	163	50.08	61.23	111.31
T_3	80	85	165	42.40	46.54	88.94
T_4	96	114	210	63.11	78.94	142.05
T_5	83	102	185	52.05	65.76	120.81
T_6	73	82	155	43.5	50.3	93.8
SEm (±)	0.894	0.785	-	0.612	0.822	-
CD (5%)	3.372	2.956	-	2.345	3.197	-

*Means of 10 replications

T. harzianum applied to cowpea seed as biopriming gave better result than talc based formulation of *T. harzianum* although in case of disease control the treatment effect was not significantly different under field condition. At the same time an organic amendment supports the greater multiplication of the antagonist and thereby increases the population density of *Trichoderma* in rhizosphere soil which are in accordance with Jahn and Puls (1998), Bhagat and Pan (2010). Soil amendments with agricultural wastes alone or in combination with biocontrol agents have been recommended for the control of soil borne pathogens that subsequently increases the yield of many crops (Liu & Hunary 2000; El-Mohamedy 2004). Among the organic based bioformulation vermicompost along with 20% mustard cake was found best. Carbendazim treated seeds showed least performance compared to other treatments. Moreover the formulated products of *Trichoderma* in organic substrates were also recorded to increase significant improvement of plant growth, vigour, biomass, yield and yield parameters. The reduction in soilborne plant diseases and subsequent enhancement in the yield of different crops after treatment with formulations of *T. harzianum* have been reported by several workers (Harman *et al.* 2004; Singh & Singh 2004).

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