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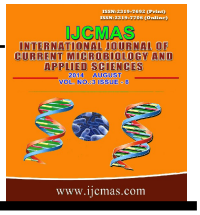

Original Research Article

Evaluation of suitable organic substrates based *Trichoderma harzianum* formulation for managing *Rhizoctonia solani* causing collar rot disease of cowpea

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Keywords

Organic substrates, *Trichoderma harzianum*, *Rhizoctonia solani*, Seedling mortality, Cowpea

A B S T R A C T

Locally available six organic substrates *eg.* spent mushroom compost (SMC), farmyard manure (FYM), vermicompost (VC), sorghum grain (SG), wheat grain (WG) and broken maize grain BMG based *Trichoderma harzianum* T5 based formulation were selected for testing their efficacy in managing *Rhizoctonia solani* causing collar rot disease of cowpea. *T. harzianum* T_5 population in spent mushroom compost (SMC) was maintained at significantly higher level than any other organic substrates considered maintaining (15 x 10^7cfu/g) substrates population upto 240 DAI. Of these six organic substrate based *T. harzianum* T_5 + *R. solani* treatments, SMC based *T. harzianum* T_5 + *R. solani* treatment was found superior to others in reduction of seedlings mortality, number of leaves, shoot and root length, shoot and root dry weight and total plant dry weight of cowpea seedlings except vigour index, whereas, wheat grain (WG) based *T. harzianum* T_5 + *R. solani* treatment was observed inferior.

Introduction

For mass production of *Trichoderm*a spp. inocula, different inorganic inert materials and organic substrates are used as carriers or delivery media during formulation. Some of the commonly used locally available substrates are agro-industrial cellulosic wastes (wheat straw, paddy straw, shelled maize cob, saw dust, paper waste, vegetable and fruit waste, coffee waste, tea waste and sugarcane bagasse), organic manures (farm yard manure, neem cake, vermiculite and spent mushroom compost) and cereal bran (wheat, grain of sorghum, wheat and pulse and rice bran, (lewis *et al*, 1991; Prakash, *et al*., 1999; Saju, *et al*., 2002; Kaviyarasan and Siva, 2007; Rini and Sulochana., 2007; Kumar and Dohroo, 2008; Mustafa, *et al*., 2009; Chaudhari *et al*., 2011; Simon and Anamika, 2011; Khandelwal, *et al*., 2012; Yadav, 2012; Babu and Pallavi, 2013, apart from these talc, lignite Mandhare and Suryawanshi, 2005) were also being used which support better shelf life of the *Trichoderma* spp as well its better

performances in management of collar rot disease of cowpea with promotion of growth as well. Collar rot disease of cowpea caused by *Rhizoctonia solani* is one of the main disease of cowpea during the seedling stage and continue upto maturity which totally collapse the plant and henceforth reduced the yield. Various chemical were available in the market for the control of this disease, but viewing the disadvantage of the negative effect of fungicide in ecology as well as the resurgence of pathogen resistance, laboratory proven efficient filamentous hyperparasitic fungus *Trichoderma isolate* (T5) were selected. The *Trichoderma* not only reduced the growth of *Rhizoctonia solani in vitro* condition and in rhizosphere but also promote the growth of the plant which thereby serving the dual benefit. They also utilize numerous mechanisms for both attacking other soil organisms and enhancing plant and root growth (Benitez *et al*., 2004; Harman, 2000; Harman *et al*., 2004a; Vinale *et al*., 2008a). Keeping in mind the following experiment was set up by selecting locally available six organic substrates *viz.* farm yard manure (FYM), vermi-compost (VC), spent mushroom compost (SMC) of *Pleurotus* mushroom, grains of maize, wheat and sorghum.

Materials and Methods

Substrates selection

Locally available six organic substrates *eg.* Farmyard manure (FYM), vermi-compost (VC), spent mushroom compost (SMC), broken maize grain (BMG), wheat grain (WG) and sorghum grain (SG) were selected for the determination of suitable substrate for *Trichoderma* multiplication and improved shelf-life at normal temperature during storage. FYM, VC and SMC were ground to powder passing 200µm sieve whereas broken maize grain, sorghum grain and wheat grain were made half boiled before use. A total of eighteen samples (six dates of observation x three replications), 25 g for each treatment were packed inside the double layered polypropylene bag and plugged with non-absorbent cotton at the mouth tightened with rubber band. All the samples were double sterilized for 1 hour at the first day, then 40 minutes at the next day for complete sterilization.Conidia from 7 days old culture of *Trichoderma harzianum* isolate T_5 which had good antagonistic potential, was inoculated with 2.1 -2.2 x 10^7 cfu/ g substrates and kept at room temperature for growth. Enumerations of *T. harzianum* T_5 population were done at 7, 30, 60, 120, 180 and 240 days after inoculation following serial dilution plate technique (Chung and Hoitink, 1990). For this purpose, five grams of each sample were diluted with 45 ml of sterile distilled water and plated on *Trichoderma* specific medium (TSM). After 5 days of incubation at 28 ± 1^OC, colonies of *T. harzianum* were counted.

Pot experiment

The experiment was conducted in 2013 with alluvial soil [sand - 40.4%, silt - 36.4%, clay - 24.3%, organic C (%) - 0.49, N (%) - 0.054, available P - 14.9 ppm and available K - 97.0 ppm] under glass house condition at the University Research Farm, Kalyani West Bengal with nine treatment combinations following completely randomized design (CRD) with three replications to evaluate the performance of different organic substrate based *Trichoderma harzianum* T_5 formulations against cowpea collar rot disease incidence and growth promotion of cowpea (*Vigna unguiculata* L. cv. Kashikanchan). Each polyethylene pot of 15 cm height x 12 cm diameter was filled with 2 Kg of alluvial soil. Then, three grams of the sand maize

medium (sand, maize and distilled water @ 37.0g, 50.0g and 13 ml respectively for 100 g) containing *Rhizoctonia solani* was mixed thoroughly with 5 - 6 cm layer of soil of each replication under treatments those were earmarked to receive *R. solani* inocula. Ten pre- soaked cowpea seeds pelleted with *Trichoderma harzianum* T_5 obtained from different organic substrates were sown treatment-wise separately. Total number of seedlings after emergence was recorded. Plants were allowed to grow up to 45 days with watering as and when required basis. Seedling height and germination percentage were recorded and vigour index was calculated as per the procedure suggested by Abdul-Baki and Anderson, 1973.Vigour index = Germination per cent x Total length of seedling (cm). The different treatments combinations are shown in Table 2

Statistical analysis

All the parameters recorded were assessed with one-way ANOVA. Duncan's multiple range test was applied when one-way ANOVA revealed significant differences (P<0.05). All statistical analyses were performed with SPSS 12.0

Results and Discussion

At 7 days after inoculation (DAI), sorghum grain substrate had maximum population of *T. harzianum* T_5 (22.5 x 10^7cfu/g) followed by broken maize grain and wheat grain while vermi-compost (12.7 x 10^7cfu/g) substrate had minimum (Table 2). *T. harzianum* T_5 population showed an increasing trend in all the six substrates up to 30 DAI and thereafter, it exhibited decreasing trend up to 240 DAI. The rate of declination of *T. harzianum* T_5 seemed to be rapid in grain based organic substrates than SMC, VC and FYM based complex substrates. The population of *T. harzianum* T_5 at 30 DAI was recorded highest again in sorghum grain (64.0 x10^7cfu/g) followed by SMC (53.0 x 10^7cfu/g), wheat grain (51.0 x 10^7cfu/g) and others. Its population in sorghum grain, wheat grain and broken maize grain substrates was minimum at 180 DAI and was not at detectable level at 10^{-7} dilution beyond 180 DAI, whereas, the same in SMC was maintained at significantly higher level than vermi-compost and farm yard manure substrates at 180 DAI and even at 240 DAI. It was evident from the results of this experiment that out of six substrates selected, *T. harzianum* T_5 population in SMC was maintained at significantly higher level than any other organic substrates considered maintaining (15 x 10^7cfu/g) population upto 240 DAI.

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In the pot experiments, reduction of seedling mortality of cowpea seedlings in different organic substrate based *T. harzianum* T_5 treatments under *R. solani* inoculation were found superior to sole inoculation with *R. solani* but inferior to control and heat killed treatments (Table 2) but heat killed treatment were *at par* with control. Of the different organic substrate based *T. harzianum* T_5 + *R. solani* treatments, WG based *T. harzianum* T_5 + *R. solani* treatment exhibited highest per cent seedling mortality (36 %) followed by SG based *T. harzianum* T_5+ *R. solani* treatment (34 %) and others but SMC based *T. harzianum* T_5 + *R. solani* treatment showed the lowest (16 %).

Number of leaves, shoot and root length, shoot and root dry weight and total plant dry weight of cowpea seedlings in six organic substrate based *T. harzianum* T_5 + *R. solani* treatments were significantly higher than sole *R. solani* inoculation and even better than heat killed inoculum and control treatments (Table - 2). Of these six organic substrate based *T. harzianum* T_5 + *R. solani* treatments, SMC based *T.*

harzianum T_5 + *R. solani* treatment was found superior to others in all the parameters considered except vigour index parameter, whereas, WG based *T. harzianum* T_5 + *R. solani* treatment was observed inferior. Seedling vigour index was observed highest in SG based *T. harzianum* T_5 + *R. solani* treatment (3239) followed by *at par* effect of SMC based *T. harzianum* T_5 + *R. solani* treatment (3174), whereas, it was noted lowest in sole *R. solani* treatment (1304). The performances of seedlings in heat killed and control treatments were better than sole *R. solani* treatment. Plant vigour index of these two treatments appeared to be *at par* with SMC based *T. harzianum* T_5 + *R. solani* treatment. It was apparent from the results that SMC based *T. harzianum* T_5 had greater capacity in the reduction of seedling mortality and augmentation plant growth promotion against *R. solani*.

Except SMC, the rate of declination of *Trichoderma* population in other substrates was faster. At 7 and 30 DAI, SG had maximum population (22.5 and 64 cfu/g) than others. At 30 DAI SMC appeared as the second best substrate after SG and followed by WG. Three grain based substrates *viz.* WG, SG and BMG supported higher population of *Trichoderma* than that in VC and FYM at 30 DAI. Tewari and Bhanu (2004) also observed maximum growth and sporulation of *Trichoderma* on grain based substrate like rice bran up to 15^{th} DAI but paddy and wheat straw substrates took the lead in sporulation at 20^{th} DAI keeping the rice bran substrate behind. SMC maintained a significantly higher population than VC and FYM up to 240 DAI, while WG, SG and BMG had non- detectable population at 240 DAI. Even at 180 DAI, VC and FYM were better organic substrates for *Trichoderma* multiplication and had better shelf-life than three grain based substrates *viz.* WG, SG and BMG. Such maintenance of longer shelf- life by FYM and vermi-compost has been reported earlier by Sarode *et al*. (1998) and Pan and Das (2010). Sarode *et al*. (1998) screened five substrates for multiplication of *Trichoderma* and detected FYM and Talc as suitable substrates for multiplication, delivery and storage of *Trichoderma* up to the period of 8 months at room temperature with perceptible loss in CFU. Pan and Das (2010) proved that vermi-compost produced high population of *Trichoderma harzianum* isolate (Th1 AN) at 120 days of incubation out of four organic substrates *e.g.* vermi-compost, leaf manure, rice bran and FYM tested.

The support of three grain based substrates *viz.* WG, SG and BMG toward higher multiplication of *Trichoderma* than VC and FYM may be due higher presence of available nutrients at the initial stage as compared to VC and FYM. When these available nutrients are exhausted, the population of *Trichoderma* falls rapidly. But in FYM, VC and SMC, nutrients are released slowly.

Among these three slow nutrient releasing substrates, SMC is unique organic substrate to *Trichoderma* because of the presence of chitin rich mushroom mycelial mat and slowly degrading lingo-cellulosic straw. Both the substrates were highly preferred and efficiently utilized by *Trichoderma* due to its ability in higher production of chitinase, ligninase, hemi-cellulase and cellulase enzymes (Knapp and Howell, 1980; Kaviyarasan and Siva, 2007; Ali *et al*., 2011). *Trichoderma* spp. decompose lingo-cellulosic substrate slowly in succession by the action of cellulase enzymes according to their requirement and release mono and di-saccharides for their further growth and development (Shin *et al*., 2000; Phutela *et al*., 2011).

Table.1 Population of *Trichoderma harzianum* (T_5) (CFU/g) in different organic substrates stored at room temperature up to 240 days after inoculation

Organic substrates	*Trichoderma harzianum* (T_5) population ($x10^7$ CFU/g sample) in organic substrates at different days of storage						
	0 day	7 days	30 days	60 days	120 days	180days	240 days
SMC	2.1	18.5 c	53.3 b	47.0 a	39.0 a	21.0 a	15
VC	2.2	12.7 e	34.0 e	30.0 b	25.0 b	7.0 b	0.9
FYM	2.1	15.7d	28.3 f	23.7 d	18.3 c	3.3 c	0.4
WG	2.2	20.1 b	51.0 c	27.3 c	8.7 d	0.1 d	ND
SG	2.1	22.5 a	64.0 a	46.2 a	10.0 d	0.9 d	ND
BMG	2.1	20.6 b	42.0 d	22.7 d	7.0 e	0.1 d	ND
SEm ±		0.2	0.6	0.5	0.5	0.4	0.1
$CD_{.05}$	NS	0.6	1.9	1.6	1.6	1.1	0.2

Spent mushroom compost= SMC, Vermicompost (VC), farmyard manure (FYM), wheat grain (WG), sorghum grain (SG), broken maize grain (BMG)

Table value of t at 5% for 12 error degree of freedom= 3.11; ND = Not detectable at 10^{-7} dilution plates; Values followed by same letter in each column do not differ significantly

Table.2 Response of six organic substrate based *Trichoderma harzianum* T_5 formulations on seedling mortality and growth promotion of cowpea against collar rot disease

Treat-ments	Per cent seedling mortality (Reduction of seedling mortality over *R. solani*)	No. of leaves	Shoot length (cm)	Root length (cm)	Shoot weight (g/plant)	Root weight (g/plant)	Total dry weight (g/plant)	Vigour index
T1	8.9 [16 def*] (31)**	14.0 a	36.4 a	6.9 a	0.91 a	0.170 a	1.08 a	3174 ab
T2	15.1 [20 cde] (27)	12.3 bcd	35.4 ab	6.5 b	0.88 ab	0.159 b	1.04 bc	2652 cd
T3	26.2 [31 bc] (16)	12.7 abc	34.2 bc	5.9 cd	0.86 ab	0.162 ab	1.02 c	2537 cd
T4	23.4 [29 bcd] (18)	12.3 bcd	33.4 c	5.6 d	0.85 b	0.156 b	1.00 c	2719 bcd
T5	32.1 [34 abc] (13)	13.0 ab	34.4 c	6.0 c	0.83 b	0.158 b	0.98 c	3239 a
T6	34.9 [36 ab] (11)	12.3 bcd	30.5 d	5.6 d	0.77 c	0.155 b	0.93 d	2407 d
T7	3.3 [9 ef] (38)	11.0 d	27.8 e	4.8 e	0.71 d	0.136 c	0.85 e	2930 abc
T8	53.3 [47 a] (0.0)	11.0 d	25.8 f	4.3 f	0.63 e	0.100 d	0.73 f	1304 e
T9	0.0 [4 f] (43)	11.3 cd	27.2 e	4.7 e	0.70 d	0.135 c	0.84 e	2979 abc
SEm±	4	0.5	0.4	0.1	0.02	0.003	0.1	155
$CD_{.05}$	12	1.4	1.2	0.4	0.05	0.008	0.2	461

T1= SMC based *Trichoderma harzianum*T_5 + *R. solani*, T2= VC based *Trichoderma harzianum*T_5 + *R. solani*, T3= FYM based *Trichoderma harzianum*T_5 + *R. solani*, T4 = BMG based *Trichoderma harzianum*T_5 + *R. solani*, T5 = SG based *Trichoderma harzianum*T_5 + *R. solani*,T 6= WG based *Trichoderma harzianum*T_5 + *R. solani* , T7 = Heat killed substrates based *Trichoderma harzianum*T_5 + *R. solani*, T8 = R. solani alone, T9 = Control

Table value of t at 5% for 18 error degree of freedom= 2.51; *R. solani =Rhizoctonia solani,* *Value within the third bracket indicates the arc- sine transformed value; ** Value within parenthesis indicates reduction of seedling mortality over *R. solani;* Values followed by same letter in each column do not differ significantly

T. harzianum T_5 population in SMC found to maintain at significantly higher level than other organic substrates considered might be due to slow release of mono and disaccharides for its further growth and development through lingo-cellulosic substrate decomposition capacity by the organism. SMC was used earlier as substrate to support the growth of bio-control agents (Andrews *et al*., 1994; Cronin *et al*., 1996; Uzun, 2004).

Kaviyarasan and Siva (2007) use spent mushroom compost derived from oyster mushroom cultivation as a carrier substrate for *Trichoderma longibrachiatum*, *T. Harzianum*, *T. viride* and *Gliocladium virens*. They found that survival rate of *Trichoderma* spp. was high in SMC as carrier substrate as compared to the commercial products.

Cowpea seeds separately bio-primed with six organic substrate based *T. harzianum* T_5 formulations were sown challenging with *R. solani* inoculation. Result showed that SMC based *T. harzianum* T_5 formulation had greater capacity in the reduction of seedling mortality and caused significant improvement in seedling growth and vigour even under challenged inoculation with *R. solani* than other organic substrate based *Trichoderma harzianum* T_5 formulations tested. The superiority of SMC based *Trichoderma harzianum* T_5 formulation over other organic formulations may be hypothesized by stating that SMC perhaps helps in the maintenance of antagonistic potential of *Trichoderma* better than other substrates through enhancement of mycoparasitism, hydrolytic enzyme production, antibiosis *etc*. The findings of Andrews *et al*. (1994), Cronin *et al*. (1996) and Uzun (2004) indicated that SMC gained disease suppression capacity by supporting growth of bio-control agents that act through antibiosis, competition and parasitism. Ability of SMC based *Trichoderma harzianum* T_5 formulation to reduce disease incidence and promote growth parameters as observed in the present experiment was noted previously by Kaviyarasan and Siva (2007) when they used SMC based *Trichoderma longibrachiatum*, *T. harzianum* and *T. viride* and *Gliocladium virens* formulations for controlling disease caused by *Rhizoctonia solani* in tomato. They mentioned that SMC based *Trichoderma* formulation not only reduced the disease but also stimulated flowering, plant height, fresh and dry shoot weight, and increased yield by 101.9, 61.5, 27.9, 38.3 and 102.5%, respectively.

The reduction of disease caused by *Rhizoctonia solani* in tomato was attributed higher chitinolytic activity by *Trichoderma* spp. Greater the potentiality of disease or pathogen reduction, the more would be the capacity of growth promotion. Therefore, it is apparent from the above mentioned discussions that SMC is not only a good substrate for multiplication and shelf-life maintenance of *T. harzianum* T_5 but also has the capacity to reduce disease incidence, improve plant growth parameters when it is used as a delivery substrate of bio-control agent, *T. harzianum* T_5.

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