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Enrichment of liquid farm inputs with *Trichoderma harzianum* for field application

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Abstract

The biocontrol agent *Trichoderma harzianum* is extensively used for the management of soil borne diseases of black pepper and cardamom in India after mixing with cow dung, composts, decomposed coffee pulp and different types of oil cakes. Clarifications are often sought by the stake holders on the possibility of integrating liquid farm inputs like biogas slurry, cow dung slurry and fertilizer solutions with the bioagent. Hence an experiment was carried out to evaluate the growth, multiplication and survival of *T. harzianum* in liquid inputs commonly available at the farm level. Biogas slurry, cow dung slurry, NPK (19:19:19) solution, Urea-SSP-MoP solution and irrigation water were inoculated with *Trichoderma* spore suspension in the proportion of 100:1. Colony forming units of *Trichoderma* and pH of the inputs were determined after 1, 2, 3, 4 & 10 days. The bioagent propagules after 10 days showed that the *Trichoderma* spore suspension (control) sustained significantly high level of population followed by biogas slurry and then cow dung slurry. It was followed by NPK (19:19:19) solution and irrigation water which were on par with each other and the population was sufficiently high enough to go for field application. Propagules in Urea-SSP-MoP solution was the least, much below the required level for field application. The pH of the Urea-SSP-MoP solution was strongly acidic and that is why it did not support the growth of *Trichoderma* and hence cannot be used for enriching with the bioagent. In all the other inputs, the pH was found to be more or less neutral. The results showed that *Trichoderma* could be mixed with liquid farm inputs like biogas slurry and cow dung slurry for field application thereby slightly reducing the labour since it is applied at the same time instead of two rounds. When *Trichoderma* is to be mixed with fertilizer solution, pH must be taken in to consideration that it should be above 5.5 as in the case of NPK solution and too acidic pH does not support the survival and growth of *Trichoderma*.

Keywords: Biogas slurry; Cow dung slurry; Disease management; Liquid fertilizer; Spices; *Trichoderma harzianum*

Introduction

Foot rot of black pepper caused by *Phytophthora capsici* is the major soil borne disease affecting the crop (Anandaraj and Sarma, 1995). Similarly, capsule rot of cardamom caused by *P. meadii* and *P. nicotianae* var. *nicotianae* and rhizome rot caused by *Pythium vexans* and *Rhizoctonia solani* results in severe crop loss (Thomas and Suseela Bhai, 1995). Application of the biocontrol agent *Trichoderma harzianum* is one of the methods to control these soil borne diseases of black pepper and cardamom (Anandaraj et al., 2003; Saju et al., 2002a; 2002b; 2003; Saju 2004; Sarma and Saju, 2004; Vijayan, 2011). *T. harzianum* employs different modes of action to combat the pathogens (Paul et al., 2005; Saju and Sarma, 2011; Saju et al., 1999; 2001). The commercial formulations of the biocontrol agent mainly in the form of coffee husk (parchment), powder and liquid are extensively used for the purpose (Siddhartha et al., 2017). In general, the living fungal spores (active substance) are incorporated in various formulations, both traditional and innovative, for applications as foliar sprays, pre-planting applications to seed or propagation material, post-pruning treatments, incorporation in the soil during seeding or transplant, watering by irrigation or applied as a root drench or dip (Kumar et al., 2014; Subash et al., 2014; Woo et al., 2014). In coffee and black pepper gardens in Karnataka, these biocontrol agent formulations are applied after enriching with cow dung, composts, decomposed coffee pulp and different types of oil cakes. In addition, *Trichoderma* is also found to be a potential antagonist of *Colletotrichum gloeosporioides* and *Pestalotiopsis* sp. infecting large cardamom cultivated in Sikkim and Darjeeling (Mahesh et al., 2014; Saju et al., 2011; 2012). Apart from the solid organic substrates, there are liquid farm inputs like biogas slurry, cow dung slurry and fertilizer solutions for foliar spray frequently prepared at the farm level for application (Saju et al., 2019). The slurries are stored in larger tanks at the point of its production and then pumped to the plots and applied with the help of hose.

Advice and clarifications are often sought by the stake holders on the possibility of enriching these slurries with biocontrol agents like *Trichoderma*. It is known that these biocontrol agents are also produced in liquid fermentation system where there are proper conditions like agitation and aeration for optimum growth and multiplication (Saju et al., 2002c). However, at the farm level these inputs in the tank are static and the multiplication of bioagents in it is not studied systematically. Convincing information is required on the growth and survival of *Trichoderma* in these liquid farm inputs so that both could be applied simultaneously thus reducing the cost of labour. Hence an experiment was carried out to evaluate the growth and multiplication of *T. harzianum* in liquid inputs available at the farm level**.**

Materials and Methods

The biocontrol agent

The strain of *Trichoderma harzianum* identified for the biocontrol of soil borne diseases of spices and maintained in the Plant Pathology laboratory of Indian Cardamom Research Institute, Regional Station, Sakleshpur, Karnataka, India was used in the study.

Preparation of spore suspension (mother culture) of *T. harzianum*

T. harzianum was grown on 100 ml potato dextrose agar (PDA) medium in flat bottles of 1000 ml capacity. After 10 days of incubation, spore suspension was prepared using distilled water. Six bottles of culture were used to prepare 1 litre of spore suspension. The number of spores per ml of the suspension was determined by serial dilution plate technique (SDPT) on rose Bengal agar (RBA) medium. This *Trichoderma* spore suspension (mother culture) was used to inoculate liquid farm inputs.

Enrichment of liquid farm inputs with *T. harzianum* and estimation of propagules at periodic intervals

Biogas slurry, cow dung slurry and chemical fertilizer solutions were used to inoculate with *T. harzianum* to study the growth and sporulation of the biocontrol gent. 100 litres of liquid farm inputs was taken in large plastic drum and 1 litre of the *Trichoderma* spore suspension was added to it (100:1) and mixed thoroughly with wooden stick. The containers with liquid farm inputs and the biocontrol agent were arranged in completely randomized design (CRD) in a permanent shed having tin-sheet roofing in the vicinity of cardamom plantation. There were six treatments with four replications each.

Treatments

T_1 Biogas slurry + *Trichoderma* spore suspension (100:1)
T_2 Cow dung slurry + *Trichoderma* spore suspension (100:1)
T_3 NPK (19:19:19), 5 g / lit + *Trichoderma* spore suspension (100:1)
T_4 Urea-SSP-MoP (3:1:2 g / lit) + *Trichoderma* spore suspension (100:1)
T_5 Irrigation water + *Trichoderma* spore suspension (100:1, control)
T_6 *Trichoderma* spore suspension (absolute control)

The biogas slurry was collected from the discharge tank of the biogas plant. Cow dung and urine from the cow shed was diluted with water to make slurry. In both the cases the slurry was of the free flowing thickness and consistency, enabling application through hose pipe. The fertilizers were dissolved in irrigation water taken in the containers. In T_5 the spore suspension was mixed with irrigation water and served as control. In T_6 the spore suspension in distilled water was maintained as such to study its longevity of survival in it and served as absolute control. The biogas slurry and cow dung slurry containers were covered with insect proof net in order to prevent the formation of maggots. The contents in each treatment were thoroughly mixed and 100 ml samples were drawn after 1, 2, 3, 4 & 10 days and colony forming units (cfu) were determined by SDPT on *Trichoderma* selective medium (Elad and Chet, 1981).

Determination of pH of the experimental mixtures of liquid farm inputs and *T. harzianum*

The pH of the experimental mixtures involving six treatments of liquid farm inputs and *T. harzianum* was determined at various intervals by using a pH meter.

Statistical analysis

The number of cfu of *T. harzianum* recorded in various treatments was converted to corresponding logarithmic values and analysed by ANOVA and the mean comparison was done by Duncan's Multiple Range Test. The pH values of the experimental mixture were also analysed by ANOVA.

Results and Discussion

Preparation of spore suspension (mother culture) of *T. harzianum*

T. harzianum was grown on 100 ml PDA medium in flat bottles of 1000 ml capacity. After 10 days of incubation, spore suspension was prepared using distilled water. Six bottles of culture were used to prepare 1 litre of spore suspension. The cfu of *Trichoderma* in the spore suspension was 42×10^8 per ml when estimated by SDPT on RBA medium.

Enrichment of liquid farm inputs with *T. harzianum* and estimation of propagules at periodic intervals

Biogas slurry, cow dung slurry and chemical fertilizer solutions were inoculated with *T. harzianum* to study the growth and sporulation of the biocontrol gent. In biogas slurry (T_1), the population of *Trichoderma* recorded just one day after inoculation (1 DAI) and 2 DAI were found to be high and thereafter slightly reduced during

3 DAI, 4 DAI and 10 DAI. However, the population recorded was on par with the recommended dose for field application. A similar trend was noted in cow dung slurry (T_2) also. In NPK solution (T_3), the population was found high only during 1 DAI and thereafter reduced as evident from the cfu values during 2 DAI, 3 DAI, 4 DAI and 10 DAI. Even then, the population recorded during 10 DAI was sufficient enough to go for field application. In Urea-SSP-MoP solution (T_4) the population of *Trichoderma* was found to be drastically reduced just 1 DAI. Even though the population showed a slight increase during 2 DAI, it again declined drastically during 3 DAI, 4 DAI and 10 DAI. Moreover, the population density was not sufficient enough to go for field application. In irrigation water (T_5) the population of *Trichoderma* maintained a uniform level throughout the period of study and the cfu count was sufficient enough to go for field application. *Trichoderma* spore suspension (T_6) in distilled water did not show any reduction in population. The cfu values of *Trichoderma* during 10 DAI in various treatments showed that the concentrated spore suspension (T_6) maintained significantly high level of population followed by biogas slurry (T_1) and then cow dung slurry (T_2). It was followed by NPK solution (T_3) and irrigation water (T_5) which were on par with each other. Population in Urea-SSP-MoP solution (T_4) was the least, much below the required count for field application (Table1).

Table 1 Growth of *Trichoderma harzianum* in various liquid farm inputs

Treatment	No of colony forming units				
	1 DAI	2 DAI	3 DAI	4 DAI	10 DAI
T_1 Biogas slurry + TSS (100:1)	$10 \times 10^{8}_{b}$ (9.0000)	$11 \times 10^{8}_{ab}$ (9.0414)	$2 \times 10^{6}_{c}$ (6.3010)	$7 \times 10^{6}_{c}$ (6.8450)	$87 \times 10^{6}_{b}$ (7.9395)
T_2 Cow dung slurry + TSS (100:1)	$6 \times 10^{8}_{b}$ (8.7781)	$5 \times 10^{8}_{b}$ (8.6989)	$8 \times 10^{6}_{b}$ (6.9030)	$18 \times 10^{6}_{b}$ (7.2552)	$25 \times 10^{6}_{bc}$ (7.3979)
T_3 N:P:K (19:19:19), 5 g / lit + TSS (100:1)	$19 \times 10^{8}_{ab}$ (9.2787)	$4 \times 10^{6}_{d}$ (6.6020)	$5 \times 10^{6}_{bc}$ (6.6989)	$9 \times 10^{6}_{d}$ (6.9542)	$58 \times 10^{6}_{c}$ (7.7634)
T_4 Urea:SSP:MoP (3:1:2 g / lit) + TSS (100:1)	$44 \times 10^{4}_{d}$ (5.6434)	$22 \times 10^{6}_{c}$ (7.3424)	$1 \times 10^{4}_{d}$ (4.0000)	$20 \times 10^{4}_{e}$ (5.3010)	$36 \times 10^{4}_{d}$ (5.5563)
T_5 Irrigation water + TSS (100:1)	$52 \times 10^{6}_{c}$ (7.7160)	$23 \times 10^{6}_{c}$ (7.3617)	$4 \times 10^{6}_{bc}$ (6.6020)	$9 \times 10^{6}_{bc}$ (6.9542)	$18 \times 10^{6}_{c}$ (7.2552)
T_6 *Trichoderma* spore suspension (TSS)	$37 \times 10^{8}_{a}$ (9.5682)	$23 \times 10^{8}_{a}$ (9.3617)	$20 \times 10^{8}_{a}$ (9.3010)	$23 \times 10^{8}_{a}$ (9.3617)	$109 \times 10^{8}_{a}$ (10.0374)
CD (p=0.05)	0.46	0.29	0.31	0.04	0.50

DAI Days after inoculation
TSS *Trichoderma* spore suspension in distilled water as mother culture
Figures in parenthesis are logarithmic values
Treatment means with same letters in a column do not differ significantly according to Duncan's Multiple Range Test at 5% probability level

Determination of pH of the experimental mixtures of liquid farm inputs and *T. harzianum*

The pH of the experimental mixtures involving six treatments of liquid farm inputs and *T. harzianum* was determined at various intervals. The pH of the treatments T_1, T_2, T_3, T_4 & T_5 showed an increasing trend towards basic side during the period of study. In T_6, such an increase was not observed and the pH was nearly neutral. However, in general, the pH of T_1, T_2 & T_3 were found to be slightly acidic or basic (more or less neutral). In T_4 the pH was strongly acidic and that is why this treatment did not support the growth of the *Trichoderma* and hence cannot be utilized for enriching with the bioagent for field application. In T_5 and T_6 the pH was slightly acidic or basic throughout the study period (Table 2).

Table 2 pH of the liquid farm inputs during the enrichment with *Trichoderma harzianum*

Treatment	pH					
	0 Hours	1 DAI	2 DAI	3 DAI	4 DAI	10 DAI
T_1 Biogas slurry + TSS (100:1)	6.7	7.0	7.3	7.8	8.0	8.6
T_2 Cow dung slurry + TSS (100:1)	6.6	6.7	7.0	7.3	7.3	7.9
T_3 N:P:K (19:19:19), 5 g / lit + TSS (100:1)	5.5	5.3	5.6	6.0	6.1	6.5
T_4 Urea:SSP:MoP (3:1:2 g / lit) + TSS (100:1)	2.9	3.2	3.5	3.6	3.5	3.9
T_5 Irrigation water + TSS (100:1)	5.6	5.9	6.1	8.3	7.0	7.2
T_6 *Trichoderma* spore suspension (TSS)	7.5	6.2	6.6	6.6	6.8	7.3
CD (p=0.05)	0.12	0.16	0.20	0.35	0.50	0.16

DAI Days after inoculation

TSS *Trichoderma* spore suspension in distilled water as mother culture

The study aimed to determine the possibility of integrating *Trichoderma* mother culture with liquid farm inputs so as to generate information on population build up in the inputs. The *Trichoderma* spore suspension (mother culture) prepared in distilled water carried 42 x 10^8 cfu per ml. This is indeed a good propagule density enabling field application for soil borne disease management in spices. However, the population development when mixed with liquid farm inputs like organic slurries and fertilizer solutions were actually a requirement for farm advisory service.

The organic slurries are routinely used in the plantation system and the liquid fertilizer solutions are used depending on the crop stage and infrastructure. Biogas slurry, cow dung slurry, NPK (19:19:19) solution, Urea-SSP-MoP solution and irrigation water were inoculated with *Trichoderma* spore suspension in the proportion of 100:1. Colony forming units of *Trichoderma* and pH of the inputs were determined after 1, 2, 3, 4 & 10 days. The bioagent propagules after 10 days showed that the *Trichoderma* spore suspension (control) sustained significantly high level of population followed by biogas slurry and then cow dung slurry. It was followed by NPK (19:19:19) solution and irrigation water which were on par with each other and the population was sufficiently high enough to go for field application. Propagules in Urea-SSP-MoP solution was the least, much below the required level for field application (Fig. 1). Studies of Nandini and Sreenivasa (2014) reported that among the enriched organic manures, the highest population (free living nitrogen fixers, Phosphorus solubilizing bacteria and *Trichoderma* sp.) was observed in enriched vermicompost followed by enriched FYM and enriched biogas spent slurry.

Similarly, in microbial population analysis of vermicompost enriched with selective biocontrol agents showed that vermicompost fortified with *T. harzianum*s recorded highest propagules after 14 days of fortification (Subashini et al., 2021). In another study, higher growth and sporulation of *T. viride* was recorded in black gram soaked water followed by 1% jaggery solution, coconut water, rice mill effluent and 1% palmyrah fruit pulp extract after 14 days of incubation in dark room at 30°C (Emerson and Mikunthan, 2015). The recommended population of *Trichoderma* for field application is 2 x 10^6 cfu per g or lit (CIBRC, 2022). In all the treatments except T_4, the population was sufficiently high enough to go for field application even 10 days after inoculation (10 DAI). In liquid fermentation systems, still higher population of *Trichoderma* has been recorded where there is constant agitation and aeration (Saju et al., 2002c). However, at the farm level, in slurry tanks, agitation and aeration is practically negligible and hence an extremely high increase in population could not be noticed.

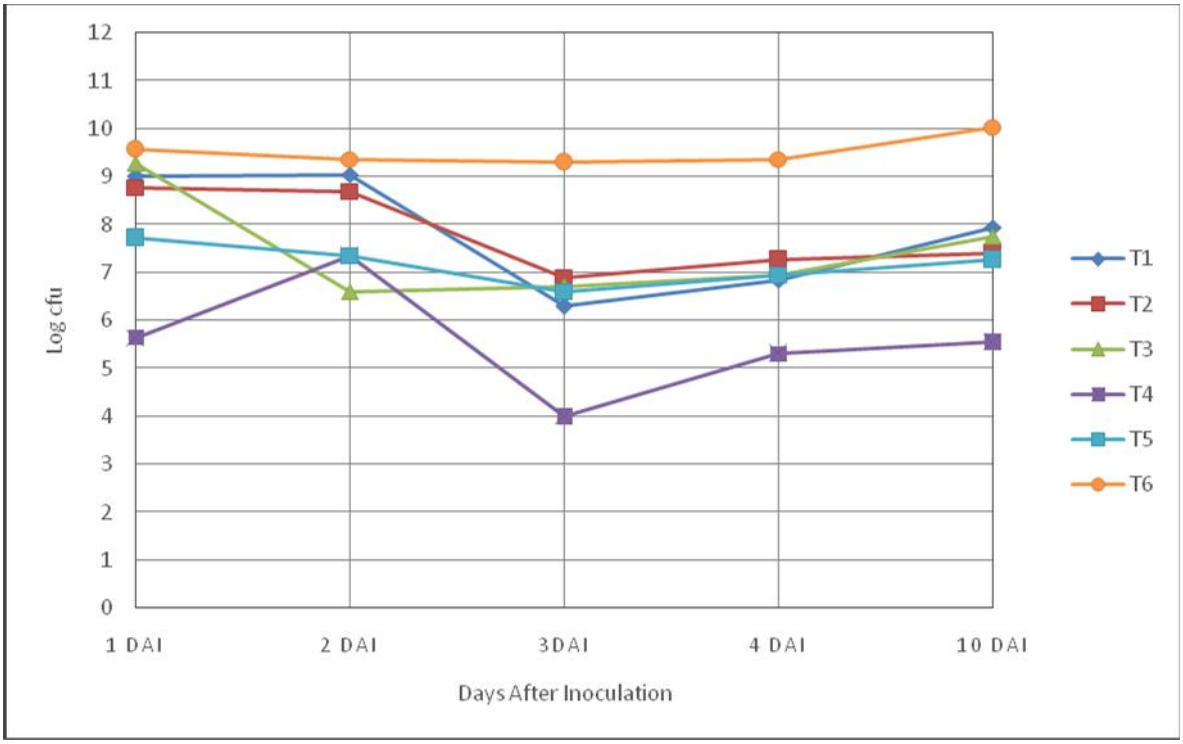

Figure 1. Propagule density of *Trichoderma harzianum* in various liquid farm inputs

The pH of the experimental mixtures involving six treatments of liquid farm inputs and *T. harzianum* was determined at various intervals (Fig. 2). pH of the growing medium greatly influences the propagule density of the bioagents in general. The present study showed that the pH of T_1, T_2 & T_3 were slightly acidic or basic (more or less neutral). In T_4 the pH was strongly acidic and this treatment did not support the growth of the *Trichoderma* and hence cannot be utilized for enriching with the bioagent for field application. However, there are reports of combined soil application of urea with *Trichoderma* for the control of *Rhizoctonia* root rot of lupine (El-Mougy and Abdel-Kader, 2014). In T_5 and T_6 the pH was slightly acidic or basic throughout the study period.

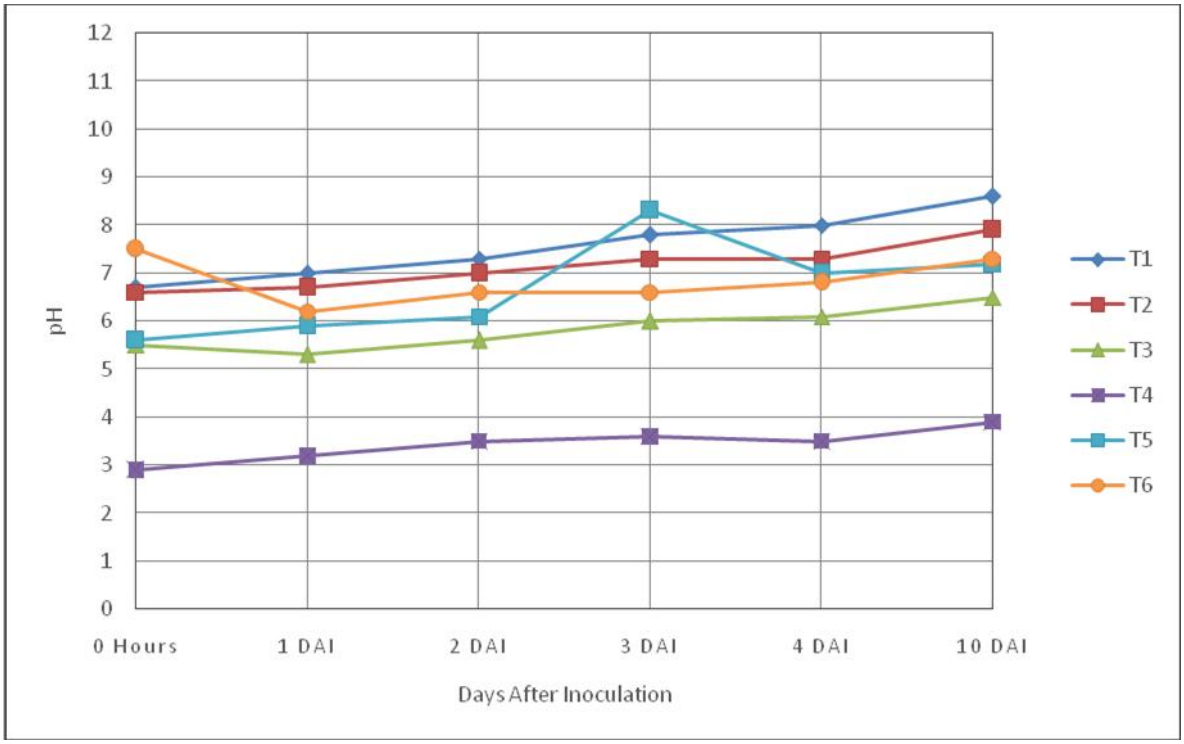

Figure 2. pH of the liquid farm inputs during the enrichment with *Trichoderma harzianum*

Conclusion

It is inferred that the *Trichoderma* could be mixed with liquid farm inputs like biogas slurry and cow dung slurry for field application as it supported the growth and survival of the biocontrol agents. Moreover, combined application of biocontrol agents and liquid farm inputs can slightly reduce the labour cost since it is applied at the same time instead of two rounds. When *Trichoderma* is to be mixed with fertilizer solution, pH must be taken in to consideration that it should be above 5.5 as in the case of T_3 and too acidic pH does not support the survival and growth of *Trichoderma*. These findings have broad functional utility for extension personnel and at the farm level where biocontrol systems are used for the management of soil borne diseases.

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