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Compatibility of *Trichoderma harzianum* (*Rifai.*) with fungicides, insecticides and fertilizers

R. SUSEELA BHAI and JOSEPH THOMAS*
Division of Crop Protection, Indian Institute of Spices Research, Calicut 673 012
**Division of Plant Pathology, Indian Cardamom Research Institute, Myladumpara 685 553*

ABSTRACT: A study was undertaken to evaluate the compatibility of commonly used agrochemicals at recommended dosages with *Trichoderma harzianum* that is being used as a biocontrol agent against capsule and rhizome rot diseases of cardamom caused by *Phytophthora meadii* and *Pythium vexans*, respectively. Three commonly used fungicides, six insecticides and NPK (75:75:150) were tested under *in vitro* and *in vivo*. Cent percent mycelial inhibition was recorded in Bordeaux mixture 1% followed by *Quinalphos* (55.84%). Other fungicides and insecticides under test were found non inhibitory at their respective recommended dosages and were at par with control, thus indicated the compatibility of *Trichoderma* with these fungicides and insecticides. *In vivo* study also showed compatibility of *T. harzianum* with chemicals and fertilizers. *Carbofuran*, *Copper oxy chloride* and *Phorate* were found highly compatible to *T. harzianum*. In addition, they were found supportive to increase the population of *T. harzianum*.

Key words: Compatibility, fertilizers, fungicides, insecticides, *Phytophthora meadii*, *Trichoderma harzianum*

The duration of active disease control can be extended by using chemical and biological control agents together in integrated management system. Even reduced amounts of the fungicide can stress and weaken the pathogen and render its propagules more susceptible to subsequent attack by the antagonist (Lorito *et al.*, 1996). Chemical protectants are effective under climatic conditions in which biological antagonists are less effective. while an active biological control agent can prophylactically colonize wounds or senescing plant tissue (Hjeljord and Tronosmo, 1998). Usually fungicide resistant or tolerant isolates of bioagents for use in integrated control are readily obtained by selection on pesticide containing media (Abd-EL Moity *et al.*, 1982). *Trichoderma* strains differ in their sensitivity to different pesticides (Koomen *et al.*, 1993). There are reports where insecticides used at recommended concentrations are inhibitory to *Trichoderma* spp. than fungicides (Tronosmo, 1989). However, the compatibility of *Trichoderma* to pesticides needs confirmation before its use in integrated management system.

Trichoderma is being used as a component in the integrated disease management of soil borne diseases of cardamom (*Elettaria cardamomum* Maton.) viz. capsule rot and rhizome rot (Thomas *et al.*, 1997; Bhai *et al.*, 1998). Similarly, some insecticides are recommended for control of insect pests such as thrips and borer for which no effective biocontrol measures have been identified. However, no information is available on the compatibility of these commonly used plant protection chemicals or fertilizers with *Trichoderma harzianum*, the biocontrol agent. Hence a study has been undertaken to test the compatibility of *T. harzianum* to commonly used and recommended dosages of fungicides, insecticides and fertilizers *in vitro* and *in vivo*.

*Corresponding author: rsbhai@rediffmail.com

MATERIALS AND METHODS

Source of the bioagent *T. harzianum*

T. harzianum (P26) isolated from the rhizosphere of cardamom and proved effective against *Phytophthora meadii* causing capsule rot and *Pythium vexans* causing rhizome rot of cardamom was used for the studies. This isolate is being maintained in the repository of Indian Cardamom Research Institute (ICRI), Myladumpara at Idukki. It was sub cultured onto Potato Dextrose Agar plates for further studies.

Fungicides, insecticides and fertilizers

Fungicides and insecticides at the recommended dosages for cardamom viz., Bordeaux mixture (BM) (1%), *Copper oxy-chloride* (0.25%), *Mancozeb* (0.25%), *Phorate* (40 g plant^{-1}), *Carbofuran* (60 g plant^{-1}), *Chlorpyriphos* (0.07%), *Quinalphos* (0.05%.), *Monocrotophos* (0.075%) and *Phosalone* (0.07%).) were used for *in vitro* and *in vivo*. Inorganic fertilizers viz. Urea, *Single super phosphate* (SSP) and *Muriate of Potash* (MOP) were used in the recommended dosage of 75:75:150kg/ha in two split doses under field and pot culture studies whereas, for *in vitro* studies, the proportionate dosage (Urea- 81.4ppm, SSP-187.5ppm, MOP-125ppm) was incorporated into the medium after sterilization.

In vitro evaluation

The above mentioned fungicides, insecticides and fertilizers in proportionate dosage were incorporated into the molten medium after sterilization and dispersed thoroughly by continuous shaking. This was poured into 90mm Petri dishes @ 15ml/plate. Mycelial plugs of 5mm cut from the growing

margin of 72h old culture of *T.harzianum was* inoculated at the centre of the Petri dish and incubated. The diameter of the colony was measured after 72h and calculated the percent growth inhibition using the formula C-T/ C x100 where C= growth in control and T=growth in treatment. The data was analyzed statistically using M S T A T C programme.

Pot culture experiment

Pot culture study was conducted in CRD with 12 treatments and 3 replications. Cardamom plants were raised in earthenware pots of 12" x12" size containing approximately 20 kg of potting mixture consisting of farm yard manure, sand and soil (1: 1: 1). These plants were applied with 200g *T. harzianum* multiplied in coffee husk having cfu of 10^8 g^{-1} The differential treatments were Bordeaux *mixture* (1%), *Copper oxychloride* (0.25%), Mancozeb (0.25%), insecticides viz. *Phorate* (20g $plant^{-1}$), *Carbofuran* (30 g $plant^{-1}$), *Chlorpyriphos* (0.07%), *Quinalphos* (0.05%), *Monocrotophos* (0.075%) and *Phosalone* (0.07%) fertilizers (Urea- 81.4ppm, SSP- 187.5ppm, MOP- 125ppm), recommended dosages of all the above fungicides, insecticides and fertilizers and control i.e. *T. harzianum* alone. The chemicals were added @ one liter/pot, 10 days after *Trichoderma* inoculation. Second round applications of treatments were given 30 days after the first application. Soil samples were collected for estimation of *Trichoderma* population twice at 3 month interval from first application. The population of *Trichoderma* was estimated by dilution plating method using *Trichoderma* specific media (Elad and Chet, 1983).

Field evaluation

The field trial was laid out in the experimental farm at Indian Cardamom Research Institute (ICRI), Myladumpara, Idukki. The trial consisted of 12 treatments as above. The experiment was in lattice design with three replications and 6 plants /treatment. Treatments were NPK 75:75:150 @ 200g $clump^{-1}$, *Bordeaux mixture* (1%) @ 2 lit $clump^{-1}$, *Copper oxychloride* (0.25%) @ 2 lit $clump^{-1}$, *Mancozeb* (0.25%) @2 lit $clump^{-1}$, insecticides viz. *Phorate* (40g $plant^{-1}$, *Carbofuran* (40g $plant^{-1}$), *Chlorpyriphos* (0.07% @100ml $clump^{-1}$), *Quinalphos* (0.05% spraying), *Monocrotophos* (0.075% spraying), *Phosalone* (0.07% spraying) and recommended dosages of all the above fungicides and insecticides and fertilizers and control with *T. harzianum* alone. *Trichoderma* multiplied in coffee husk having cfu of $3x10^8$ was applied @ 250g $clump^{-1}$ to all the plants . The differential treatments were imposed one week after *Trichoderma* application. Soil samples were collected for estimation of *Trichoderma* population after the application of different treatments twice at one month interval. The experiment was conducted for two consecutive years during 1999 and 2001.

RESULTS AND DISCUSSION

In vitro evaluation

In the *in vitro* bioassay, the growth of *T. harzianum* was recorded after 72h. The results showed significant difference in the growth among the various treatments. Among the fungicides Bordeaux mixture 1% was found highly inhibitory (100%) to *Trichoderma* when compared to *Copper oxy-chloride* (0.25%) and *Mancozeb* (0.25%). Among the insecticides *quinalphos* (0.05%.) showed an inhibition 55.84% followed by *Monocrotophos* 0.075% (12. 7%) and *Chlorpyriphos* 0.07% (8.7%). All other treatments including NPK were at par with control showing the compatibility of *Trichoderma* with these fungicides and insecticides. Even *Copper oxy-chloride, phorate and phosalone* greatly encouraged the growth (90mm) of *Trichoderma* (Table 1).

Table 1. Inhibition of *Trichoderma harzianum in vivo* in media amended with plant protection chemicals and fertilizers

Treatments		Growth at 72h (diameter)	Inhibition(%)
T1	NPK (75:75:150)	88.13	2.08
T2	*Bordeaux mixture* (1%)	0.00	100.00
T3	*Copper oxy-chloride* (0.25%)	90.00	0
T4	*Mancozeb* (0.25%)	86.10	4.24
T5	*Phorate* @ 20g $plant^{-1}$	*90.20*	*0.0*
T6	*Chlorpyriphos* 0.07%	82.18	8.69
T7	*Quinalphos* 0.05%	39.74	55.84
T8	*Monocrotophos* 0.075%	78.43	12.69
T9	*Phosalone* 0.07%	90.00	0
T10	Control	90.00	-
	LSD 0.05%	0.16	

Pot culture evaluation

When recommended dosages of fertilizer, fungicides and insecticides were incorporated into potted plants fortified with *T. harzianum*, no significant difference in population of *Trichoderma* was noticed between treatments and control. It was interesting to note that fungicides like *Copper oxychloride* enhanced the population level. Three months (90 days) after the first application a significant increase in population was noticed with *Copper oxychloride* and phorate (log e= 5.61 and 5.55 cfu g^1) respectively as against log e= 4.54 in control. The other fungicides and insecticides were also found compatible when compared to control. However *Quinalphos* and *Chlorpyriphos* showed comparatively lower cfu than other treatments. A substantial increase in population was noticed after 180 days in all the treatments except *Copper oxychloride* and *Phorate*. After 180 days *Carbofuran* showed the maximum population followed by *Copper oxy-chloride* even though there is a decrease from initial level (Table 2a). But the overall results very clearly indicated the compatibility of the bioagent with the commonly used plant protection chemicals and fertilizers at recommended dosages (Table 2b).

Field evaluation

The overall results of field evaluation (Table 3) showed that there is no significant difference in population level of *Trichoderma* between different treatments when applied as per recommended schedule individually and together in the integrated system for the control of diseases and pests as well as for crop management in cardamom. *Phorate* @ 40g $plant^{-1}$ and *Copper oxychloride* 0.25% showed the

Table 2a. Population *Trichoderma harzianum* in the soil treated with Plant Protection chemicals and fertilizers (Pot culture) (Three months interval from treatment imposition*)

Treatments		Population density (log cfu g^{-1} soil)	
		90 days *	180 days*
T1	NPK (75:75:150)	4.67 de	5.08 cd
T2	*Bordeaux mixture* 1%	4.95 c	5.33 bc
T3	*Copper oxy-chloride* 0.25%	5.61 a	5.49 ab
T4	*Mancozeb* 0.25%	4.76 cde	5.14 cd
T5	*Phorate* @ 20g $plant^{-1}$	5.55 b	5.29 bc
T6	*Carbofuran* @ 30g $plant^{-1}$	4.90 cd	5.63 a
T7	*Chlorpyriphos* 0.07%	4.48 e	5.10 cd
T8	*Quinalphos* 0.05%	4.26 e	5.19 bcd
T9	*Monocrotophos* 0.075%	4.70 cde	5.20 bcd
T10	*Phosalone* 0.07%	4.76 cde	5.21 bcd
T11	Recommended schedule of pesticide, fungicides and fertilizer	4.73 cde	5.33 bc
T12	Control (*T. harzianum* alone)	4.54 e	5.32 bc

Table 2b. Population of T. *harzianum* in the soil treated with Plant protection chemicals and fertilizers (Pot culture experiment)

Treatments		*Trichoderma* population log $cfug^{-1}$ one month after application		
		1999-2000	2000-2001	Pooled data
T1	NPK 75:75:150	3.80	3.90	3.85
T2	*Bordeaux mixture* 1%	4.34	4.18	4.27
T3	*Copper oxy-chloride* 0.25%	3.89	4.10	4.01
T4	*Mancozeb* 0.25%	4.11	3.78	3.97
T5	*Phorate* @ 20g plant-11	4.04	3.87	3.96
T6	*Carbofuran* @ 30g plant-1	3.99	4.13	4.06
T7	*Chlorpyriphos* 0.07%	4.16	4.17	4.16
T8	*Quinalphos* 0.05%	3.92	4.06	4.00
T9	Monocrotophos 0.075%	4.01	4.14	4.08
T10	*Phosalone* 0.07%	3.87	4.08	3.99
T11	Normal practice-(Recommended dosages of PP chemicals &fertilizer)	3.75	4.57	4.33
T12	Control (*T. harzianum* alone)	3.75	4.26	4.07
		NS	NS	NS

maximum population (log_e= 4.70 and 4.68 cfu g^{1}, respectively) which clearly indicated that these chemicals can be applied along with *Trichoderma* with out having any inhibitory effect on the latter. Similar trend was observed during both the years. The increase in population may be due to the reduction in natural soil fauna such as mycophagus arthropods, which feed on fungal propagules in soil (Stephen *et al.*, 2000). But there is comparatively a reduction in population during the second year in all the treatments including control. This may be due to the heavy monsoon prevailed during the period. However, the pooled data for the two years also showed that the commonly used agrochemicals are compatible with the *T. harzianum* at the

Table 3. Population of *T. harzianum* in the soil treated with Plant Protection chemicals and fertilizers (Field experiment)

Treatments		*Trichoderma* population log $cfug^{-1}$ one month after application		
		1999-2000	2000-2001	Pooled data
T1	NPK 75:75:150	4.15	4.16	4.16
T2	*Bordeaux mixture* 1%	4.55	4.23	4.42
T3	Copper oxy-chloride 0.25%	4.68	4.31	4.54
T4	*Mancozeb* 0.25%	4.40	4.16	4.30
T5	*Phorate* @ 20g $plant^{-1}$	4.70	3.91	4.47
T6	*Carbofuran* @ 30g $plant^{-1}$	4.45	4.22	4.35
T7	*Chlorpyriphos* 0.07%	4.45	3.90	4.26
T8	*Quinalphos* 0.05%	4.37	3.78	4.17
T9	*Monocrotophos* 0.075%	4.59	4.21	4.44
T10	*Phosalone* 0.07%	4.57	3.94	4.36
T11	Normal practice-(Recommended dosages of PP chemicals &fertilizer)	4.62	3.95	4.41
T12	Control (*T. harzianum* alone)	4.55	3.51	4.28
		NS	NS	NS

recommended dosages for cardamom (Table 3). In our field studies *Copper oxychloride* was found compatible with *T. harzianum* which is supported by Vinale *et al.* (2004). They also reported the compatibility of *T.harzianum* and *T. atroviride* with *Copper oxy-chloride.*

Stephen *et al.* (2000) studied the compatibility of *Phorate* and *Chlorpyriphos* with *T. harzianum in vitro* and in soil at different concentrations of each chemical considering the recommended dose 6-36ppm a.i for *Phorate* and 10-40 ppm a.i for *Chlorpyriphos.* They observed that Phorate and *Chlorpyriphos* could be safely applied with *T. harzianum* for the management of *Phytophthora* foot rot, nematodes and mealy bugs on black pepper. Subsequently, *Metalaxyl, Mancozeb, Copper oxychloride*, and *Quinalphos* up to 300 ppm did not affect the population of *T. harzianum* (IISR 1369) in soil and hence would be relatively tolerant to field concentrations of above pesticides (Saju, 2005) and suggested that these pesticides could be integrated along with *T. harzianum* (IISR 1369). So also application of urea, rock phosphate, *Muriate* of *potash* and NPK together did not affect the survival of population of *T. harzianum* IISR 1369. It showed higher population over control.

Experiments were also conducted to determine the compatibility of biocontrol agents with commercially effective chemicals against White root rot (*Dematophora necatrix*) of apple in India (Gupta and Sharma, 2004). They evaluated different combinations of *Carbendazim*, Mancozeb and *Phorate* and biocontrol agents, such as *T. viride, T. harzianum, Gliocladium virens* and *Enterobacter aerogenes*, which were found effective under *in vitro* conditions and in pots. Of the various chemicals tested under *in vitro* conditions, *Carbendazim* was inhibitory to all the fungal antagonists whereas, Mancozeb and *Phorate* were least inhibitory at 200 ppm. *T. harzianum* has already been used in the integrated control programme consisting of two

sprays with a benzimidazole or dicarboximide fungicide with more consistent control than the use of antagonist alone (Gullino *et al.*, 1995). Roberti *et al.* (2006) tested *Clonostachys rosea*, *T. atroviride*, *T. harzianum*, *T. longibrachiatum* and *T. viride* to evaluate their *in vitro* sensitivity towards five fungicides (carboxin, guazatine, prochloraz, thiram and triticonazole) and four herbicides (chlorsulfuron, chlorotoluron, flufenacet and pendimethalin). None of the antagonists showed any mycelial radial growth inhibition in presence of the herbicides at field dose, except for T. *longibrachiatum*. Their results demonstrated how an integration of microorganisms with pesticides makes the control of wheat foot rot possible.

The result of the present study is also in agreement with these earlier works. Thus, it is inferred that *Trichoderma harzianum* can be integrated with the routinely used fungicides and insecticides in cardamom.

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