Compatibility of *Trichoderma viride* and *Pseudomonas fluorescens* with plant protection chemicals and fertilizers in cardamom

M.K. Dhanya*, K.B. Anjumol, M. Murugan and K.B. Deepthy

Cardamom Research Station, Pampadumpara, Kerala Agricultural University, Idukki 685553, Kerala , India.

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Abstract

An *in vitro* experiment was conducted with the objective to find out the suitability of combination of plant protection chemicals (insecticides and fungicides) and fertilizers (urea, urea granules, single super phosphate, muriate of potash, diammonium phosphate, bone meal and micronutrient mixture) with antagonists like *Trichoderma viride* and *Pseudomonas fluorescens* used in the integrative cardamom farming situation in Kerala. Thirty three chemicals were tested for their compatibility individually and in combinations. *Trichoderma viride* showed good compatibility (100 percent) with insecticides (imidacloprid, spinosad, chlorantraniliprole, flubendiamide and acephate), fungicides (potassium phosphonate and fosetyl aluminium) and fertilizers (urea, urea granules, single super phosphate, muriate of potash and micronutrient mixture). Incompatibility of *T. viride* was observed with insecticides (quinalphos and *Azadirachta indica* + *Pongamia pinnata* oil mixture) and fungicides (carbendazim, hexaconazole, potassium phosphonate + hexaconazole mixture and captan + hexaconazole mixture). Hundred per cent compatibility was noticed for *Pseudomonas fluorescens* with insecticides (*pongamia* and neem oil mixture, acephate, quinalphos, chlorantraniliprole, dimethoate, imidacloprid and flubendiamide), fungicides (hexaconazole and potassium phosphonate + hexaconazole mixture) and fertilizers (SSP, MOP, urea, bone meal and urea granules) at the recommended concentration. Insecticides like chlorpyriphos, acetamiprid, carbosulfan, and fungicides such as fosetyl aluminium, copper hydroxide, COC, cymoxanil + mancozeb mixture as well as micronutrient mixture significantly inhibited the growth of *P. fluorescens.* For integrated pest management in small cardamom farming, these chemicals along with the respective antagonists are recommended so as to reduce the environmental risk due to toxic pesticides and increase the sustainability of cardamom farming.

Keywords: Botanicals, Cardamom, Compatibility, Fertilizers, Fungicides, Insecticides, *Pseudomonas fluorescens, Trichoderma viride*

Introduction

With the view of reducing environmental risk and increasing agricultural sustainability of key and sensitive agro ecosystems, the integration of synthetic chemicals and biocontrol agents have been the subject of research in the recent years. Recommended doses of insecticides along with bio control agents show promising effect on the management of various plant pests than the chemicals alone (Vinit et al., 2012). Combining a fungicide tolerant biocontrol agent with respective fungicides has improved the extent of disease control and reduced the quantity of fungicides required for effective management (Buck, 2004). Therefore, the combined use of biocontrol agents and chemical pesticides has enticed much attention as a way to obtain synergistic or additive effects in the control of soil-borne pathogens (Locke et al., 1985).

India has been well accepted as the "Land of spices" even though the productivity of some spices is less than those of other Asian and neo tropical countries.

*Author for correspondences: Phone: 9447388215, Email: dhanya_mk2000@yahoo.co.in

One of the major spices in India is the small cardamom (*Elettaria cardamomum* Maton) which is grown under intensive management. The production and productivity of this crop has been affected by several pathogens like *Pythium, Phytophthora, Fusarium* and *Rhizoctonia* and also insects pests such as shoot borer (*Conogethes punctiferalis* G.), thrips (*Sciothrips cardamomi*), root grubs (*Basilepta fulvicorne*) and root knot nematode (*Meloidogyne* sp.). Hence the crop is heavily sprayed with toxic pesticides on regular basis. To minimize the pesticide usage and load in the environment and produce farmers prefer IPM practices to sole chemical application.

Since there is a ban for red labeled pesticides in Kerala, growers are using yellow, blue and green labeled insecticides as well as blue and green labeled fungicides along with specific biocontrol agents. *P. fluorescens* and *T. viride* are the most commonly used biocontrol agents by cardamom farmers along with chemical pesticides. Scientific information and evidences on the compatibility of pesticides and fertilizers with these biocontrol agents is scanty for cardamom. Therefore, this *in vitro* study has been made to evaluate the compatibility of common biocontrol agents *P. fluorescens* and *T. viride* with the frequently used chemical pesticides and fertilizers at recommended doses.

Materials and Methods

Kerala Agricultural University isolates of *P. fluorescens* and *T. viride* that are already proven to be effective against major diseases (capsule rot, clump rot and *Fusarium* rot) of cardamom were used in this study. The details of fungicides, insecticides and chemical fertilizers recommended and used for rainfed cardamom are given in Table 1-3. The data generated in this study were analyzed using MSTAT and DMRT (Duncans Multiple Range Test).

In vitro compatibility of T.viride with insecticides, fungicides and fertilizers

Respective recommended concentration of each chemical as given in Table 1-3 was added to molten sterile potato dextrose agar media(PDA) which was later poured on to separate sterile Petri dishes. Three replications were maintained in each treatment under completely randomized design. The PDA without chemicals/fertilizers served as control. Mycelial discs of 5mm size were cut from the growing margin of 72 h old culture of *T. viride* and inoculated at the centre of the media in the Petri dishes and incubated at 28^0C. The diameter of

Table 1. Insecticides used under *in vitro* compatibility studies

Treatments	Insecticides Chemical name (Trade name)	Dose l^{-1} water
T1	Imidacloprid (Maharaja 17.8 SL)	0.5ml
T2	Carbosulfan (Marshal 25EC)	2 ml
T3	Chlorpyriphos (Tricel 20 EC)	1.5 ml
T4	Chlorantraniliprole (Coragen 18.5 SC)	0.3 ml
T5	Lambda cyhalothrin (Karate 5 EC)	0.6 ml
T6	Plant extract of *Pinus sylvestris* (Pinex plus)	1 ml
T7	Flubendiamide (Fame 39.35 SC)	0.1ml
T8	Fipronil (Agadi 5 SC)	1.5 ml
T9	Quinalphos (Ekalux 25 EC)	2 ml
T10	Dimethoate (Tagor 30 EC)	1.5 ml
T11	*Azadirachta indica* + *Pongamia pinnata* mixture(Actin plus)	2.5 ml
T12	*Pongamia* oil + *Neem* oil(1:1) mixture (*Poneem)*	2 ml
T13	Thiamethoxam (Actara 25 WG)	0.2 g
T14	Acephate (Acefex 75 SP)	1.5 g
T15	Spinosad (Tracer 45 SC)	0.2 ml
T16	Acetamiprid (Proud 20 SP)	0.4 g

Table 2. Fungicides used under *in vitro* compatibility studies

Treatments	Fungicides : Chemical name (Trade name)	Dose l^{-1} water
T1	Potassium phosphonate (Blue wave 40%)	3 ml
T2	Hexaconazole (Roshan plus 5 SC)	1 ml
T3	Potassium Phosphonate + Hexaconazole (3:1) mixture (F- control 40% and Roshan plus 5 SC)	3 ml
T4	Bordeaux mixture	1 %
T5	Cymoxanil8+ Mancozeb 64 (Moximate 64 WP)	2 g
T6	Copper oxychloride (Blue trox 50 WP)	2 g
T7	Copper hydroxide (Isacide101 77 WP)	2 g
T8	Carbendazim (Bavistin 50 WP)	1 g
T9	Fosetyl- Al (Aliette 80 WP)	1g
T10	Captan 70 WP+ Hexaconazole 5 WP (Taquat 5 WP)	2 g

Table 3. Fertilizers used under *in vitro* compatibility studies

Treatments	Fertilizers	Dose(ppm)
T1	Urea	135.7
T2	Urea granules	135.7
T3	Single super phosphate (SSP)	312.5
T4	Muriate of potash (MOP)	208.3
T5	Diammonium phosphate (DAP)	10000
T6	Bone meal	347.2
T7	Micronutrient Mixture (Zn, B, Mn etc)	5000

colony was measured after six days and percent growth inhibition was worked out using the formula C-T/C x 100, where C is the growth of biocontrol agent in control and T is the growth in media containing chemicals/fertilizers (Suseela and Thomas, 2010).

In vitro compatibility of P. fluorescens with insecticides, fungicides and fertilizers

Corresponding concentration of each chemical/ fertilizer was added to molten sterile nutrient agar media and then poured on a separate sterile Petri dish. One day old (24 h*) P. fluorescens* culture was streaked on to the respective plates and incubated at 28±2^{0}C for 24-48 h and observed for their susceptibility/tolerance to the test chemicals. The medium without pesticide/fertilizer served as control. To quantify the growth on solid media, a score chart was prepared (Vidhya et al., 2012). The growth of *P. fluorescens* in media containing agrochemicals was further confirmed by turbidometric method. To ascertain the tolerance or susceptibility, 200µl of bacterial culture (24h old) was inoculated in 10 ml of sterile peptone - water in test tubes containing the matching concentration of agrochemicals (Table 1-3). The inoculated tubes were incubated on a rotary shaker at 30^{0}C and 180 rpm. The cell growth in each treatment was measured by optical density (OD) at 610 nm after 24 h inoculation in comparison with control.

Results and Discussion

In vitro compatibility of T. viride with insecticides, fungicides and fertilizers

Among selected insecticides imidacloprid, spinosad, chlorantraniliprole, flubendiamide and acephate were 100 percent compatible with *T. viride;* whereas, acetamiprid, thiamethoxam and *poneem* showed an respective inhibition of 15.5%, 15.5% and 17.49%. Fipronil and dimethoate exhibited 25% inhibition while all other insecticides had more than 40% inhibition to the test antagonist. Among them, quinalphos and actin plus showed highest inhibition of 60.6% and 57.7% correspondingly (Table 4). Tronosmo (1989) has reported that insecticides used at recommended concentrations were more inhibitory to *Trichoderma* spp. than fungicides. Kranthi and Rajasekhar (2010) have observed that among insecticides, the second-generation synthetic pyrethroids like lambda cyhalothrin were less harmful than the conventional

Table 4. *In vitro* compatibility of insecticides, fungicides and fertilizers against *T. viride*

Insecticides	Per cent inhibition (Mean)*	Fungicides	Per cent inhibition (Mean)*	Fertilizers	Percent inhibition (Mean)**
T1	0.5[g]	T1	0.5[f]	T1	0.7[b]
T2	54.8[bc]	T2	90[a]		
T3	43.9[d]	T3	90[a]	T2	0.7[b]
T4	0.5[g]				
T5	52.5[c]	T4	43.9[d]		
T6	51.4[c]			T3	0.7[b]
T7	0.5[g]	T5	20.9[e]		
T8	25.4[e]	T6	50.3[c]		
T9	60.6[a]			T4	0.7[b]
T10	25.2[e]	T7	59.4[b]		
T11	57.7[ab]			T5	3.6[a]
T12	17.5[f]	T8	90[a]		
T13	15.5[f]			T6	3.4[a]
		T9	0.5[f]		
T14	0.5[g]				
T15	0.5[g]	T10	90[a]	T7	0.7[b]
T16	15.5[f]				
Control	0.5[g]	Control	0.5[f]	Control	0.7[b]
CD (0.05)	4.2	CD (0.05)	2.91	CD (0.05)	0.26
CV%	10.2	CV%	3.52	CV %	10.54

* Arc sine transformed values ** Square root transformed values

In each column figures followed by same letter do not differ significantly according to DMRT.

insecticides like monocrotophos and quinalphos. Singh et al. (2014) found that chlorantraniliprole and chlorpyriphos were compatible with *Trichoderma* spp. Soumik et al. (2010) have agreed that the insecticide quinalphos showed toxicity even at low concentration (10 ppm) which indicated the high incompatibility of this chemical with *T. harzianum*. Bagwan (2010) reported that 5% neem oil enhanced the growth of *Trichoderma*. Bheemaraya et al. (2012) showed that imidacloprid and dimethoate were compatible with *T. harzianum*, while quinalphos, carbosulfan and combination chemical of profenophos and cypermethrin were not. The results were in agreement with these earlier findings.

Among fungicides tested, two, viz., potassium phosphonate and fosetyl aluminium were found to be 100 percent compatible with *T. viride*. It was also observed that when potassium phosphonate + hexaconazole mixture was used, there was an inhibition of 90% to *T. viride*. Hexaconazole, carbendazim and captan + hexaconazole mixture also gave 90% inhibition. Amongst other fungicides, a mixture of cymoxanil and mancozeb (1:8) resulted in least inhibition (20.9%). Copper containing fungicides such as BM, Kocide and COC resulted in high inhibition levels (43.92%, 59.44% & 50.32% in that order) (Table 4). Silimela and Korsten (2001) have brought to notice that the efficiency of biocontrol agents could further be improved when applied with the recommended fungicide at a lower concentration. Soumik et al. (2010) have observed some incompatibility of hexaconazole with *T. harzianum*. Shahida et al. (2010) have studied and reported that *T. harzianum* and *T. viride* were compatible with potassium phosphonate and mancozeb and also found more than 65% and 100% inhibition by COC and BM respectively. Kranthi and Rajasekhar (2010) have expressed that the fungicides carbendazim, mancozeb and tridemorph had negative impact on

the growth of *T. viride*. Pandian et al. (2013) claimed that copper hydroxide at the rate 2 g l^{-1} was inhibitory to *T. viride* under *in vitro* condition. Gaur and Sharma (2010) have seen compatibility of *T. viride* with various fungicides and reported that COC, mancozeb, fosetyl-Al and cymoxanil 8% + mancozeb 64% mixture showed moderate to good compatability. The findings of this *in vitro* investigation also gave results in line with that of earlier studies. Among fertilizers, urea, urea granules, MOP, SSP and micronutrient mixture had cent per cent compatibility with *T. viride*, but sporulation was reduced in case of SSP and MOP treated plates. DAP and bone meal treatments had registered a minimum incompatibility of 3.6% and 3.4% respectively (Table 4). Application of urea, rock phosphate, MOP and NPK together did not affect the survival of *T. harzianum* (Saju, 2004). Shylaja and Rao (2012) have confirmed that urea and MOP were totally compatible with *T. harzianum* as SSP had significantly inhibited its growth. Kranthi and Rajasekhar (2010) insisted that MOP and SSP have shown good compatibility with *T. viride*. Our results are in accordance with the earlier published findings.

In vitro compatibility of P. fluorescens with insecticides, fungicides and fertilizers

The results of the study have shown that *P. fluorescens* was compatible with *poneem,* acephate, quinalphos and chlorantraniliprole (111-166%) followed by dimethoate, flubendiamide and imidacloprid (96-103%) and the growth was better compared to control. All other insecticides like chlorpyriphos, carbosulfan and acetamiprid showed maximum inhibition (21-29% growth) followed by pine oil and fipronil (45-50%). The growth of the antagonists on the solid media containing above chemicals has shown similar findings (Table 5). There are only few reports about the compatibility studies on *P. fluorescens* with insecticides. Gokil

Table 5. *In vitro* compatibility of insecticides, fungicides & fertilizers against *P. fluorescens*

Insecticides	OD (610 nm) & Growth on solid media	Fungicides	OD (610 nm)& Growth on solid media	Fertilizers	OD(610 nm) & Growthon solid media
T1	0.47^{e}(V)	T1	0.39^{e}(G)	T1	0.49^{abc}(V)
T2	0.13^{j}(S)	T2	0.54^{a}(V)	T2	0.47^{cd}(V)
T3	0.12^{j}(S)				
T4	0.51^{d}(V)	T3	0.47^{b}(V)	T3	0.51^{a}(V)
T5	0.42^{fg}(V)				
T6	0.21^{i}(M)	T4	0.28^{d}(M)	T4	0.51^{a}(V)
T7	0.44^{ef}(V)				
T8	0.23^{i}(M)	T5	0.11^{e}(S)	T5	0.44^{d}(V)
T9	0.61^{c}(V)				
T10	0.47^{e}(V)	T6	0.11^{e}(S)		
T11	0.27^{h}(M)			T6	0.48^{bcd}(V)
T12	0.76^{a}(V)	T7	0.09^{e}(S)		
T13	0.39^{g}(G)	T8	0.39^{c}(G)	T7	0.11^{e}(S)
T14	0.66^{b}(V)	T9	0.07^{e}(S)		
T15	0.42^{fg}(V)	T10	0.28^{d}(M)	Control	0.46^{cd}(V)
T16	0.09^{j}(S)				
Control	0.46^{e}(V)	Control	0.46^{b}(V)		
CD (0.05)	0.04	CD (0.05)	0.06	CD (0.05)	0.04
CV %	5.8	CV%	11	CV%	4.8

S: Slight growth, M: Moderate growth, G: Good growth, V: Very Good growth

In each column figures followed by same letter do not differ significantly according to DMRT.

(2013) reported that the insecticide quinalphos was compatible with *P. fluorescens* up to 500 ppm. According to Keshgond and Naik (2013), carbosulfan and neem seed oil showed a minimum inhibition with *P. fluorescens* whereas nimbicidine and eucalyptus leaf extract showed complete inhibition.

In vitro evaluation of fungicides with *P. fluorescens* showed that its growth in hexaconazole was the highest compared to control (117%) followed by potassium phosphonate + hexaconazole mixture (102%) but it was on par with control, where as fosetyl- Al, COC, copper hydroxide and cymoxanil + mancozeb mixture inhibited growth to the tune of 15-24% (Table 5*). P. fluorescens* was found to be more tolerant to fungicides than fungi and this might be due to the reason that some bacteria might use pesticides as nutrient source and hence can tolerate higher concentrations of chemicals (Aislabie and Jones, 1995). According to Shahida et al. (2010), *P. fluorescens* was compatible with potassium phosphonate , but incompatible with BM. Keshgond and Naik (2013) have noticed that carbendazim had minimum inhibition when mancozeb and captan showed complete inhibition of *P. fluorescens*. According to Gokil (2013) fungicides such as tricyclazole and carbendazim were compatible with *P. fluorescens* up to 2000 ppm. Mohiddin and Khan (2013) found that *P. fluorescens* was compatible with fungicides like thiram, mancozeb, captan and carbendazim. Maheshwari (2013) also had reported a good compatibility of *P. fluorescens* with carbendazim, thiram and mancozeb. A study by Pandian et al. (2013) had proved that *P. fluorescens* was compatible with lower concentration of copper hydroxide (0.5 g l^{-1}). On the contrarary, in the study, copper hydroxide had recorded very high incompatibility that could be due to the higher concentration (2 g l^{-1}) used in the experiment.

Among fertilizers SSP and MOP resulted in the highest growth (111%) followed by urea and bone meal (102-107%). The growth in the media containing urea granules (101%) and DAP (96%) was on par with control. The least growth (24%) was observed in micronutrient containing media (Table 5). These results showed that SSP, MOP, urea and bone meal enhanced the growth of *P. fluorescens*. Bagyalakshmi et al. (2012) conducted an *in vitro* compatibility study using indigenous PGPRs with fertilizers in tea and reported that MOP (3%) and rock phosphate (up to 10.5%) had enhanced the growth of PGPR, while urea (4%) inhibited its growth considerably.

The results of the study have clearly showed that imidacloprid, spinosad, chlorantraniliprole, flubendiamide, acephate, potassium phosphonate, fosetyl aluminium, urea, urea granules, MOP, SSP and micronutrient mixture were highly compatible with *T. viride*. In the case of P. *fluorescens,* pongamia + neem oil mixture, acephate, quinalphos, chlorantraniliprole, dimethoate, imidacloprid, flubendiamide, hexaconazole, potassium phosphonate + hexaconazol mixture, SSP, MOP, urea, bone meal and urea granules were 100% compatible at the respective concentrations. This information can therefore be used for selecting appropriate chemicals with their dosages for application along with bioagents in farmer's field. However further investigations are recommended for field application.

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