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Compatibility of mutant isolates of *Trichoderma* spp. with agrochemicals

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ABSTRACT: Two stable mutants, one each of *Trichoderma viride* (TvM_1) and *Trichoderma harzianum* (ThM_1), obtained through gamma radiation were tested for their compatibility with different pesticides in order to fit them in integrated disease management for the control of fusarial wilt of chilli. Both the fungal mutants showed high compatibility carbendazim (0.1%), fipronil (0.2%), imidacloprid (0.025%) and fluchloralin (0.33%). TvM_1 showed compatibility with captan (0.25%), copper oxy chloride (0.15%), phosalone (0.1%) and butachlor (0.2%) and ThM_1 was compatible with mancozeb (0.125%) and phosalone (0.1%). Mancozeb (0.25%), copper oxy chloride (0.3%), dicofol (0.5%), pendimethalin (0.66%) and alachlor (0.4%) were found to be highly inhibitory to the radial growth of the mutants TvM_1 and ThM_1.

KEY WORDS: Agrochemicals, compatibility, *Trichoderma harzianum* mutant 1 (ThM_1), *T. viride* mutant 1 (TvM_1)

INTRODUCTION

Biocontrol agents when effective and compatible with plant protection inputs like pesticides can be dovetailed with integrated crop production practices. Application of chemicals for the control of diseases has now become controversial because of the rising costs involved and related adverse effects on the environment. Integration of pesticides and biocontrol agents has been the subject of research over the years. Integration of biological and chemical control methods has the potential to control plant pathogens with minimal interference in the natural biological equilibrium (Papavizas, 1985). Biological approach can be successful if the biocontrol agents are compatible with fungicides and biopesticides (Papavizas, 1985). Integrated management of soil borne pathogens is one way of reducing the severe impact of various pesticides on the ecosystem. The present study was conducted to evaluate the compatibility of biocontrol agents with commonly used fungicides, insecticides and herbicides used in chilli crop, which could in future be recommended as possible combinations in integrated disease management program of wilt pathogen.

MATERIALS AND METHODS

Fungal mutants *T. viride* (TvM_1) and *T. harzianum* (ThM_1) were maintained on potato dextrose agar (PDA) amended with rose bengal medium after exposing them to four different doses (50, 75, 100 and 125kg) of gamma radiation at Gamma Radiation Unit, Rajendranagar, Hyderabad, Andhra Pradesh, India, as per Mukherjee and Mukhopadhay (1993).

Commercial formulations of four fungicides, insecticides and herbicides each, which are commonly used for the control of different biotic stresses in chilli crop in Andhra Pradesh, *viz.*, carbendazim (Bavistin 50%WP), copper oxy chloride (Tagcop 50%WP), mancozeb (Indofil M-45 75%WP) and captan (Captaf 50%WP); imidacloprid (Confidor 17.8%SL), fipronil (Regent 5%SL), phosalone (Zolone 35%EC), dicofol (Kelhane 18.5%EC); alachlor (Lasso 50%EC), butachlor (Thunder 50%EC), pendimethalin (Stomp 30%EC) and flucloralin (Basalin 45%EC) were selected. These were tested for the growth inhibition of fungal mutants (TvM1 and ThM1) by poisoned food technique on PDA amended with antifungal antibiotic, Rose Bengal medium (Nene and Thapliyal, 1993). The pesticides were tested at two doses, i.e., recommended and another at half of the recommended dosages.

Each 100ml portion of the medium was dispensed into a 250ml Erlenmeyer conical flask and autoclaved at 121°C for 30 minutes. It was then cooled to about 45°C. Stock solutions of the pesticides prepared in sterilized distilled water were incorporated into each flask to provide different levels of doses. Each flask was shaken well and poured into sterilized Petri dishes (90mm). Medium without pesticide served as control. Each plate was centrally inoculated with a 8mm inoculum disc from 7-day-old culture of respective fungal mutants TvM_1 and ThM_1. The inoculated dishes were incubated at 25 $\pm$ 1°C. Observations on radial growth of colony (mm) of the biocontrol agents in the petri-plates treated with different pesticides at different doses were recorded on 8th day of incubation. Four replicates were maintained for each treatment including one set of control separately for recommended and half of the recommended dosages. Per cent growth inhibition over control was calculated using two-way analysis of variance and interpreted at 5 per cent level of significance.

RESULTS AND DISCUSSION

Sensitivity of the mutants TvM_1 and ThM_1 against fungicides

Among the four fungicides tested for compatibility, all of them significantly inhibited the mycelial growth of the mutants TvM_1 and ThM_1 at their recommended and half the recommended dosage (Table 1). Mancozeb at 0.25% inhibited the radial growth of TvM_1 to a maximum extent of 82.16 per cent followed by COC at 0.3% and captan at 0.25% with 81.86 and 68.12, respectively.

Mancozeb at 0.125% recorded a maximum per cent inhibition of 63.82 followed by copper oxy chloride (54.87) at 0.15% and captan (54.59) at 0.125%, respectively. The results obtained are in agreement with findings of Bhat and Srivastava (2003) who stated that mancozeb exhibited fungistatic action against *T. viride* while copper oxy chloride inhibited the growth of *Trichoderma* spp. to a maximum extent. However, carbendazim was found to be least effective and best compatible towards the mutant TvM_1 both at 1% and 0.05% with 51.82 and 41.66 per cent inhibitions compared to the rest of the fungicides tested.

In case of mutant ThM_1, a maximum of 88.85 and 65.69% inhibitions was exhibited by copper oxy chloride both at 0.3% and 0.15% respectively. The next effective fungicide was mancozeb (64.16%) and captan (63.25%) at recommended doses without any significant difference between these two fungicides in inhibiting the radial growth of the mutant ThM_1, while at half the recommended dosage, next to copper oxy chloride, highest per cent inhibition of the mutant was exhibited by captan (62.49%) followed by mancozeb (58.53%), respectively.

ThM_1 was least inhibited by carbendazim with (54.41%) and (52.73%) both at 0.1% and 0.05%,

Table 1. ***In vitro*** **compatibility of** ***T. viride*** **(mutant TvM_1) and** ***T. harzianum*** **(mutant ThM_1) with fungicides**

Fungicide	Dosage (%)	*T. viride* mutant-1	*T. harzianum* mutant-1
Carbendazim 50%WP	0.1 0.05	51.82 (46.04) 51.66 (45.95)	54.41 (47.53) 52.73 (46.56)
Copper oxy chloride 50%WP	0.3 0.15	81.86 (64.83) 54.87 (47.80)	88.85 (70.56) 65.69 (54.18)
Mancozeb 75%WP	0.25 0.125	82.16 (65.12) 63.82 (53.03)	64.16 (53.22) 58.53 (49.92)
Captan 50%WP	0.25 0.125	68.12 (55.76) 54.59 (47.64)	63.25 (52.73) 62.49 (52.24)
Control		0.00 (0.00)	0.00 (0.00)
LSD (P=0.05)		3.28	2.76

Figures in parentheses are transformed values

Table 2. ***In vitro*** **compatibility of** ***T. viride*** **(mutant TvM_1) and** ***T. harzianum*** **(mutant ThM_1) with insecticides**

Name of the Insecticide	Dosage (%)	*T. viride* mutant-1	*T. harzianum* mutant-1
Imidacloprid 17.8%SL	0.025 0.0125	51.20 (45.68) 51.15 (45.65)	53.34 (46.91) 52.11 (46.21)
Fipronil 5%SL	0.2 0.1	51.17 (45.67) 50.60 (45.34)	50.60 (45.34) 50.30 (45.17)
Phosalone 35%EC	0.2 0.1	65.64 (52.64) 54.71 (47.71)	69.21 (56.32) 52.28 (46.31)
Dicofol 18.5%EC	0.5 0.25	94.19 (76.25) 92.73 (74.73)	98.63 (83.47) 96.64 (79.55)
Control		0.00 (0.00)	0.00 (0.00)
LSD (P=0.05)		2.77	2.18

Figures in parentheses are transformed values

respectively. As such the fungicide was found to be compatible compared to rest of the fungicides tested. These results are in agreement with the findings of Girija and Umamaheshwaran (2003) who reported the compatibility of *T. virens* with carbendazim at 100ppm concentration under *in vitro* conditions.

Sensitivity of the mutants TvM_1 and ThM_1 against insecticides

Of the four insecticides tested for their sensitivity towards the mutant TvM_1, significantly highest per cent inhibition of 94.19 and 92.73 was recorded by dicofol at 0.5 % and 0.25% dosages followed by phosalone (65.64) and (54.71) at 0.2% and 0.1% dosages, respectively (Table 2). Imidacloprid at 0.025% dosage, inhibited the radial growth of TvM_1 to an extent of 51.20 and fipronil inhibited the mutant to a minimum extent of 51.17% at 0.2% dosage.

Table 3. ***In vitro*** **compatibility of** ***T. viride*** **(mutant TvM_1) and** ***T. harzianum*** **(mutant ThM_1) with herbicides**

Name of the Herbicide	Dosage (%)	*T. viride* mutant 1	*T. harzianum* mutant 1
Alachlor 50%EC	0.4 0.2	79.31 (63.14) 73.93 (59.30)	96.18 (79.51) 89.47 (71.15)
Butachlor 50%EC	0.4 0.2	62.57 (52.28) 55.32 (48.06)	69.96 (56.79) 69.20 (56.33)
Pendimethalin 30%EC	0.66 0.33	92.70 (74.49) 82.61 (65.62)	93.85 (76.14) 90.36 (74.76)
Flucloralin 45%EC	0.33 0.165	59.90 (50.73) 54.72 (47.72)	60.15 (50.86) 54.56 (47.62)
Control		0.00 (0.00)	0.00 (0.00)
LSD (P = 0.05)		3.93	NS

Figures in parentheses are transformed values

While at half the recommended dosage, the per cent inhibition of the mutant by imidacloprid was 51.15 and with fipronil the per cent inhibition was 50.60%, which were on par indicating that there is no significant difference between these two insecticides in inhibiting the radial growth of the mutant TvM_1. Fipronil inhibited the mutant to 51.17 and 50.60 per cent at 0.2% and 0.1% dosages respectively and was found to be least effective and best compatible with the mutant compared to other insecticides tested.

In case of ThM_1, dicofol exhibited highest 98.63 and 96.64 per cent inhibitions at .5% and 0.25% dosages. At recommended dosage next to dicofol, maximum 69.21% inhibition of the mutant was recorded by phosalone, followed by imidacloprid (53.34%), respectively. At half the recommended dosage, it was phosalone with 52.28 per cent inhibition and imidacloprid with 52.12 per cent inhibition, respectively. While the per cent inhibition rates of the mutant by imidacloprid at recommended (53.34%) and half the recommended (52.12%) dosage and fipronil at recommended (50.60%) and half the recommended doses (50.30%) and phosalone at half the recommended (52.28%) dosage were on par, there was no significant difference between imidacloprid, fipronil and phosalone at their respective dosages in inhibiting the radial growth of the mutant ThM_1. Similar results were obtained by Bhat and Sabalpara (2001), Desai and Srikant (2004), Tiwari *et al.* (2004), Vijayaraghavan and Abraham (2004), with organophosphorus group of insecticides against the radial growth of *T. viride* and *T. harzianum*. In view of minimum radial growth inhibition of the mutant ThM_1 by fipronil both at 0.2% and 0.1% dosages compared to the three other insecticides tested, it was found to be highly compatible showing its fungistatic action.

Sensitivity of the mutants TvM_1 and ThM_1 against herbicides

Of the four herbicides tested against the mutant TvM_1, pendimethalin at 0.66% and 0.33% significantly inhibited the radial growth of TvM_1 to a maximum extent of 92.70 per cent and 82.61 per cent, followed by alachlor (79.31%) (73.93%) and butachlor (62.57%) (55.32%) at 0.4% and 0.2% dosages, respectively. The radial growth of mutant TvM_1 was least inhibited by fluchloralin with per cent inhibition rates of 59.90 and 54.72 at 0.33% and 0.165% dosages, respectively. There was no significant difference between the herbicides, butachlor and fluchloralin in inhibiting the radial growth of mutant TvM_1, since per cent inhibition rates at half the recommended dosage were at on par. However, the radial growth of the mutant TvM_1 was least inhibited by fluchloralin at its recommended and half the recommended dosages, respectively (Table 3).

For the mutant ThM_1 maximum inhibition (96.18) per cent of the mutant was recorded by alachlor at 0.4%, followed by pendimethalin (93.85) at 0.66% and butachlor (69.96) at 0.4% dosage. At 0.33% dosage, pendimethalin inhibited radial growth of the mutant to an extent of 90.36 per cent followed by alachlor and butachlor with 89.47 and 69.20 per cent inhibition at 0.2% dosage, respectively. These results are in agreement with the findings of Jayaraj and Radhakrishnan (2000) who reported that alachlor, butachlor and pendimethalin, significantly reduced the sporulating and cellulase producing ability of *T. harzianum*, especially at their higher concentrations. Jaworska and Dluzniewska (2002) also stated that pendimethalin affected the growth and biological activity of *T.harzianum* and *T. viride* and strongly inhibited the growth rate of the antognistic fungi. Cent per cent inhibition of hyphal growth of *T. harzianum* by alachlor was observed by Desai and Srikant (2004) and Abdel-Mallek *et al.* (1994). In view of the minimum inhibition rates of the mutant ThM_1 at recommended (60.15 %) and half the recommended dosages (54.56 %) in fluchloralin amended medium compared to the rest of the herbicides tested, it was found to be most compatible. The present investigations revealed, using of mutant biocontrol agents together with compatible agrochemicals against the target disease, help in reducing pesticide cost and also inhibits the adverse affects caused by the over utilization of chemicals on the environment.

REFERENCES

Abdel-Mallek, A. Y., Hamida, S. K. and Omar, S. A. 1994. Fungal succession and decay of herbicide treated wheat straw. *Folia Microbiologica*, **39**: 561-566.

Bhat, N. M. and Srivastava, L. S. 2003. Evaluation of some fungicides and neem formulations against six soil borne pathogens and three *Trichoderma* spp. *in vitro*. *Plant Disease Research, Ludhiana*, **18**: 56-59.

Bhat, T. K. and Sabalpara, A. N. 2001. Sensitivity of some bio-inoculants to pesticides. *Journal of Mycology and Plant Pathology*, **31**: 267.

Desai, S. A. and Srikant, K. 2004. Effect of fungicides, insecticides and weedicides on the growth and sporulation of native *Trichoderma harzianum* Rifai. *Karnataka Journal of Agricultural Sciences*, **17**: 57-62.

Girija, V. K. and Umamaheswaran, 2003. Basal rot of balsam and its management through bioagents. *Plant Disease Research*, **18**: 52-55.

Jaworska, M. and Dluzniewska, J. 2002. Activity *in vitro* of some herbicides on the fungi of *Trichoderma* genus. *Progress in Plant Protection*, **42**: 633-635.

Jayaraj, J. and Radhakrishnan, N. V. 2000. Tolerance of *Trichoderma harzianum* to certain herbicides *in vitro*. *Indian Journal of Weed Science*, **32**: 74-76.

Mukherjee, P. K. and Mukhopadhyay, A. N. 1993. Induction of stable mutants of *Gliocladium virens* by gamma-irradiation. *Indian Phytopathology*, **46**: 393-397.

Nene, Y. L. and Thapliyal, P. N. 1993. *Fungicides in Plant Disease Control* (3rd ed.), pp. 691. In: Nene, Y.L. and Thapliyal, P.N. (Eds.). Oxford and IBH Publishing Company Private Limited, New Delhi, 691 p

Papavizas, G. C. 1985. *Trichoderma* and *Gliocladium*: Biology, ecology and potential for biocontrol. *Annual Review of Phytopathology*, **23**: 23-54.

Tiwari, R. K. S., Rajput, M. L., Singh, A. and Thakur, B. S. 2004. Nontarget effect of insecticides on the mycelial growth of *Trichoderma harzianum* (Rifai). *Indian Journal of Plant Protection*, **32**: 140-141.

Vijayaraghavan, R. and Abraham, K. 2004. Compatibility of biocontrol agents with pesticides and fertilizers used in black pepper gardens. *Journal of Mycology and Plant Pathology*, **34**: 506-510.

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