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COMPATIBILITY OF BIOCONTROL AGENTS WITH FUNGICIDES

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ABSTRACT

The present research was conducted to study the compatibility of *Trichoderma harzianum* strain TCMS-14 and *Pseudomonas fluorescence* strain Psf 173 under *in vitro* conditions at recommended dosage, half of the recommended dosage and one-fourth of the recommended dosage with eight fungicides. Out of which four were contact fungicides *viz*. sulphur, thiram, zineb and mancozeb and rest four were systemic fungicides *i.e.* dimethomorph, pyraclostrobin, metalaxyl and propiconazole. The *Trichoderma harzianum* strain TCMS-14 was found to be completely compatible with sulphur (@625ppm, 1250ppm and 2500 ppm), zineb (@375ppm, 750ppm and 1500ppm), dimethomorph and metalaxyl (@500ppm, 1000ppm and 2000ppm) showing zero per cent inhibition where as propiconazole (@31.2ppm,62.5ppm and 125ppm) was found to be completely incompatible showing 100 percent inhibition at all the three dosages. The *Pseudomonas fluorescence* strain Psf 173 was found to be completely compatible with propiconazole (@31.2ppm, 62.5ppm and 125ppm) showing growth similar to control whereas completely incompatible with mancozeb (@625ppm, 1250ppm and 2500ppm) showing no growth on comparison with control. The information generated from this study would be helpful in increasing the efficacy of biocontrol agents by avoiding the use of incompatible insecticides, while adapting integrated pest management programme.

INTRODUCTION

Fungicides are deleterious to the environment and also harmful for the soil productivity and human and animal health and are also not practicable owing to high cost of chemicals and environmental pollution. Due to the disadvantages of fungicides, integrated disease management programs are applied, in which judicious and recommended use of fungicides and their integration with biocontrol agents is favored. The idea of combining Bio Control Agent (BCA) with fungicide is for the development or establishment of desired microbes in rhizosphere (Papavizas and Lewis, 1981). Biological control offers a novel approach when applied either alone or in combination with other management practices without the demerits of chemical control (Papavizas, 1985; Mukhopadhyay, 1987). Biocontrol agents are living entities and they need to establish themselves in the soil, to grow and counter attack the pathogens through either one of more mechanisms *viz*. mycoparasitism, antibiosis, competition and plant growth promotion *etc*. There lies a probability that supplementation of specific compounds which helps in establishment and growth of the bioagents in the soil, provide a competitive advantage to the introduced biocontrol agents and improve its biocontrol potential. There are many examples in plant disease management systems where application of fungicides at low doses were beneficial for bioagents and enhanced disease control was achieved (Frances *et al.,* 2002; Buck, 2004). In some cases it has been found that duration of active disease control could be extended by using chemical and biological control agents together in integrated management system. However, biocontrol agents and fungicides are not always compatible. Combined application of biocontrol agents with commonly used fungicides and insecticides may result either in synergism / antagonism between the two. Combination of biocontrol agents with reduced level of pesticides promotes the degree of disease suppression without risk on non-target organism similar to that achieved with full dose of fungicide application (Benitez, 2004). The *Trichoderma harzianum* was found to be compatible with mancozeb (Mc Lean *et al*., 2000). The higher tolerance in the *Trichoderma harzianum* was recorded against metalaxyl MZ up to 0.5% (Sushir *et al*., 2008). Carbendazim was comparatively less inhibitory to the bioagents and also gave satisfactory inhibition to the pathogen *Sclerotia oryzae* (Prakash *et al.,* 2012). The *Trichoderma* was found to be compatible with mancozeb (1000ppm) and incompatible with propiconazole (25ppm) and the *Pseudomonas* was found to be compatible with thiophanate methyl (96.07%) at 50 ppm followed by mancozeb, carbendazim, copper oxychloride and propiconazole and incompatible with hexaconazole at 25 ppm (Basha *et al.,* 2010). One pesticide has been reported as compatible to a particular biocontrol agent in one study whereas other has reported otherwise. Further in recent past many molecules are very effective at relatively lower doses and their compatibility is yet to be worked out. Keeping in view the above facts, the present study was designed with an objective to study the compatibility between biocontrol agents and several contact and systemic fungicides at different doses. The paper deals with the estimation of compatibility of *Trichoderma harzianum* strain TCMS-14 and *Pseudomonas fluorescence* strain Psf-173with fungicides.

MATERIALS AND METHODS

The present study was conducted at Biocontrol Laboratory, Department of Plant Pathology, G.B Pant University of Agriculture and Technology, Pantnagar. *Trichoderma harzianum* strain TCMS-14 and *Pseudomonas fluorescence* strain Psf-173 provided by the same laboratory were used to conduct the experiments. The fungal BCA was cultured on PDA and bacterial BCA on Nutrient Agar media and maintained at 27 ± 1ºC and 28 ± 1ºC, respectively. Seven days old culture of *Trichoderma harzianum* and 2 days old culture of *Pseudomonas fluorescence* were used in all the studies.

Estimation of compatibility of *Trichoderma harzianum* strain TCMS-14 with fungicides –

Eight fungicides *viz*. Sulphur (Wokovit 80%WG), Thiram (Thiram 75%WP), Zineb (Indofil Z-78 75%WP), Mancozeb (Indofil M-45 75%WP), Metalaxyl (Galaxy35%WP), Pyraclostrobin (Headline 20%WG), Propiconazole (Tilt 25%EC) and Dimethomorph (Acrobat 50%WP) were tested against TCMS-14 under *in vitro* conditions at recommended dosage, half of the recommended dosage and one-fourth of the recommended dosage by using "Poison Food Technique" (Schmitz, 1930). Stock solution of 3000 ppm concentration of each fungicide was prepared in sterilized distilled water. The requisite quantity of fungicide was incorporated into molten PDA separately, mixed thoroughly by vigorous shaking of flasks just before pouring into petriplates. Twenty millilitre PDA was poured in each petriplate. The medium was allowed to solidify and then each petriplate was centrally inoculated with mycelia disc of 5mm diameter from 7 day old culture of *T. harzianum* strain TCMS-14. Petriplates were incubated at27 ± 1ºC for 4days. The PDA petriplates without any insecticide, inoculated with the 5mm disc of *T. harzianum* strain TCMS-14 served as check. Three replications were kept for each treatment. The observation on radial growth of mycelium was taken by measuring the colony diameter (mm) vertically and horizontally at right angle and mean colony diameter was calculated. The percent mycelial growth inhibition of *T. harzianum* strain TCMS-14 over control was calculated by using following formula:

$$I = C - \frac{T}{C} \times 100$$

Where C = Colony diameter (mm) of fungus in control.

T = Colony diameter (mm) of fungus in Treatment.

I = Percent Inhibition of mycelial growth.

The fungicides which showed ≥ 50 percent reduction in mycelial growth were considered incompatible whereas, those showing < 50 percent reduction in mycelial growth were considered compatible (Singh *et al*., 2012).

Estimation of compatibility of *Pseudomonas fluorescence* strain Psf-173 with fungicides –

The *Pseudomonas fluorescence* strain Psf-173 were tested under *in vitro* conditions with eight fungicides *viz*. Sulphur (Wokovit 80%WG), Thiram (Thiram 75%WP), Zineb (Indofil Z-78 75%WP), Mancozeb (Indofil M-45 75%WP), Metalaxyl (Galaxy35%WP), Pyraclostrobin (Headline 20%WG), Propiconazole (Tilt 25%EC) and Dimethomorph (Acrobat 50%WP) at recommended doses, half of the recommended doses and one-fourth of the recommended doses with the help of the procedure described by Shanmugam and Narayanasamy (2008). Stock solution of 3000 ppm concentration of each fungicide was prepared in sterilized distilled water. The requisite quantity of fungicide was incorporated into molten Nutrient Agar (NA) separately, mixed thoroughly by vigorous shaking of flasks just before pouring into petriplates. Twenty millilitre NA was poured in each petriplate. The medium was allowed to solidify and then each petriplate was streaked with the 3-4 days old culture of *Pseudomonas fluorescence* strain Psf-173 in three replications and the plates were incubated for 48 hours. To measure the compatibility, growth of strain Psf-173 of *Pseudomonas fluorescence* on fungicide amended media was rated as + + + (Good); + + (Moderate); + (Poor); and – (No growth) and compared with growth of *Pseudomonas fluorescence* strain Psf-173 on non-amended fungicide NA plates.

The data was analyzed statistically at the computer centre of G.B. Pant University of Agriculture and Technology, Pantnagar, by using STPR programme for Completely Randomized Design (CRD). The treatments were compared by the means of critical differences (CD) at 5% level of significance.

RESULTS AND DISCUSSION

Data present in Table-1 revealed that sulphur, zineb, dimethomorph and metalaxyl were found to be completely compatible as they show zero percent inhibition of mycelial growth of *Trichoderma harzianum* strain TCMS-14 at all the tested concentrations. Pyraclostrobin (@ 25ppm, 50ppm and 100ppm), Thiram (@ 500ppm and 1000ppm) and mancozeb (@625ppm) was found to be moderately compatible where as the mycelial growth of TCMS-14 was inhibited by thiram (@2000ppm), mancozeb (@1250ppm and 2500ppm) and propiconazole (@31.2ppm, 62.5ppm and 125ppm).

Estimation of compatibility of *Pseudomonas fluorescence* strain Psf-173 with fungicides-

The results revealed that out of the eight fungicides tested propiconazole (@31.2ppm, 62.5ppm and 125ppm), metalaxyl (@500ppm) and zineb (@ 375ppm) was found to be completely compatible with Psf-173 as the growth is exactly same as that of control whereas thiram (@500ppm and 1000ppm), metalaxyl (@2000ppm and 1000ppm) and pyraclostrobin (@50ppmand 100ppm) was found to be moderately compatible with Psf-173 as the growth is slightly less than the control. Mancozeb (@625ppm, 1250ppm and 2500ppm), sulphur (@ 625ppm, 1250ppm and 2500 ppm), dimethomorph (@ 500ppm, 1000ppm and 2000ppm), thiram (@2000ppm), zineb (@ 750ppm and 1500ppm) and pyraclostrobin (@ 25ppm) were found to be incompatible with *Pseudomonas fluorescence* strain Psf-173.

The results of the present study revealed that sulphur, zineb, dimethomorph and metalaxyl was found to be compatible with *Trichoderma harzianum* strain TCMS-14 which is in accordance with the previous findings that *T.harzianum* is more tolerant to metalaxyl (Nallathambi *et al*., 2009; Mukhopadhyay *et al*., 1986; Mukherjee *et al*., 1989, Papavizas *et al.,* 1982). Desai *et al*. (2002) have reported that *T. harzianum*

Table 1: Effect of different fungicides on radial growth of *T. harzianum* strain TCMS-14

Fungicides	Concentration (ppm)	Colony diameter(mm)	Growth inhibition (%)
Sulphur 80% WG	2500	90.0	0.0
Sulphur 80% WG	1250	90.0	0.0
Sulphur 80% WG	625	90.0	0.0
Thiram 75%WP	2000	42.0	46.6
Thiram 75%WP	1000	46.0	51.1
Thiram 75%WP	500	47.8	53.1
Zineb 75%WP	1500	90.0	0.0
Zineb 75%WP	750	90.0	0.0
Zineb 75%WP	375	90.0	0.0
Mancozeb 75%WP	2500	26.3	29.2
Mancozeb 75%WP	1250	40.5	45.0
Mancozeb 75%WP	625	45.1	50.1
Dimethomorph 50%WP	2000	90.0	0.0
Dimethomorph 50% WP	1000	90.0	0.0
Dimethomorph 50%WP	500	90.0	0.0
Pyraclostrobin 20%WG	100	45.0	50.0
Pyraclostrobin 20%WG	50	47.6	52.8
Pyraclostrobin 20%WG	25	48.6	54.0
Metalaxyl 35%WP	2000	90.0	0.0
Metalaxyl 35%WP	1000	90.0	0.0
Metalaxyl 35%WP	500	90.0	0.0
Propiconazole 25%EC	125	0.0	100.0
Propiconazole 25%EC	62.5	0.0	100.0
Propiconazole 25%EC	31.2	0.0	100.0
Control	—-	90.0	0.0
S.Em±	—-	1.16	—-
CD at 5%	—-	3.47	—-

Table2 : Effect of different fungicides on radial growth of *Pseudomonas fluorescence* strain Psf-173

Fungicides	Concentration (ppm)	Growth
Sulphur 80% WG	2500	+
Sulphur 80% WG	1250	+
Sulphur 80% WG	625	+
Thiram 75%WP	2000	+
Thiram 75%WP	1000	+ +
Thiram 75%WP	500	+ +
Zineb 75%WP	1500	+
Zineb 75%WP	750	-
Zineb 75%WP	375	+ + +
Mancozeb 75%WP	2500	-
Mancozeb 75%WP	1250	-
Mancozeb 75%WP	625	-
Dimethomorph 50%WP	2000	+
Dimethomorph 50% WP	1000	+
Dimethomorph 50%WP	500	+
Pyraclostrobin 20%WG	100	+ +
Pyraclostrobin 20%WG	50	+ +
Pyraclostrobin 20%WG	25	+
Metalaxyl 35%WP	2000	+ +
Metalaxyl 35%WP	1000	+ +
Metalaxyl 35%WP	500	+ + +
Propiconazole 25%EC	125	+ + +
Propiconazole 25%EC	62.5	+ + +
Propiconazole 25%EC	31.2	+ + +
Control	—-	+ + +

Growth of *Pseudomonas fluorescence* strain Psf-173 in Nutrient Agar amended with various concentrations of fungicides: + + + = Good; + + = Moderate; + = Poor; and - = No growth.

strain C52 was sensitive to Mancozeb which is in accordance with the present investigations, however, our results are contradictory with the findings of Mc Lean *et al*. (2000), Tewari *et al*. (2005) and Bagwan *et al*. (2010) who reported that Mancozeb (0.2%) was comparatively safer against *T. harzianum*. This difference might be due to the different strains used in the studies. The present study reported that *Pseudomonas fluorescence* strain Psf-173 was found to be compatible with metalaxyl and mancozeb which is in accordance with the finding of Mohiddin *et al*. (2013). *Pseudomonas* was found to be compatible with mancozeb, carbendazim and propiconazole (Basha *et al.,* 2010) which is in accordance with the present findings.

On the basis of present *in vitro* experiments it can be concluded that metalaxyl and zineb at lower dose are compatible with biocontrol agents and can be used in combination with biocontrol agents for the management of various diseases. Moreover, the use of combination of fungicide tolerant biological control agents with reduced levels of fungicide in IPM programme would result in disease suppression similar to that achieved with full dosage of fungicides (Monte, 2004).

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