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Ergosterol Biosynthesis Inhibitor - Propiconazole Offers Possibility of Integration of Bioagents and Insecticides in Pest Management Practices

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Ergosterol biosynthesis inhibitor – propiconazole was compatible with bacterial biocontrol agents *viz.*, *Pseudomonas* and *Bacillus* under *in vitro* conditions. The compatibility assessed using propiconazole amended nutrient and King's B broth at different concentration (250, 500, 750, 860 and 1000 ppm) by turbidimetic method and it was observed that the bacterial growth was not affected by propiconazole even at the highest concentration of 1000 ppm. The turbidity increased with increase in incubation time as compared to control. Further, this fungicide was physically and biologically compatible with three insecticides namely chlorpyriphos, dicofol and methyl-demeton at their respective recommended dosage under laboratory and glasshouse conditions.

Key words: Ergosterol biosynthesis inhibition, propiconazole, *Pseudomonas, Bacillus* and insecticides

Ergosterol biosynthesis inhibitors commonly known as EBIs represent the largest group of modern fungicides having diverse range of chemical structure but similar biochemical mode of action and either prevent or cure a broad spectrum of fungal disease at lower rates (Zarn *et al*., 2003). They are primarily sterol demethylation inhibitors (DMIs), which represent the most important group of systemic fungicides, and are applied commonly for the control of rusts, powdery mildews and scabs. Most fungi in ascomycetes, basidiomycetes and fungi imperfecti are sensitive to this compound. Propiconazole is the most popular EBIs fungicide belonging to the group triazole. The length and breadth of activity of this fungicide has resulted in versatile uses against many pathogens (Jayaraj *et al*., 2005; Gopinath *et al*., 2006).

Inspite of this, recent school of thoughts on appearance of fungicide resistant strains coupled with insurge of residue levels through food web has warranted for safety uses of fungicides. Development of fungicide resistance has been demonstrated in more than 100 pathogenic fungi. Resistance to triazole group of fungicides was noticed in case of *Erisiphe graminis* in barley by Wolfe and Fletcher (1981). Further, Steva *et al*. (1990) reported triazole resistrant strain of *Uncinula necator* in case of grapes. Integration of biocontrol agents (BCA) with these effective fungicides may offer a possibility of reducing the doses and thereby delaying or avoiding development of resistant strains. The compatibility of fungicide with bioagents forms a prime criterion for the successful integration. In many instances, compatibility of *Pseudomonas* with fungicide has been documented (Kishore *et al*., 2005; Anand *et al*., 2010) Besides disease control, the combination of biocontrol agents with fungicide yielded an increased multiplication and survival of BCA on phylloplane (Kishore *et al*., 2005; Anand *et al*., 2010). Thus in our study compatability of propiconazole was tested.

Intensive agricultural practices call for multiple operations to be done simultaneously. It is always a practice to apply pesticide for the control of both insects and diseases. An ideal character of an efficient fungicide is, it should be compatible with commonly used insecticide so that both insecticide and fungicide can be delivered simultaneously. In a number of cases, synergistic action of insecticide and fungicide against pests has been noticed. This is an added advantage if fungicides are compatible with insecticide. Anand *et al.* (2007) reported synergistic action of azoxystrobin 25 SC with dimethoate against thrips population in chilli. Similarly, Sendhilvel (2003) reported synergestic action of azoxystrobin and monocrotophos against flea beetle, mealy bug, downy mildew and powdry mildew disease. Keeping this in mind, the study was contemplated to understand possibility of integration of propiconazole with biocontrol agents and insecticide in the delivery systems.

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Materials and Methods

Source of fungicide and insecticides

The commercial grade of fungicide propiconazole 25 EC and insecticides *viz;* chlorphyriphos 25 EC, dicofol 30 EC and methyl-demeton *30* EC were purchased from pesticide market Coimbatore, Tamil Nadu, India.

Isolation of biocontrol agents

Antagonistic bacteria namely *Bacillus subtilis* and *Pseudomonas fluroscens* were isolated from the rhizosphere soil by serial dilution method on Nutrient agar medium and King's B medium, respectively. They were incubated at 30 °C for 24 h. Colonies with characteristics of *B. subtilis* and *P. fluroscens* were subcultured individually and purified by streaking them on their respective medium. The identification was done as per the methods recommended in the laboratory guide for identification of plant pathogenic bacteria published by the American Phytopathological Society (Schaad, 1992).

Compatibility studies

Compatibility test between bio control agents and propiconazole

The bacterial biocontrol agents *viz* ., *P. fluorescens* and *B. subtilis* were tested for their compatibility with propiconazole under *in vitro* conditions through turbidimetry method as described by Archana *et al*. (2012). One ml of the bacterial culture was transferred to a 250 ml sidearm flask containing 50 ml of King's B (KB) and Nutrient broth for *P. fluorescens* and *B. subtilis*, respectively. Propiconazole at five different concentrations *viz.,* 100, 250, 500, 750 and 1000 ppm was added separately to 250 ml sidearm flask containing 50 ml of Nutrient broth with one ml of the bacterial culture. The control was maintained without addition of propiconazole and bio control agents in KB and Nutrient broth, respectively. The flasks were incubated at 28 ± 2 °C in a psychotherm shaker. Three replications were maintained in each concentration. The optical density value of the culture broth was determined in spectrophotometer at 610 nm at regular intervals of 16 hrs.

Compatibility of propiconazole with insecticides under in vitro conditions

Preparation of standard hard water

Standard hard water is defined as water, which provides a hardness of 342 ppm calculated as calcium carbonate. For getting this hardness, 304 mg of anhydrous calcium chloride and 139 mg of magnesium chloride were dissolved in distilled water. This solution was used to prepare insecticide solutions for all tests.

Emulsion stability test for physical compatiblity

The test was carried out for chlorphyriphos 25 EC, dicofol 30 EC and *methyl-demeton 30* EC as prescribed by Indian Standard Specification for emulsion stability test (ISI, 1964). To 75 to 80 ml of standard hard water kept in a beaker at 30 °C, the insecticide and fungicide were added by means of Mohr's type pipette. The insecticide and fungicide mixture was added to the standard hard water @ 25 to 30 ml pouring directly into the beaker and not along the sides of the beaker. The contents of the beaker were stirred with a glass rod at four revolutions per second during the addition. The diluted emulsion was made up to 100 ml mark with water which was transferred immediately to a clean dry graduated cylinder. Then the cylinder with its contents was kept at 30 °C in a thermostat for 30 min. The creaming matter at the top and sedimentation at the bottom were observed. The creamy/sedimentation above 2ml was considered as unstable.

Biological compatibility under glasshouse conditions

Biological compatibility was tested using chilli as test plant. Chilli seedlings of variety K2 were raised in mud pots of size 30 x 45 cm having fresh soil containing sand, red soil and farmyard manure in 3:1:1 ratio and arranged on slab in a row. The pots were watered periodically. After 40 days of planting the experimental chilli seedlings in pots were sprayed with mixture of insecticides chlorpyriphos @ 1.5 ml l_{-1}, dicofol @ 2.5 ml l_{-1} and methyl-demeton @ 2.0 ml l_{1} with the five different concentrations of propiconazole (0.25 ml l_{-1}, 0.50 ml l_{-1}, 0.75 ml l_{-1}, 0.86 ml l_{-1} and 1.0 ml l_{-1}) to test the compatibility. The experiment was conducted with five replications and the seedlings were observed for leaf injury on first, third and fifth day after spray through naked eye.

Statistical analysis

The data generated from various experiments of this study were statistically analyzed by DMRT with IRRISTAT software. The data with per cent values were subjected to arc sine transformation.

Results and Discussion

Isolation of biocontrol agents

Isolation of bacterial biocontrol agents from rhizosphere using specific media yielded one *Bacillus* and one *Pseudomonas* isolate. The serrated colony with dull white colour was observed in case of *Bacillus*. On the other hand shiny glistening colony with pigmentation was observed in case of *Pseudomonas*. Further, based on Grahms reaction and biochemical tests these were identified as *B. subtilis* and *P. fluorescens* (Data not shown). In a similar way Prabuling (2011) also isolated bacteria from rhizopshere soil using specific media.

Compatibility test between propiconazole and biocontrol agents

Table 1. Compatibility of propiconazole with *P. fluorescens* and *B. subtilis*

Concentration of propiconazole	*OD value at 610 nm at different hrs											
	P. fluorescens						*B. subtilis*					
(ppm)	16 hr	32 hr	48 hr	64 hr	80 hr	96 hr	16 hr	32 hr	48 hr	64 hr	80 hr	96 hr
250	0.1136$_a$	0.1209$_a$	2.0013$_a$	2. 3019$_a$	2.2323$_a$	2.1043$_a$	0.1210$_a$	0.1306$_a$	2.0014$_a$	2.3219$_a$	2.2623$_a$	2.2043$_a$
500	0.1152$_a$	0.1216$_a$	2.0025$_a$	2. 3035$_a$	2.2359$_a$	2.1057$_a$	0.1225$_a$	0.1319$_a$	2.0035$_a$	2.3245$_a$	2.2638$_a$	2.2058$_a$
750	0.1166$_a$	0.1234$_a$	2.0048$_a$	2.3057$_a$	2.2378$_a$	2.1068$_a$	0.1243$_a$	0.1338$_a$	2.0056$_a$	2.3267$_a$	2.2659$_a$	2.2077$_a$
860	0.1178$_a$	0.1247$_a$	2.0061$_a$	2.3077$_a$	2.2389$_a$	2.1077$_a$	0.1268$_a$	0.1357$_a$	2.0074$_a$	2.3284$_a$	2.2673$_a$	2.2086$_a$
1000	0.1189$_a$	0.1258$_a$	2.0077$_a$	2.3093$_a$	2.2396$_a$	2.1092$_a$	0.1276$_a$	0.1364$_a$	2.0089$_a$	2.3297$_a$	2.2689$_a$	2.2098$_a$
Control	0.1125$_a$	0.1201$_a$	2.0008$_a$	2.3015$_a$	2.2013$_a$	2.1032$_a$	0.1185$_a$	0.1296$_a$	2.0010$_a$	2.3215$_a$	2.2615$_a$	2.2012$_a$

*Mean of three replication

In a column, means followed by common letters are not significantly different at the 5 % level by DMRT

Propiconazole with different concentration was evaluated *in vitro* for its compatibility with the bacterial biocontrol agents, *P. fluorescens* (Pf 2) and *B. subtilis* (Bs 7). The growth of bacteria in propiconazole amended broth (250, 500, 750, 860 and 1000 ppm) was assessed by turbidimetic method and the results are presented in table 1. The bacterial growth was not affected by propiconazole even at the highest concentration of 1000 ppm. There was no significant difference in OD value of the bacterial growth tested at all the concentrations. The turbidity increased with increase in incubation time as in control. The possible reason for compatibility is might be due to lack of absence of ergosterol biosynthesis mechanism in bacteria which is common for cell wall synthesis in higher fungi. Similar results of compatibility of *Pseudomonas* and *Bacillus* with azoxystrobin 23 SC was reported by Archana *et al.* (2012). The results were in agreement with findings of Anand *et al*. (2007) who reported the compatibility of *Pseudomonas* with azoxystrobin. Further, *Pseudomonas aeruginosa* isolate GSE 18 and isolate GSE 19 were tolerant to chlorothalonil up to 2000 µg/ml (Kishore *et al.,* 2005).

Compatibility test between propiconazole and insecticides

Physical compatibility - Emulsion stability

The results on the investigations carried out to study the physical compatibility in terms of emulsion stability revealed that recommended dose of insecticides chlorpyriphos @ 1.5 ml l^{-1}, dicofol @ 2.5 ml l^{-1} and methyl-demeton @ 2.0 ml l $_1$ with propiconazole at five different concentrations *viz.,* 250, 500,750, 860 and 1000 ppm had not produced any creamy matters (or) sediment more than 2.0 ml at the top or bottom of the 100 ml measuring cylinder. The results confirmed the physical compatibility of the above insecticides with propiconazole. The results of present study are in agreement with findings of Sendhilvel (2003), Anand *et al.* (2007) and Archana *et al.* (2012) who reported the physical compatibility of azoxystrobin with insecticides.

Biological compatibility

The biological compatibility of propiconazole at all the concentrations *viz.,* 250, 500, 750, 860 and 1000 ppm with the commonly used insecticides *viz*., chlorpyriphos @ 1.5 ml l^{-1}, dicofol @ 2.5 ml l^{-1} and methyl-demeton @ 2.0 ml l $_1$ was analysed under glass house conditions. All the above insecticides used exhibited good compatibility with propiconazole with all the concentration and produced no phytotoxic injury under glass house conditions. The results of the experiment are in line with findings of Bagwan (2010) who reported the compatibility of insecticides with fungicides. Hence, all the above insecticides can be combined with propiconazole 20 EC during application.

Thus in conclusion since propiconazole is compatibile with both biocontrol agents and insecticides and this offers possibility of integrating these pesticides in the delivery system thereby aiding in reducing cost incurred in the protection measures.

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