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Interaction of fungicides with bio-control agents

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Abstract
Compatibility tests were conducted for triazole fungicides against entomopathogens, *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium lecanii* @ 1, 10, 100, 1000, 10000ppm doses. Tebuconazole was highly toxic and completely inhibited the growth of all entomopathogenic fungi tested @ 1000 and 10,000ppm but with *Lecanicillium lecanii* growth was completely inhibited even at 100ppm. Difenaconazole and tricyclazole fungicides were less toxic to *Beauveria bassiana* and *Metarhizium anisopliae* but to *Lecanicillium lecanii* complete inhibition was recorded @ 100, 1000 and 10,000ppm and similarly 100 percent inhibition in tricyclazole was noticed only at 10,000ppm dose. Myclobutanil has a significant effect on the growth of all the fungi tested at 10,000ppm. Propiconazole fungicide was less toxic to *Metarhizium anisopliae* but was highly toxic to *Beauveria bassiana* and *Lecanicillium lecanii* @ 1000 and 10,000ppm treatments indicating that the triazole fungicides are not compatible with the entomopathogenic fungi tested @ 1000ppm and 10000ppm.

Keywords: Triazoles, entomopathogens, compatibility, fungi

Introduction
Entomopathogenic fungi as biological control agents show promise in reducing insect pest populations and damage in different agro ecosystems [1]. Alternative to chemical pesticides, entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin, was found to be promising biological control agent against several insect pest populations [2, 3]. The success of using entomopathogens in integrated pest control stratagies depends on its conidial survival in environment [4]. The fungicides are routinely applied to control plant diseases, but many fungicides with broad spectra of activity may adversely affect the efficacy of *Beauveria bassiana* that occurs in the natural environment [5]. The conidial survival can be affected by the environmental factors [6] or application of biopesticides/chemicals including fungicides which are commonly used to control the disease [7, 8]. Similarly the other entomopathogenic fungus, *Metarhizium* species are cosmopolitan and reported to infect more than 300 arthropod species belonging to several insect orders [9]. These fungi are also adapted to live as saprophytes as well as symbionts in the plant rhizosphere [10] which make the soil their major habitat. In vitro growth inhibition of these entomopathogens in the presence of fungicides is a useful criterion for testing its sensitivity. Studies on compatibility of fungicides with entomopathogenic fungus will be useful for farmers at the time of application to select suitable fungicides and entomopathogens in integrated pest management [1, 11, 12]. This alternative strategy may lead to decrease in the use of non compatible fungicides to control diseases and also increase the control efficacy of fungus and minimize the environmental contamination [13, 14, 15, 16]. So the objective of the present study was to evaluate compatibility with widely used entomopathogenic fungi, i.e., *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium lecanii* against triazole group of fungicides that have broad spectrum activity against plant diseases which can serve as a guideline for field use of the entomofungus as the toxicity of such pesticides to fungal entomopathogens may vary with fungus species and strain, chemical nature of the active ingredient, mode of action, product formulation, and recommended label rate [8].

Materials and methods
The entomofungus, *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium lecanii* were isolated from Kadapa region of Andhra Pradesh, India and the spores were stored on anhydrous sterile silica gel crystals at -20^{O}C [17]. The spores were retrieved on Sabourad Dextrose Agar supplemented with 0.2% yeast extract medium [18] and incubated at 25 ± 2^{O}C until dense sporulation was observed.

The spores were harvested using sterile spatula into sterile water containing 0.02% tween 20. The conidial concentration in the suspension was calculated using Neubauer chamber under the microscope. The suspensions were diluted until the desired concentration. The procedure for compatibility was carried out by poison food technique [19]. The fungicides chosen for the experiments were the most frequently used by farmers and described in Table 1 for compatibility tests. SDAY was prepared and sterilized in autoclave at 15 lb and the fungicide were sterilized under U.V light radiation of 30 min and these fungicides were tested at five concentrations i.e. 1,10,100,1000 and 10,000ppm which were incorporated into the autoclaved SDAY medium after cooling. The SDAY without fungicide was served as control. The 15 days old culture disc (4mm) of entomopathogen was inoculated on to the medium and four replications were maintained for each treatment. These petriplates were incubated at 25 ±2°C and observation was recorded at every 24 h interval to a maximum of 10 days. The percent inhibition was calculated using the formulation suggested by Vincent [20].

$$PI = \frac{C-T}{C} X100$$

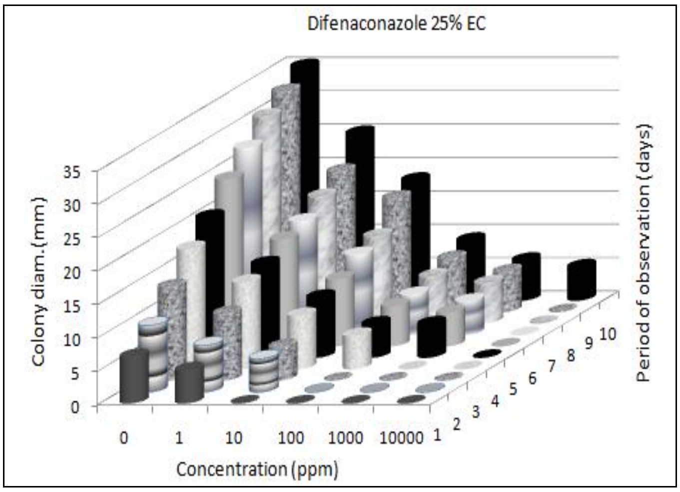
Where PI = percent inhibition of the growth
C = diameter of the fungal growth in control and
T = diameter of the fungal growth in treatment

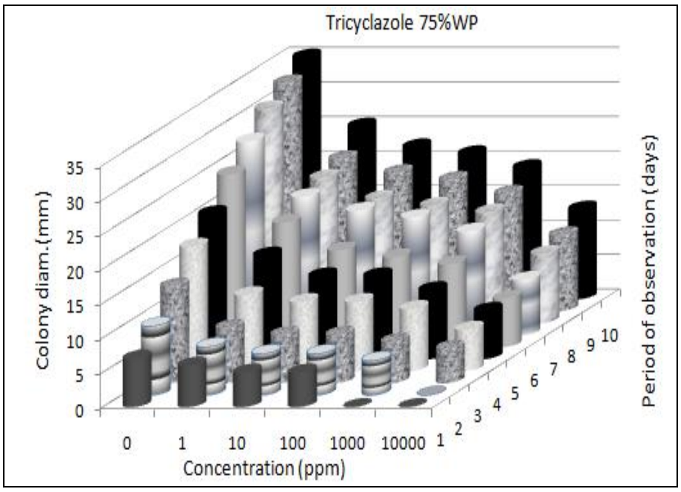
Table 1: List of Fungicides along with their manufacturer source.

Active ingredient	Formulation (%)	Commercial Product Name	Manufacturer
Difenaconazole	25 EC	Score®	Syngenta India limited
Tricyclazole	75 SP	Logik®	PI Industries ltd
Hexaconazole	5 SC	Contest plus®	New Chemi Industries
Propiconazole	25EC	Tilt®	Syngenta India limited
Tebuconazole	25.9EC	Folicur®	Bayer India Ltd.
Myclobutanil	10WP	Index®	Nagarjuna Agrichem Limited

Results and discussion

***Beauveria bassiana*:** *Beauveria bassiana* showed growth on medium containing hexaconazole @1 and 10ppm but no growth was observed when hexaconazole was used @100, 1000 and 10,000ppm. In 10ppm treatment, growth of *Beauveria bassiana* showed negative correlation with concentration of hexaconazole up to 2 days but on 10th day mean radial growth of 5.8mm was observed (Fig. 1). In hexaconazole, 100, 1000 and 10,000ppm treatments showed 100 percent inhibition. Shafa Khan *et al.* [21] findings proved that fungicides, bordeaux and hexaconazole are highly toxic to *B. bassiana* by completely inhibiting germination, vegetative growth and spore production. Similar findings by Gnanaprakasam Antony Raj *et al.* [22] reported that hexaconazole inhibited 100% spore germination and 80% vegetative growth inhibition of *Beauveria bassiana* fungus in all the concentrations tested. Difenaconazole and tricyclazole were less toxic to *Beauveria bassiana* compared to other fungicides i.e., hexaconazole, propiconazole, tebuconazole and myclobutanil. Difenaconazole @1ppm noticed 28% inhibition which is on par with Tricyclazole @1ppm. Difenaconazole @ 10, 100, 1000 and 10,000ppm, readings were noticed 48, 74, 82 and 85 percent inhibition respectively, where as in tricyclazole @ 10, 100, 1000 and 10,000 noticed 37, 40, 45 and 62 percent inhibition, respectively. Propiconazole and tebuconazole were found to be more effective and completely inhibited the growth of *Beauveria bassiana* at 1000 and 10,000 ppm and showed 100% inhibition, but very minute mycelial growth was observed @1, 10 and 100ppm. In conformity, Loureiro *et al.* [23] revealed that the fungicides viz. thiophanate methyl cartap, tebuconazole, metalaxyl and mancozeb inhibited the germination, vegetative growth and sporulation of *B. bassiana*. Myclobutanil plates showed maximum mean radial growth of fungus i.e., 15.7mm, 13.1mm and 6mm at 1, 10 and 100ppm, respectively on 10 day of incubation and a very minute growth of *Beauveria bassiana* was observed at 1000ppm whereas in 10,000ppm the growth of fungus was significantly reduced and 100 percent inhibition was recorded.





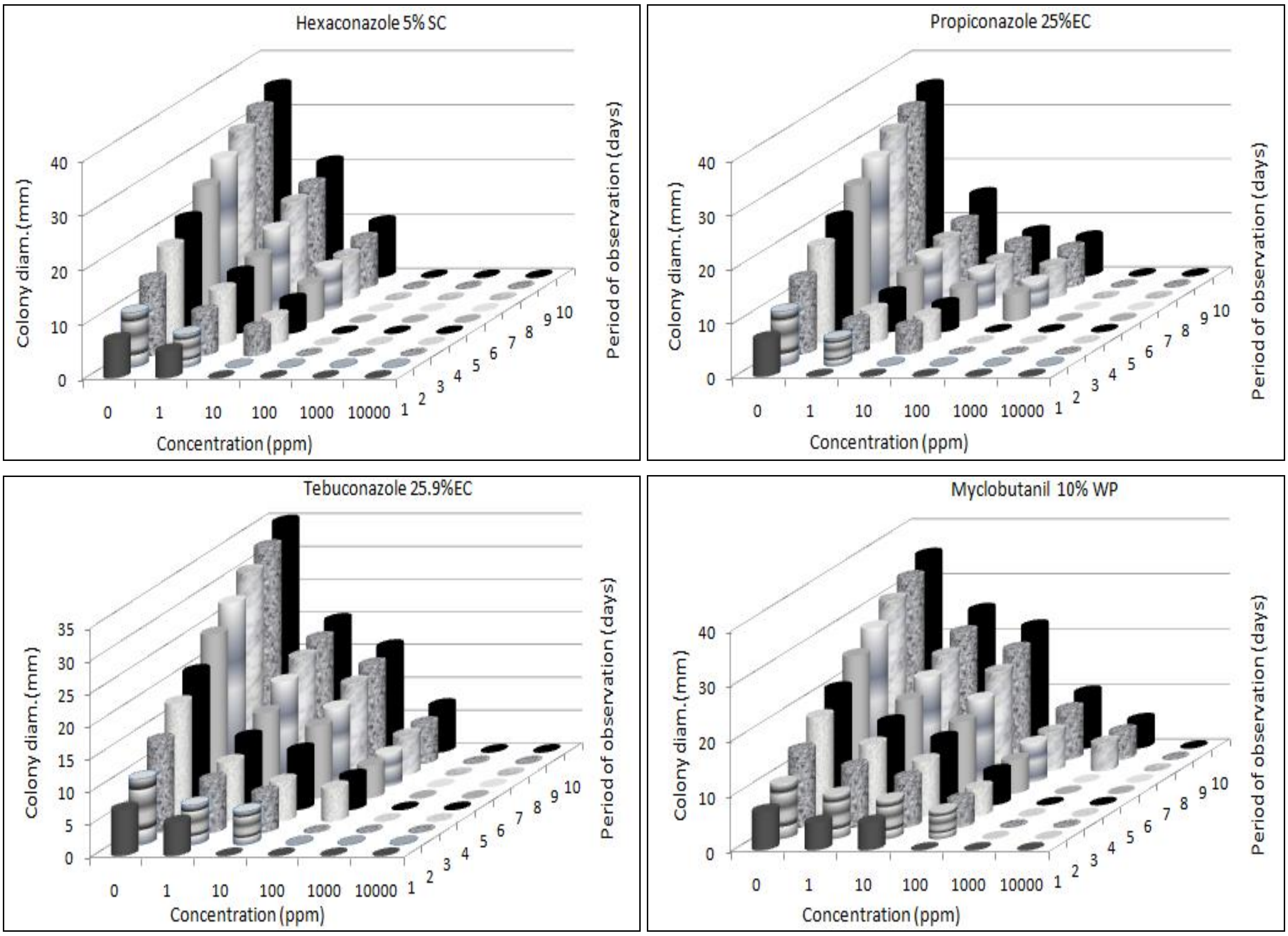


Fig 1: Effect of fungicides on *In vitro* growth of *Beauveria bassiana*

Metarhizium anisopliae: A negative correlation was observed between concentration of hexaconazole and the growth of *M. anisopliae* up to 7 days of incubation. Growth in 100 and 1000ppm treatments started after 72 h (3 days) and 144h (6 days), respectively of incubation and in 10000ppm after 168 h (7 days) of incubation (fig. 2). Growth of the fungus in plates containing hexaconazole @ 10ppm (21mm) or higher concentration was significantly less than the control (36mm) even after 10 days of incubation. The concentration of difenaconazole showed a negative correlation with growth of *M. anisopliae* during 10 days of incubation. Growth in 1 and 10ppm started within 24 h of entomopathogen inoculation, in 100 ppm after 48h whereas in 1000ppm after 72h and in 10000ppm after 120h (5days) of incubation. No growth of *M. anisopliae* was observed in tebuconazole @ 1000 and 10,000ppm treatment but growth in 1 and 10ppm started after 48h and 1000ppm after 72h of incubation. A significant negative correlation between the growth of *M. anisopliae* and concentration of tebuconazole was evident during 10 days of incubation but effect in 100 and 1000ppm was higher than the effect of difenaconazole, tricyclazole, hexaconazole, propiconazole and myclobutanil. Results were found in the earlier reports of Rachappa *et al*. [24] that fungicides hexaconazole, propiconazole were highly toxic to *Metarhizium ansiopliae* followed by Rodrigo Alves da Silva [25] proved that the fungicides difenoconazole (69 mL ha^{-1}), propiconazole (75 mL ha^{-1}), trifloxystrobin (313 g ha–1) and azoxystrobin (56 mL ha–1) were the most harmful products to all biological stages of *M. anisopliae* and they should not be applied together with this fungus in tank mixing. Use of tricyclazole @ 1 (36mm), 10 (34mm) and 100 ppm (31mm) showed the growth of *M. anisopliae* that was on par with control after 10 days of incubation, whereas growth in 1000 and 10000ppm started after 48h and 72h of incubation but very much reduced compared to control. Suppression in growth was less in 1, 10 and 100ppm treatment but use of tricyclazole @ 1000ppm or more showed significant suppression in growth of *M. anisopliae*. *M. anisopliae* grew easily in medium containing myclobutanil @ 1, 10 and 100ppm after 10 days of incubation whereas growth in 1000ppm treatment started after 144h (6 days) of incubation with only 5mm and no growth of this entomopathogen was observed in myclobutanil @ 10000pm.

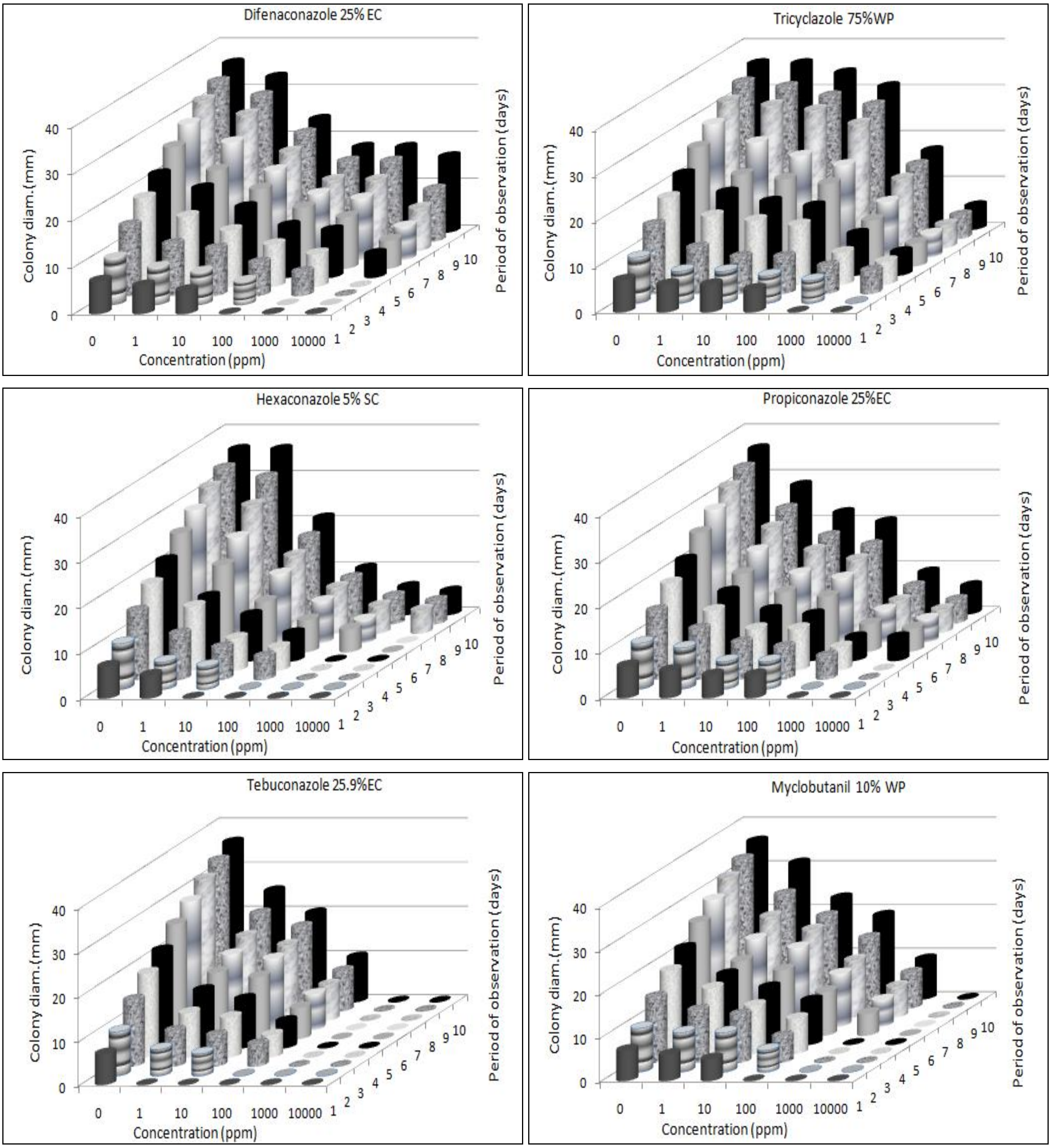


Fig 2: Effect of fungicides on *In vitro* growth of *Metarhizium anisopliae*

Lecanicillium lecanii: *L. lecanii* showed growth on medium containing difenaconazole @1 and 10ppm but no growth was observed when difenaconazole was used @100, 1000 and 10000ppm even 10 days after incubation (fig.3). In 1 and 10ppm treatments, mean radial growth was 18.1mm and 13mm, respectively compared to control which has 19.1mm (table 1). Tebuconazole proved to be highly toxic and very little growth of *L. lecanii* was observed in treatments containing tebuconazole @ 1 and 10ppm and no growth was observed at 100ppm or higher concentrations. A negative correlation was observed between concentration of hexaconazole and the growth of *L. lecanii*. Growth in 10 and 100 ppm treatments started after 48 and 96h of incubation and no growth was observed in 1000 and 10000ppm even after 10 days of incubation. Complete inhibition of growth of *L. lecanii* was observed where tricyclazole was used @ 10000ppm and no growth was observed after first 48h of incubation in tricyclazole @ 1000ppm. The growth of *L. lecanii* was on par with control in plates containing tricyclazole @1 and 10ppm after 10 days of incubation, however the growth of *L. lecanii* suppressed significantly where tricyclazole was used @ 1000ppm or more. Propiconazole @100, 1000 and 10000ppm treatment completely prevented the growth of *L. lecanii* but growth started after 48h of incubation where propiconazole was used @ 100ppm and significant suppression in growth was observed at 1 and 10ppm even after 10 days of incubation. Growth of *L. lecanii* showed negative correlation with the concentration of myclobutanil where growth started after 120 h (5 days) of incubation in 100ppm with a sharp decline compared to growth in 1 and 10ppm treatment after 10 days of incubation. Myclobutanil @ 1000 and 10000ppm completely inhibited the growth of *L. lecanii*.

In the present study, tebuconazole was highly toxic to all the entomopathogenic fungi tested at higher doses @ 1000 and 10000ppm and *Lecanicillium lecanii* was highly susceptible entomopathogenic fungi followed by *Beauveria bassiana* and *Metarhizium anisopliae* and in conclude none of the triazole fungicides tested were not all compatible (table 1) with these fungi even at a dose of 100ppm which is much lesser than the general field recommended dose (1000 ppm). In the triazole fungicides (difenoconazole, propiconazole), ergosterol biosynthesis is inhibited; consequently preventing fungal cell membranes formation[26].

Our results are in agreement with the mode of action of these triazoles group fungicides which reduced conidial germination, mycelial growth and conidiation. It was also reported that triazole fungicides were found to be more toxic to entomopathogenic fungi[27] and that was once again proved in the present investigation.

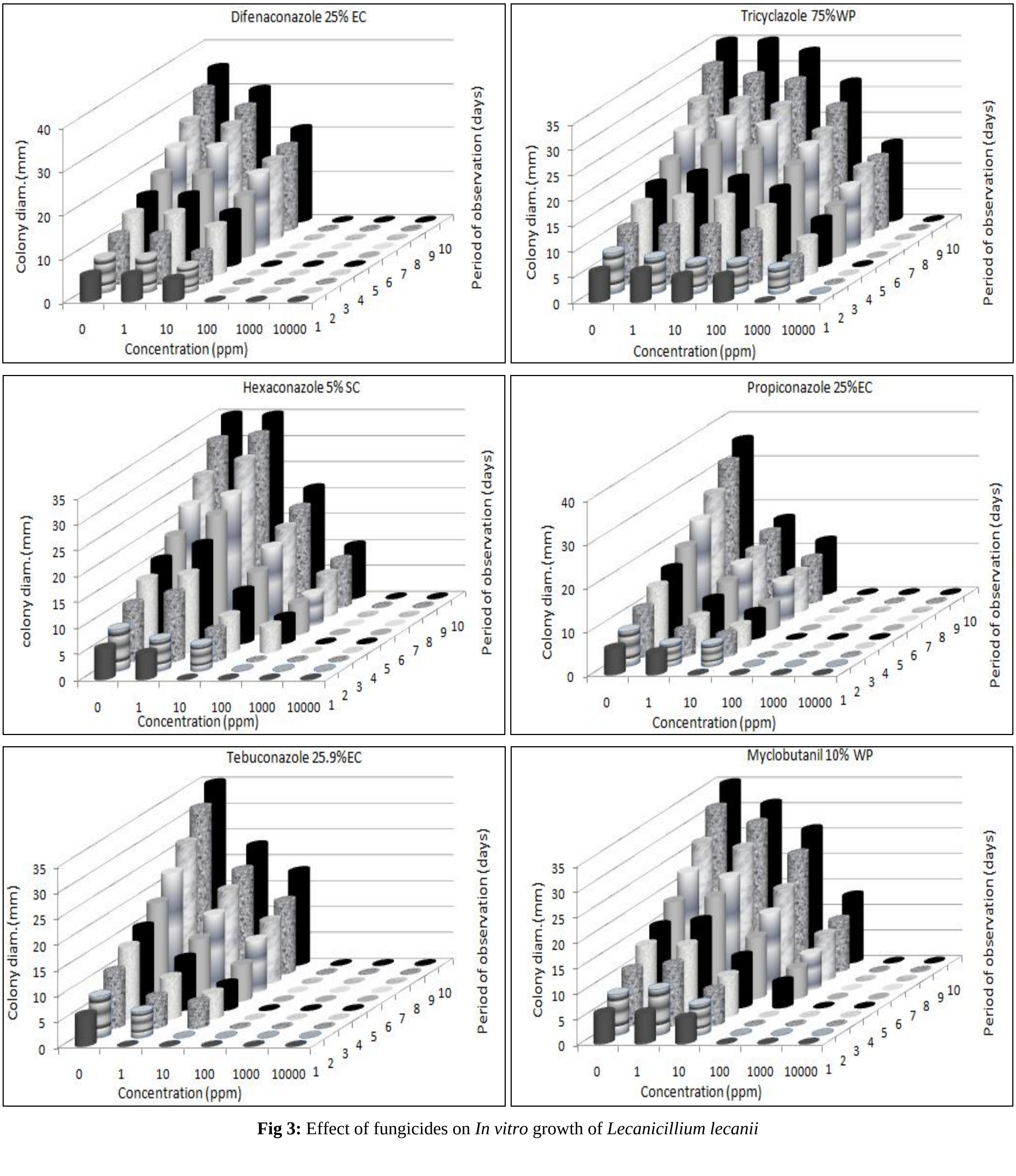


Fig 3: Effect of fungicides on *In vitro* growth of *Lecanicillium lecanii*

Our results are in agreement with the mode of action of these triazoles group fungicides which reduced conidial germination, mycelial growth and conidiation. It was also reported that triazole fungicides were found to be more toxic to entomopathogenic fungi [26] and that was once again proved in the present investigation.

Conclusion

It can be seen from results of this study that fungicides of triazole compounds namely, difenaconazole, tricyclazole, hexaconazole, propiconazole, tebuconazole and myclobutanil are not compatible with the three entomopathogenic fungi, *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium lecanii* tested and not to be recommended in field as tank mix spray for insect pests management.

Table 1: Mean radial growth and percent Inhibition of fungicides on entomopathogens

Entomopathogenic fungi	Fungicides / Doses (ppm)	Mean Radial Growth (mm)						Percent Inhibition (10 day)				
		1	10	100	1000	10000	Control	1	10	100	1000	10000
Beauveria bassiana	Difenaconazole 25EC	14.7	9.7	4.6	3.3	0.5	22.2	28	48	74	82	85
	Tricyclazole 75SP	15.3	13.2	12.7	10.9	6.7	22.2	28	37	40	45	62
	Hexaconazole 5EC	12.5	5.8	Nil	Nil	Nil	22.2	40	71	100	100	100
	Propiconazole 25EC	8.1	5.1	3.0	Nil	Nil	22.2	57	77	80	100	100
	Tebuconazole25.9EC	12.5	9.4	3.9	Nil	Nil	22.2	42	54	80	100	100
	Myclobutanil 10WP	15.7	13.1	6.0	1.5	Nil	22.2	28	37	71	85	100
Metarhizium anisopliae	Difenaconazole 25EC	19.8	15.3	10.9	9.6	5.4	23	8	33	50	50	55
	Tricyclazole 75SP	20.1	18.7	17.2	9.7	4.0	23	0	5.5	13	52	86
	Hexaconazole 5EC	18.7	11.2	6.2	2.8	1.5	23	0	41	72	83	86
	Propiconazole 25EC	16.7	12.6	11.6	5.2	3.1	23	22	38	44	75	83
	Tebuconazole25.9EC	13.5	11.8	5.8	Nil	Nil	23	30	44	72	100	100
	Myclobutanil 10WP	17.5	14.6	9.9	3.5	Nil	23	13	36	47	75	100
Lecanicillium lecanii	Difenaconazole 25EC	18.1	13.0	Nil	Nil	Nil	19.1	14	40	100	100	100
	Tricyclazole 75SP	19.6	18.7	15.9	9.1	Nil	19.1	0	8.3	22	57	100
	Hexaconazole 5EC	20.4	11.2	4.9	Nil	Nil	19.1	0	40	71	100	100
	Propiconazole 25EC	10.3	6.7	Nil	Nil	Nil	19.1	51	65	100	100	100
	Tebuconazole25.9EC	11.7	7.6	Nil	Nil	Nil	19.1	34	48	100	100	100
	Myclobutanil 10WP	18.6	13.0	5.0	Nil	Nil	19.1	11	25	62	100	100

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