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Effect of spinosad 45 SC on growth and Development of Entomopathogenic Fungi *Metarhizium anisopliae* and *Beauveria bassiana*

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ABSTRACT: An experiment was conducted to assess the *in vitro* effect of spinosad 45 SC (Tracer ®) on the entomopathogenic fungi *viz.*, *M. anisopliae* and *B. bassiana.* The insecticide was applied at various concentrations and the field dose and higher doses adversely affected colony development, sporulation and spore germination. The effect was significantly higher on *B. bassiana* than on *M. anisopliae* and the effect increased with the dosage of insecticide used.

Key words: Spinosad 455C, entomopathogenic fungi, sporulation, inhibition.

INTRODUCTION

Concerns in the use of entomopathogenic fungi as alternative pest control agents are increasing even where chemical pesticides have been used as the main agents for controlling pests and diseases in crop production (Jeong Jun and Kyu Chin, 2007). The entomopathogenic fungus, *Metarhizium anisopliae* and *Beauveria bassiana* are the facultative insect pathogens with significant host range and host specificity. They are registered as biopesticides with a broad host range and used for management of several insect pests (Amutha *et al.,* 2010). The insecticide and entomopathogenic fungi are often used in combination for managing insect

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pests of crops, especially vegetables due to their low toxicity and low residual effect. Such use of microbial pesticides combined with pesticides in pest management practices requires detailed compatibility study. The use of incompatible insecticides may inhibit the development and reproduction of the pathogens, affecting the result in IPM practices. Keeping this in view, present investigation was taken up to study the effect of spinosad 45 SC on growth and development of *M. anisopliae* and *B. bassiana* under *in vitro* condition.

MATERIALS AND METHODS

The study was conducted at the Kerala Agricultural University, College of Agriculture, Padanakkad, Kasaragod, Kerala, India. Poisoned food technique (Nene and Thapliyal, 1997) was adopted for the experiment. Radial growth of the test isolates were recorded 10, 12 and 14 days after inoculation. Per cent inhibition of growth over control was calculated using the formula (Vincent, 1927), I=C-T/C×100. Where I = per cent inhibition, C = growth of micro organism in untreated medium and T = growth of micro organism in treated medium.

After *in vitro* effect of spinosad on *M. anisopliae* and *B. bassiana*, sporulation assay was carried out using Martins' rose Bengal agar media. The media was prepared using 5 g peptone, 10 g dextrose, 20 g agar, one gram KH_2PO_4, 0.5 g $MgSO_4.H_2O$, 30 mg Rose Bengal and one litre distilled water. All the ingredients except agar were dissolved in 500 ml distilled water while 20 g agar was dissolved in 500 ml distilled water by boiling. They were then mixed thoroughly and to this mixture Rose Bengal dye was added. This was then poured in to two 500 ml conical flasks and plugged with cotton and sterilized in autoclave at 121.0 ^{0}C for 15 to 20 minutes.

The spore suspension of entomopathogenic fungi were prepared by serial dilution, using *M. anisopliae* and *B. bassiana* growth in different treatments. One milliliter spore suspension of the test fungus was poured in a sterilized petri plate. Thereafter, 20 ml molten and cooled Rose Bengal agar medium was poured in to the petri plate containing spore suspension under aseptic condition and was allowed to solidify and was incubated at 28±1^{0}C in an incubator. Number of colonies of the test isolates was recorded after 10 to 14 days of incubation. The spore germination inhibition was assessed following the method of Peterson (1941). The number of germinated spores in the field was recorded 12 hr after incubation under a compound microscope (40 X objective). From each replication, five microscopic fields were observed following the method of Yashoda (1998), for converting average number of spores germinated per microscopic field.

Per cent spore germination inhibition was calculated using the formula developed by Verma and Singh (1987), I=C-T/C×100. Where I = per cent inhibition, C = number of spores germinated in control and T = number of spores germinated in treatment.

RESULTS AND DISCUSSION

Minimum percent of inhibition (5.23) of colony diameter of *M. anisopliae* was found in samples

treated with 0.0018 % (recommended concentration) concentration of spinosad 45 SC at 14 days after inoculation, while the maximum (26.75) per cent inhibition was found in samples treated with 0.0054 % of spinosad 45 SC. The spinosad 45 SC at the concentration of 0.0036 % recorded 10.44 per cent inhibition of colony diameter of *M. anisopliae.* The present findings were not in agreement with the earlier report of Asi *et al.* (2010) that, spinosad 45 SC was safe to conidial germination and growth of *M. anisopliae*. The present study revealed that, spinosad 45 SC inhibits *M. anisopliae* colony and hence it is not safe for conidial germination (Table 1).

The lowest (23.22) per cent of inhibition of colony diameter of *B. bassiana* was found in sample treated with 0.0018 % concentration of spinosad 45 SC at 14 days after inoculation and it was on par with 0.0036 % concentration with 29.62 per cent inhibition. The highest (47.76)

Table 1. Effect of spinosad 45 SC on growth of *M. anisopliae* in *in vitro* culture

Treatments	Inhibition of colony diameter of *M. anisopliae* (cm)*			Per cent inhibition of colony diameter of *M. anisopliae* *		
	10 DAI	12 DAI	14 DAI	10 DAI	12 DAI	14 DAI
T_1 : Spinosad 45 SC @ 0.0018%	3.35	3.47	4.07	4.24	4.73	5.23
T_2 : Spinosad 45 SC @ 0.0036%	3.27	3.32	3.85	6.41	8.86	10.44
T_3 : Spinosad 45 SC @ 0.0054%	2.65	2.72	3.15	24.22	25.20	26.75
T_4 : Control	3.50	3.65	4.30	-	-	-
SE @0.05 %	0.13	0.14	0.13	1.03	1.79	0.81
CD @0.05 %	0.04	0.04	0.04	3.31	5.79	2.61

*Mean of four replications DAI- Days After Inoculation

per cent inhibition of colony diameter was found in samples treated with 0.0054 % concentration at 14 days after inoculation. The present finding is not in agreement with Amutha *et al.* (2010) who reported that, spinosad 45 SC is slightly toxic to *B. bassiana.* The present study revealed that there was drastic reduction of colony growth of *B. bassiana* when treated with recommended dosage of spinosad 45 SC (Table 2).

All the tested concentration of spinosad 45 SC gave 100 per cent inhibition of sporulation and spore germination on *B. bassiana,* but in the case of *M. anisopliae,* minimum (3.76 per cent) inhibition of sporulation and (26.82 per cent) spore germination was found in the 0.0018 % concentration and highest inhibition of (17.86 per cent) sporulation and (82.73 per cent) spore germination was recorded in samples with 0.0054 % concentration. The present findings were not in agreement with the earlier report of Akbar *et al.* (2012) where they reported that spinosad was compatible with *M. anisopliae* and was found safe to conidial germination and growth of the fungi. The result of present study shows that, the spinosad inhibits conidial germination

Table 2. Effect of spinosad 45 SC on growth and development of B. bassiana

Treatments	Inhibition of colony diameter of *B. bassiana* (cm)*			Per cent inhibition of colony diameter of *B. bassiana* *		
	10 DAI	12 DAI	14 DAI	10 DAI	12 DAI	14 DAI
T1 : Spinosad 45 SC @ 0.0018%	2.00	2.55	2.95	8.82	20.20	23.22
T2 : Spinosad 45 SC @ 0.0036%	1.82	2.35	2.70	16.79	26.36	29.62
T3 : Spinosad 45 SC @ 0.0054%	1.67	1.72	2.00	23.65	46.03	47.76
T4 : Control	2.20	3.20	3.85	-	-	-
SE @0.05 %	0.15	0.14	0.25	2.39	1.82	2.72
CD @0.05 %	0.04	0.04	0.08	7.65	5.82	8.71

*Mean of four replications DAI- Days After Inoculation

and growth of *M. anisopliae* and hence it is not safer to the fungi. The spore germination of *B. bassiana* was totally inhibited by the spinosad and hence the current studies are not in accordance with the results of Rajanikanth *et al.* (2010) who reported that *B. bassiana* is compatible with spinosad (Table 3 and 4).

From the study, it was clear that both the fungi tested are not compatible with the spinosad 45 SC in the laboratory condition. This need to be ascertained under field condition, since, the field condition may have lower dose of insecticide due to drift and further break down of insecticide.

It is inferred that the insecticide spinosad 45 SC inhibits the growth of both the pathogens. The adverse effect is higher in *B. bassiana* than on *M. anisopliae*. The adverse effect is

Table 3. Effect of spinosad 45 SC on sporulation of entomopathogenic fungi

Treatments	Mean count of colony *		Inhibition of sporulation (%)*	
	M. anisopliae	*B. bassiana*	*M. anisopliae*	*B. bassiana*
T_1 : Spinosad 45 SC @ 0.0018%	312.50	0.00	3.76	100.00
T_2 : Spinosad 45 SC @ 0.0036%	290.00	0.00	10.69	100.00
T_3 : Spinosad 45 SC @ 0.0054%	266.75	0.00	17.86	100.00
T_4 : Control	324.75	0.00	-	-
SE @0.05 %	3.83	-	0.36	-
CD @0.05 %	1.24	-	1.16	-

*Mean of four replications

Table 4. Effect of spinosad 45 SC on spore germination of entomopathogenic fungi

Treatments	*M. anisopliae*		*B. bassiana*	
	Germinated spores (%)	Inhibition of spore germination (%)*	Germinated spores (%)	Inhibition of spore germination (%)*
T1 : Spinosad 45 SC @ 0.0018%	11.24	26.82	00.00	100.00
T2 : Spinosad 45 SC @ 0.0036%	36.05	44.65	00.00	100.00
T3 : Spinosad 45 SC @ 0.0054%	47.65	82.73	00.00	100.00
T4 : Control	65.13	-	30.50	-
SE @0.05 %	0.45	0.81	-	-
CD @0.05 %	1.41	2.49	-	-

*Mean of four replications

lowest in field dose of the insecticide and increases with higher doses. With *M. anisopliae* the growth, sporulation and spore germination are significantly lower than in control but the differences are not very high. Since the response is seen as dose dependent by lowering the dose in field use it may be possible to nullify the adverse effect of combining treatments. Regarding *B. bassiana* which shows high growth suppression and 100 per cent suppression on sporulation and spore germination even at field level of the insecticide, a combined use may not be advantageous.

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