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Research Note

Effect of selected pesticides on the growth parameters of *Metarhizium anisopliae* (Metch.) Sorokin

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ABSTRACT: *In vitro* compatibility studies were conducted with the green muscardine fungus, *Metarhizium anisopliae* (Metch.) Sorokin and commonly used pesticides. The test insecticides *viz.,* spinosad, indoxacarb, novaluron and cartap hydrochloride after 21 days recorded 6.42, 5.86, 5.74 and 5.30 cm radial growth of *M. anisopliae*, respectively and all these treatments were on par with each other and with that of control. The conidial concentration per cm of *M. anisopliae* in spinosad amended media was highest and lowest in cartap hydrochloride. The conidial viability of *M. anisopliae* in spinosad, indoxacarb, novaluron and cartap hydrochloride treated media was 89.2, 84.6, 80.4 and 77.4 per cent, respectively. No significant difference in radial growth of *M. anisopliae* was found with tebuconazole, azoxystrobin and chlorothalonil, whereas total inhibition of radial growth was observed in propiconazole treated medium. Significant reduction of *M. anisopliae* conidial concentration per cm was recorded in tebuconazole and azoxystrobin treated media.

KEY WORDS: *Metarhizium anisopliae,* spinosad, indoxacarb, novaluron, cartap hydrochloride, tebuconazole, azoxystrobin, chlorothalonil, propiconazole

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It is reported that 700 (Charnley, 1989) to 750 species (Carruthers and Soper, 1987) of fungi are pathogenic to insects, but only about a dozen species have been exploited so far for insect control. They can perpetuate in target insect (Ferron, 1978) while their safety to non target organisms, ease and economical feasibility for *in vitro* mass culture makes them one of the preferred options among microbials. Under Indian conditions four entomopathogenic fungi were reported promising *viz., Beauveria bassiana* (Bals.) Vuillemin, *Metarhizium anisopliae* (Metch.) Sorokin, *Nomuraea rileyi* F Samson and *Verticillium lecanii* Viegas.

The *M. anisopliae* is widely distributed fungus with a broad host range. Over 100 species of insects belonging to different orders are known to be infected by this fungus. Chemical pesticides being synergistic/ antagonistic among themselves, may have antagonistic or synergistic effects on the potentiality of *M. anisopliae* and may influence natural epizootics. Such situation warrants only the compatible insecticides and/or fungicides to be used in combination with these microbial agents to derive the fullest potential of the organism with least environmental pollution along with cost effectiveness. Therefore, for successful establishment of entomopathogenic fungi in IPM programmes, its compatibility with insecticides and fungicides is very important to manage the insect pests.

The strains of *M. anisopliae* were obtained from National Bureau of Agriculturally Important Insects (NBAII), Bangalore and single spore isolation procedure was followed till pure cultures were established on the Sabouraud's Dextrose Agar medium with yeast extract (SDAY). The pure cultures thus obtained were further cultured and preserved on SDAY slants as well as in Paraffin oil for further experimental purpose (Rombach *et al.,* 1986).

Inoculation of the medium with mycelial mat

Circular discs of 10 mm diameter were cut from vigorously grown culture of *M. anisopliae* using a sterile cork borer and such discs were placed in the middle of each Petri plate on the medium amended with insecticide at recommended concentration. Medium inoculated with

the fungus without insecticide served as control. All these steps were carried out under aseptic conditions inside an inoculation chamber. These plates were incubated at 25 ± 1°C for 21 days. The *M. anisopliae* cultured on SDAY medium was studied for various biological properties such as radial growth, conidia per unit area, viability of conidia and time taken for germination of 50 per cent spores.

Radial growth

The radial growth of *M. anisopliae* was measured using a measuring scale at an interval of 7, 14 and 21 days and compared with control.

The radial growth, number of conidia per unit area and viability of conidia were also recorded following the procedures and compared with control using the formula.

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

Where,

R = Per cent reduction of radial growth / conidia per unit area / conidial viability.

C = Radial growth / conidia per unit area / conidial viability of fungi grown on control or untreated medium.

T = Radial growth / conidia per unit area / conidial viability of fungi grown on insecticide treated medium.

Conidia per unit area

The circular disc of 10 mm diameter was cut randomly from the ten days old uniformly grown culture plates. Each disc was placed in a test tube containing 10 ml of distilled water. The spores present in the disc were allowed to disperse uniformly in the water by rotating the test tube on a vortex for one minute. Proper care was taken to avoid spillage of the suspension while rotation. The suspension was serially diluted and the spores were counted with the help of an improved Neubaur Haemocytometer under a compound microscope at 40 x magnification and number of spores present per ml was calculated using the formula.

No. of spores / ml = Total no. of spores in 5 randomly selected big squares of haemocytometer x 5 x 10^4 (Aneja, 1996).

The readings thus obtained were computed to 10 ml to determine the number of conidia per unit area of 10 mm diameter disc.

Conidial viability and time taken for 50 per cent germination of spores

The conidia were harvested from the uniformly grown culture plates with the help of a fine camel hair brush into sterile distilled water and filtered through double layered muslin cloth.

A 500 µl of the spore suspension was placed in the cavities of a cavity slide containing 1000 µl of SDAY medium. The slides were placed in a Petri plate containing a moistened filter paper at its bottom and were incubated at a temperature of 25 ± 1°C and RH of 95 per cent. The slides were observed after every 2 interval under microscope till 50 per cent of the conidia visible in any of the focused region germinated. This was recorded as TG_{50}, the time taken for germination of 50 per cent of spores. The germination of conidia was recorded after 24 hrs of incubation and the per cent spore germination was calculated using the formula

$$G = \frac{N}{T} \times 100$$

Where,

G = Per cent spore germination.

N = Number of spores germinated.

T = Total number of spores observed.

Effect of selected insecticides on growth parameters of *M. anisopliae*

Radial growth

The radial growth of *M. anisopliae* recorded at 7 Days after inoculation (DAI) on media amended with spinosad, indoxacarb and novaluron were 4.62, 4.34 and 4.16 cm, respectively, and they were on par with each other, while the cartap hydrochloride amended medium recorded lowest radial growth of 3.58 cm which was significantly different from other treatments including control (4.80 cm) and was on par with novaluron (Table 1).

After 14 DAI, *M. anisopliae* recorded a radial growth ranging from 5.28 to 7.00 cm. The highest radial growth among treatments was in control (7.00 cm) which significantly differed from other insecticides *viz.,* spinosad (6.26 cm) indoxacarb (5.52 cm), novaluron (5.50 cm)

Table 1: Effect of selected insecticides on the growth of *Metarhizium anisopliae*

Insecticides	Field recommended concentration *(%)*	Radial growth (cm)				Conidial concentration / cm x 10^7	Conidial viability (%)	TG_{50} (hrs)
		7 days	14 days	21 days	Mean			
Indoxacarb	0.015	4.34 (12.01)[a]	5.52 (13.58)[c]	7.72 (16.12)[b]	5.86 (13.91)[a]	3.02 (10.00)[d]	84.6 (66.96)[c]	11.0
Spinosad	0.0135	4.62 (12.39)[a]	6.26 (14.48)[b]	8.40 (16.84)[a]	6.42 (14.58)[a]	3.78 (11.21)[b]	89.2 (70.93)[bc]	9.5
Novaluron	0.0100	4.16 (11.75)[ab]	5.50 (13.55)[c]	7.56 (15.95)[b]	5.74 (13.76)[a]	3.65 (11.01)[bc]	80.4 (63.97)[cd]	11.0
Cartap hydrochloride	0.0500	3.58 (10.87)[b]	5.28 (13.27)[c]	7.06 (15.40)[c]	5.30 (13.19)[a]	3.57 (10.89)[c]	77.4 (61.71)[d]	13.2
Control		4.80 (12.64)[a]	7.00 (15.34)[a]	8.66 (17.11)[a]	6.82 (15.03)[a]	4.79 (12.64)[a]	94.8 (77.49)[a]	7.6
SEm ±		0.30	0.21	0.16	1.25	6.80	1.69	
C.D. *P* = 0.05		0.90	0.62	0.48	3.96	0.20	4.92	

Figures in parentheses are angular transformed values
Figures indicated by same alphabets are not significantly different from each other as per DMRT
SEm± : Standard Error of Mean

and cartap hydrochloride (5.28 cm). The radial growth of *M. anisopliae* on media treated with spinosad differed significantly from indoxacarb, novaluron and cartap hydrochloride that are on par with each other.

Spinosad with 8.40 cm radial growth was found to be significantly superior over other insecticidal treatments at 21 DAI. That was on par with control (8.66 cm), whereas indoxacarb, novaluron and cartap hydrochloride treated media recorded a radial growth of 7.72, 7.56 and 7.06 cm respectively. Indoxacarb and novaluron treatments were on par with each other, wherea cartap hydrochloride was significantly lower when compared to other insecticidal treatments.

The overall radial growth of *M. anisopliae* on spinosad, indoxacarb, novaluron and cartap hydrochloride treated media recorded 6.42, 5.86, 5.74 and 5.30 cm respectively and all these treatments were on par with each other and also with that of control which recorded 6.82 cm radial growth.

The overall per cent inhibition of radial growth over control was lowest on spinosad (5.77%) and highest in cartap hydrochloride (22.82%). All the insecticidal treatments were on par with each other and also to that of control which indicates that all the insecticides were exhibiting similar effect on per cent inhibition of radial growth of *M. anisopliae.*

Conidial concentration

M. anisopliae on spinosad and novaluron contaminated media recorded a conidial concentration of 3.78 and 3.65 x 10^7 /cm respectively and were on par with each other, whereas cartap hydrohchloride and indoxacarb recorded conidial concentration of 3.57 and 3.02 x 10^7 /cm, respectively as against 4.79 x 10^7 /cm in control (Table 1). The conidial concentration on cartap hydrochloride treated medium with *M. anisopliae* was on par with novaluron treatment, whereas, indoxacarb treated media was significantly lower when compared with other treatments. The lowest per cent reduction of conidial concentration over control was recorded in spinosad (21.08%), whereas highest was in indoxacarb (36.95%).

Conidial viability

The per cent conidial viability obtained in spinosad and indoxacarb treated media was 89.2 and 84.6 per cent respectively, which were on par with each other and differed significantly from control (94.8%). The conidial viability of 80.4 and 77.4 per cent respectively was recorded in novaluron and cartap hydrochloride treated

media which were on par with each other and significantly differed with other treatments.

Time taken for 50 per cent spore germination

Among the media amended with insecticide, *M. anisopliae* recorded a lowest duration of 9.50 hrs in spinosad when compared with other insecticidal amendments ndicating the rapid germination of conidia (Table 1). The indoxacarb + *M. anisopliae and* novaluron + *M. anisopliae* treatment recorded 11.0 hrs for their 50 per cent spore germination. The longest duration of 13.2 hrs (TG_{50}) was recorded in cartap hydrochloride treatment when combined with *M. anisopliae* as against the least duration of 7.6 hrs in control.

These results are in concurrence with the findings of Mietkiewski and Gorski (1995), who reported that dimethoate and pirimicarb inhibited the growth of *M. anisopliae* at ten times the recommended doses, while the fungal colonies obtained were 85 per cent of the size of control even at the highest concentration of alpha – cypermethrin indicating lesser inhibitory action.

Gupta *et al.* (2002) also found *M. anisopliae* to be tolerant to azadirachtin at all the concentrations (10, 1000 and 2000 ppm), while the fungus was found to be sensitive to insecticides *viz.,* chlorpyriphos, endosulfan, monocrotophos and quinalphos, wherein fungal inhibition occurred with the increasing concentrations of insecticides. Neves *et al*. (2001) reported that the neonicotinoid insecticides (acetamiprid, imidacloprid and thiamethoxam) did not affect the conidial germination, conidial production and vegetative growth of *M. anisopliae*.

Effect of selected fungicides on growth parameters of *M. anisopliae*

Radial growth

The radial growth of *M. anisopliae* recorded at 7 DAI showed no significant difference in tebuconazole (1.82 cm) and azoxystrobin (1.62 cm) contamination, while, chlorothalonil amended medium recorded 1.04 cm radial growth which was significantly lower than the above treatments (Table 2). The *M. anisopliae* radial growth was totally inhibited in propiconazole amended medium. However, all the fungicidal amendments differed significantly with control (2.42 cm).

At 14 days after the fungicidal contamination the radial growth of *M. anisopliae* was 2.94 and 2.80 cm in tebuconazole and azoxystrobin treated media, respectively and were on par, while, chlorothalonil contamination recorded 1.96 cm radial growth which was significantly different with the above treatments. The radial growth was totally inhibited in case of propiconazole contaminated medium as against 4.16 cm in control.

The tebuconazole and azoxystrobin recorded 3.80 and 3.64 cm radial growth of *M. anisopliae* respectively which were on par with each other and differed significantly with control (7.18 cm) and chlorothalonil (3.06 cm). The total inhibition of *M. anisopliae* radial growth with propiconazole fungicide treated media continued even after 21 DAT which is significantly lower when compared to above treatments.

The overall radial growth of *M. anisopliae* recorded was 2.85, 2.68 and 2.02 cm in tebuconazole, azoxystrobin and chlorothalonil treatments respectively and there was no significant difference among them and also with that of control. The total inhibition of *M. anisopliae* radial growth was recorded in propiconazole treated medium only, whereas control recorded 4.58 cm overall radial growth which significantly differed with the above treatments.

Conidia per unit area

Tebuconazole and azoxystrobin treatments recorded 2.39 and 2.19×10^7 / cm respectively of conidial concentration, while chlorothalonil recorded 1.97×10^7 / cm conidial concentration as compared to control (4.21×10^7 /cm). In propiconazole treated medium 100 per cent inhibition of conidial germination was recorded. There was significant difference among all the treatments tested against *M. anisopliae* (Table 2).

The cent per cent reduction of conidial concentration over control was recorded in propiconazole, whereas lowest reduction of 43.23 per cent was recorded in tebuconazole treated medium.

Conidial viability

The per cent conidial viability obtained in tebuconazole (73.8%), azoxystrobin (64.8%) and chlorothalonil (60%) amended media differed significantly among them and was significantly lower than the control (85.8%). The propiconazole recorded 100 per cent loss of conidial viability (Table 2).

Time taken for 50 per cent spore germination

The TG_{50} for conidia of *M. anisopliae* in media amended with fungicides was found to be lowest in chlorothalonil (12.8 hrs) followed by tebuconazole (13.2 hrs) and azoxystrobin (13.8 hrs). No spore germination was recorded for *M. anisopliae* in propiconazole amendment (Table 2).

Table 2: Effect of selected fungicides on the growth of *Metarhizium anisopliae*

Insecticides	Field recommended concentration *(%)*	Radial growth (cm)				Conidial concentration / cm x 10^7	Conidial viability (%)	TG_{50} (hrs)
		7 days	14 days	21 days	Mean			
Propiconazole	0.1	0.00 (0.00)[d]	0.00 (0.00)[d]	0.00 (0.00)[d]	0.00 (7.99)[a]	0.00 (0.00)[e]	0.00 (0.00)[e]	–
Chlorothalonil	0.2	1.04 (5.51)[c]	1.96 (7.94)[c]	3.06 (10.07)[c]	2.02 (7.99)[a]	1.97 (8.06)[d]	60.0 (50.80)[d]	12.8
Azoxystrobin	0.1	1.62 (7.27)[b]	2.80 (9.62)[b]	3.64 (10.99)[b]	2.68 (9.31)[a]	2.19 (8.50)[c]	64.80 (53.62)[c]	13.8
Tebuconazole	0.1	1.82 (7.72)[ab]	2.94 (9.86)[b]	3.80 (11.24)[b]	2.85 (9.62)[a]	2.39 (8.89)[b]	73.80 (59.25)[b]	13.2
Control		2.42 (8.94)[a]	4.16 (11.7)[a]	7.18 (15.53)[a]	4.58 (12.08)[a]	4.21 (11.853)[a]	85.80 (67.94)[a]	10.7
SEm ±		0.49	0.31	0.11	1.21	7.38	1.00	
C.D. ($P = 0.05\%$)		1.46	0.93	0.33	3.81	0.21	2.96	

Figures in parentheses are angular transformed values
Figures indicated by same alphabets are not significantly different from each other as per DMRT
C.D. (0.05%): critical difference at 5 per cent level
SEm± : Standard Error of Mean

The results are concurrent with the findings of Gardner and Kinard (1998), James and Elzen (2001), Xu *et al*. (2002) and Bhattacharya *et al*., (2004) wherein they observed no detrimental effects of imidacloprid on *B. bassiana*. The expression of low inhibition in the biological properties of *B. bassiana* strains may be due to the presence of emulsifiers and other additives in the formulated products of insecticides. Generally, wettable powders and flowable formulations cause no inhibition and often increase colony counts whereas, emulsifiable concentrate formulations frequently inhibit *B. bassiana* germination (Anderson *et al*., 1989). Adjuvants in wettable powders and flowable formulations may act as mild abrasives and break up agglomerations of conidia, which would improve the field performance of *B. bassiana*.

The results of the present study suggest that the insecticides spinosad and indoxacarb can be used with *M. anisopliae* in pest management. This combination would give an added advantage where the insecticide pathogen mixtures introduce multiple mortality factors against the target pest with insecticide making the insect physiology weak to a desired degree which makes it much more susceptible to the attack of the entomopathogens (Fedorinehik, 1974) and also delay the chances of expression of resistance to new insecticides (Georghiou, 1983). This approach in pest management was explored by Steinkraus (1996) and Brown *et al*. (1997) who found that the combination of imidacloprid and *B. bassiana* yielded greater control of adult tarnished plant bugs in cotton over the use of either of them alone. Ali *et al.* (2007) reported that imidacloprid was highly compatible with *B. bassiana* (isolate DEB1008) and flufenoxuran is not compatible with *B. bassiana.*

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