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Effect of insecticides and fungicides on growth and sporulation of *Metarhizium rileyi* (Farlow) Samson

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Abstract
The effect of different newer insecticide molecules and some commonly used fungicides were evaluated on the growth and sporulation of *Metarhizium rileyi* through poisoned food technique. The different pesticides *viz.*, Spinetoram 11.7 SC, Flubendiamide 480 SC, Chlorantraniliprole 18.5 SC, Emamectin benzoate 5 SG, Spinosad 45 SC, Thiodicarb 75 WP, Difenthuron 50 WP, Lufenuron 5.4 EC, Profenophos 50 EC, Carbendazime, Propiconazole, Azoxystrobin, Tebuconazole and Copper oxy chloride were used. The insecticides and fungicides were mixed into the SMAY media and a circular disc of *M. rileyi* culture was procured by a cork borer and placed in the center of the poisoned media and observed for its growth and sporulation. The colony diameter, growth inhibition (%) and spore yield were recorded. The fungicides carbendazime, propiconazole showed complete growth inhibition (100%), insecticide Emamectin benzoate 5 SG showed a maximum inhibition of 77.37%. While, Azoxystrobin, Tebuconazole recorded low growth inhibition of 8.17% and 12.36% respectively. The insecticides Profenophos 50 EC and Chlorantraniliprole 18.5 SC showed lower growth inhibition of 2.46% and 19.84% respectively. The spore yield was gravely affected by $0.12x10^8$ spores/ml by thiodicarb while in control $6.24x10^8$ spores/ml was recorded. The spore yield was not affected in the treatments Chlorantranilirpole 18.5 SC and Azoxystrobin.

Keywords: Insecticides, fungicides, *Metarhizium rileyi*, colony diameter, spore yield, growth inhibition

Introduction
Entomopathogenic fungi cannot replace the need for chemical pesticides in all commercial production systems. Insecticides may be needed to suppress a rapidly expanding pest population or to control pests not targeted by an entomopathogenic fungus. Fungicides are often be required to control plant diseases, but many fungicides having broad spectrum of activity, can adversely affect the efficacy of entomopathogenic fungi. Herbicides, botanical pesticides and plant growth regulators are also used extensively in most agroecosystems to harness the benefit of these and therefore their compatibility with mycopathogens becomes dessessive for combined use. While the potential inhibitory effects of pesticides on the entomopathogenic fungus cannot be ignored. Also, there are numerous examples where the application of chemical pesticides has enhanced the efficacy of entomopathogens against insect pests (Quintela and McCoy, 1997, Kruger and McCoy, 1997, Kaakeh *et al.*, 1997, GARDNER and Kinard, 1998) [1-4]. The mycopathogen, to be considered as an integral part of plant protection has to meet the requirement of compatibility with many other measures recommended for management of pests, diseases and weeds.

Metarhizium rileyi is one such mycopathogen, whose occurrence in different geographical areas has been reported in many studies and is being used as microbial bioagent against various noctuid pests infesting different crops like soybean, cabbage, groundnut, castor, potato, etc. (Patil *et al*. 2003; Manjula *et al*. 2003, 2005) [5-7]. Therefore, successful implementation of biological agents like entomopathogenic fungi like *Metarhizium rileyi* along with newer insecticidal molecules will reduce the insecticidal load in an IPM programme. The use of pesticides could be changed in ways to minimize their effect on bioagents. The practice of IPM procedures with combined application of biocontrol and chemical methods have been proved to be having a positive effect on bioagents. Mainly usage of selective pesticides and also the ones showing synergistic effect when applied together with microbial pesticides are perhaps the most powerful tool to implement in IPM programmes (David Orr, 2009) [8]. Therefore, keeping this in view, present investigations were taken-up to understand the effect of pesticides on *M. rileyi* in *in-vitro*.

Material and Methods

The experiment was conducted in Entomology Laboratory, Crop Protection Section, ICAR- Indian Institute of Oilseeds Research, Hyderabad during 2019-21. The standard media used for culturing *M. rileyi* was Sabouraud's Maltose Agar Medium fortified with 1 per cent yeast extract. The components used in preparing SMAY medium were enlisted below:

Table 1: SMAY medium components

Ingredients	Quantity (g/ml)
Agar	20 g
Peptone	10 g
Maltose	40 g
Yeast extract	5 g
Chloramphenicol	80 mg/l
Distilled water	1000 ml

The newer insecticide molecules and fungicides were tested under laboratory conditions to identify their inhibitory effect on radial growth and sporulation when cultured on toxicant incorporated media by poisoned food technique. The insecticides and fungicides used were mentioned in Table 2.

Table 2: List of insecticide and fungicide used alone and in combinations for physical and biological compatibility tests

Insecticide/Fungicide	Dose
Chlorantraniliprole 18.5 SC	0.3 ml/l
Diafenthiuron 50 WP	1.2 g/l
Emamectin Benzoate 5 SG	0.45 g/l
Flubendiamide 480 SC	0.3 ml/l
Lufenuron 5.4 EC	0.6 ml/l
Profenophos 50 EC	2 ml/l
Spinetoram 11.7 SC	0.7 ml/l
Spinosad 45 SC	0.4 ml/l
Thiodicarb 75 WP	1 g/l
Carbendazim 50 WP	1 g/l
Propiconazole 25 EC	1 ml/l
Azoxystrobilin 25 SC	1 ml/l
Tebuconazole	1 ml/l
Copper oxy chloride 50 WP	2 g/l

The experiment was conducted by preparing SMAY media and the media was melted and allowed to cool down till it was warm before pouring into petri dishes while the insecticides and fungicides were added to the medium and mixed by gentle shaking for attaining homogeneous mixture. Then, the media was poured evenly into the petri dishes and allowed to solidify. 60ml of media was used for three petri plates. The fully-grown culture of *M. rileyi* was cut into circular discs of 10mm diameter with the help of cork borer. The discs were placed in the center of the petri dish with the fungal mat facing the surface of media. The observations were recorded at 7 days after inoculation on radial growth, percent growth inhibition and to understand the effect of pesticides on sporulation the spores were harvested on 8 days after inoculation. The spore counts were taken with the help of Neubauer Hemocytometer

Results

9 insecticides and 5 fungicides were assayed for their growth inhibitory property. Overall inhibition of fungal growth by insecticides ranging from 2.46 to 100 per cent. The results indicate that fungicides were highly inhibitory (100%) and toxic to the mycopathogen followed by insecticides (77.37%). Insecticides were less toxic (2.46%) to fungus (Table 3).

All the insecticides and fungicides inhibited the growth of fungus significantly. Among them carbendazim, propiconazole, emamectin benzoate, spinosad and copper oxy chloride were found highly detrimental to fungus by retarding the growth totally (Table 3). Whereas, the degree of inhibition in other tested insecticides and fungicides ranged from 2.46 to 49.82 percent. Azoxystrobin, Tebuconazole, Chlorantraniliprole, Spinetoram were found to be comparatively safe to the fungus (8.17%, 12.36%, 19.84% and 7.32% inhibition) followed by Flubendiamide (34.85%). Spore count per plate was assessed in all the treatments. As there was no growth in carbendazim, propiconazole, no spores were produced whereas, profenophos 50 EC showed less growth 2.46% inhibition, and there was no spore development. While, Chlorantraniliprole, Flubendiamide, Azoxystrobin and Tebuconzole higher conidial load of $2.7x10^8$, $1.17x10^8$, $2.43x10^8$ and $1.87x10^8$ conidia per plate among the insecticides and fungicides.

Moderate growth inhibition was recorded in Lufenuron and diafenthuron with 49.82% and 42.27% inhibition. While, the spore yielded was $0.78x10^8$ and $0.55x10^8$ conidia per plate respectively.

Table 3: Effect of insecticides and fungicides on growth and sporulation of *M. rileyi*

Treatments	Colony diameter (mm)	Growth inhibition (%)	Spore yield ($x10^8$)
Chlorantraniliprole 18.5 SC	32.00 (5.68)	19.84	2.7
Diafenthurion 50 WP	23.00 (4.77)	42.27	0.55
Emamectin benzoate 5 SG	9.00 (2.96)	77.37	0.26
Flubendiamide 480 SC	26.00 (5.13)	34.85	1.17
Lufenuron 5.4 EC	20.00 (4.47)	49.82	0.78
Profenophos 50 EC	39.00 (6.28)	2.46	-
Spinetoram 11.7 SC	37.00 (6.11)	7.32	0.9
Spinosad 45 SC	19.00 (4.35)	52.32	0.81
Thiodicarb 75 WP	11.33 (3.37)	71.55	0.12
Carbendazime	0.00 (0.71)	100.00	-
Propiconazole	0.00 (0.71)	100.00	-
Azoxystrobilin	36.67 (6.09)	8.17	2.43
Tebuconazole	35.00 (5.95)	12.36	1.87
Copper Oxy Chloride	12.50 (3.61)	68.75	0.5
Control	40.00 (6.36)	-	6.24
S.Em	0.230	-	-
CD	0.670	-	-

Figures in parenthesis () were square root transformed.

On the other hand, Chlorantraniliprole and Spinetoram were found safe to the fungus by inhibiting only 19.84 and 7.32 percent growth, respectively. Among the insecticides, emamectin benzoate found more detrimental by retarding its growth significantly (77.37%) over others, followed by Thiodicarb (71.55%) and Spinosad (52.32%). Conversely, chlorantraniliprole, spinetoram, flubendiamide and profenophos were found comparatively less detrimental (34.85 to 2.46% inhibition) to the fungal growth. Within, the growth inhibitory insecticides, lufenuron and difenthuron were relatively lesser growth inhibition (49.82 to 42.27%). Spore yield among insecticides depends upon its inhibitory action on colony growth. It varied from $0.12x10^8$ to $2.7x10^8$ conidia per plate. Obviously conidial count was less in thiodicarb treated plate has its inhibitory action was strong and it did not allow fungus to grow and sporulate with only $0.12x10^8$ conida per plate. On the contrary in chlorantraniliprole and azoxystrobin treated plates spore load was highest among treatments ($2.7x10^8$ and $2.4x10^8$ conidia per plate). Spore yield in other insecticides was proportionate to colony growth except in profenophos which showed less growth inhibition but there was no sporulation. While, carbendazime and propiconazole complete inhibited growth thereby no recorded sporulation.

Discussion

Li and Holdom (1994) [9] found complete growth inhibition in propiconazole, carbendazim and flusilazole at field recommended dose. High toxicity of carbendazim and mancozeb to *N. rileyi* has been reported by Kulkarni (1999) and Hegde (2001) [10, 11]. On the contrary, carbendazim and mancozeb, were safe to the fungus (*N. rileyi*). (Gopalkrishnan and Mohan 2000) [12] concur the present findings. Chlorantranilirpole, Spinetoram, Azoxystrobin and Tebuconazole were comparatively safe to the *M. rileyi* under laboratory studies needs confirmation under field situation. It would be better if they are used in sequence with time lag either before or after the mycopathogen application. Toxicity of insecticides and fungicides will also reduce as days progress after application. This leads to the assumption that application of the mycopathogen is possible after certain safe period, which again needs more detailed study under field conditions

The observed difference could be due to inherent variability of chemicals to biological creatures. Compatibility of chemical pesticides with the mycopathogen have been studied by Fargues (1975) and Anderson *et al*. (1989) [13, 14], Mohammed *et al*. (1987) [15]; Castinerias *et al*. (1991) [16]. Hassan and Charnely (1989) [17] revealed the in-consistent interaction between fungus and insecticides. Li and Holdam (1994) [9] observed fungicides and insecticides as more deleterious than other insecticide to the mycopathogen. They observed extremely detrimental effect of chlorpyriphos, tempephos and malathion to mycelial growth and sporulation of *M. anisopliae*, while carbamate insecticides like carbofuran, methomyl and oxamyl were moderately toxic. Pyrethroids and insect growth regulators were safe to the development stages of fungus.

Results of the present study thus reveal that except few (emamectin benzoate, thiodicarb, spinosad, profenophos, carbendazime, propconazole and copper oxy chloride) all other insecticides and fungicides can be safely used along with the mycopathogen. However, laboratory results on artificial media may not be reproducible in field as there will be degradation of toxicants. chlorantraniliprole, spinetoram, azoxystrobin and tebuconazole can be mixed with the fungus to get enhanced effect but needs field confirmation. For others, as discussed earlier with fungicides, safe interval between insecticide spray and the mycopathogen inoculation decides the effectiveness and strategy development for the conjunctive or supplementary use of mycopathogens.

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