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Compatibility of *Metarhizium* (*Nomuraea*) *rileyi* rice bran oil formulationwith insecticides

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Abstract
The compatibility studies of *Metarhizium* (*Nomuraea*) *rileyi* rice bran oil formulation with four insecticides, regarding mycelial growth revealed considerably novaluron recorded higher percent inhibition of 34.67 and lufenuron recorded lower percent inhibition of 23.56. Regarding sporulation, in spinosad treatment, the highest of 0.81×10^8 conidia ml^{-1} was observed.

Keywords: *Metarhizium* (*Nomuraea*) *rileyi,* rice bran oil formulation, compatibility, insecticides, conidia ml^{-1}

Introduction
Pest management involving biocontrol agents is assuming prominence and has been considered as an important and safe strategy in insect population reduction. Among the several micro-organisms *viz*., bacteria, fungi, virus, protozoans and nematode, entomopathogenic fungi fills an extremely important niche for control of insect pests. It is one of the important groups of bio-agents that associate with the insects living in diverse habitats, including fresh water, soil surface and aerial location. Entomopathogenic fungi are often reported for causing high level of epizootics in nature and are the most versatile biological control agent and environmentally safe (Carruthers*etal.* 1987) [4].

It is found worldwide and associated with insects and phytophagous mite populations, contributing to biological control of these arthropods on several economically important crops.

The knowledge of the compatibility between entomopathogenic fungi and pesticides may facilitate the choice of proper products for integrated pest management (IPM) program considering the fungus as an important pest control agent.

Integration of selected strain of entomopathogenic fungi with selective insecticides can improve the control efficiency, besides decreased amount of insecticides, required it also minimize the risks of environmental contamination and delay the expression of insecticide resistance in insect pests (Ambethgar, 2009) [1].

Materials and Methods
The compatibility of *M. rileyi* rice bran oil formulations with four insecticides at field recommended doses was carried out. Lufenuron (Cigna) 5.4% EC, novaluron (Rimon) 10% EC, emamectin benzoate (Xplode) 5% SG and spinosad (Tata Taffin) 45% SC along with an untreated control were studied.

Disc inoculation method
To study the radial growth of *N. rileyi,* in the laminar airflow chamber onto insecticides poisoned SMAY medium, 5 mm disc of *M. rileyi* (NNR-5) are introduced with the help of sterile incinerated cork borer. Different treatments were compared with respect to relative toxicity to *M. rileyi* growth.

The percent inhibition was measured by using the formula:

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

Where in,

I = Percent inhibition of mycelial growth

C = Colony diameter of pathogen in control (mm)

T = Colony diameter of pathogen in treatment (mm)

Spore suspension inoculation method

The SMAY medium of 100 ml present in 250 ml capacity conical flasks was melted on hot plate and allowed to reach to Luke warm temperature. Then 0.03 ml of spinosad, 0.05 g of emamectin benzoate, 0.01 ml novaluron and 0.01 ml lufenuron were introduced into separate conical flasks lukewarm SMAY medium. One ml of NNR-5 (Rice bran oil + 5 g *M. rileyi*+ Triton X-100 (0.1%)) rice bran oil formulation was pippeted into Petri plate. Then SMAY medium containing insecticides was poured into Petri plates @ 20 ml/plate. For each insecticide, five replications were maintained along with untreated control. The Petri plates were incubated after sealing with parafilm and maintained in the incubator at 25°C. After 15 days of inoculation, the spores were harvested into 50 ml of water and the data in terms of spore count ml^{-1} was recorded with the help of Neubaur haemocytometer. Thus, total spore count produced in individual Petri plates was assessed.

Results and Discussions

Effect of insecticides on growth of *M. rileyi* through disc inoculation method

Inhibitory effect of commonly used and safer four insecticides on growth of *M. rileyi* was studied through disc inoculation method. The mean growth of the mycelium with different insecticides observed was 68.80 mm in lufenuron, 58.80 mm in novaluron, 62.80 mm in emamectin benzoate, 66.80 mm in spinosad and in control it was 90 mm (Table 1).

The percent inhibition growth of *M. rileyi* was less than 35 in all the tested insecticides. Among the tested four insecticides, novaluron recorded relatively higher percent inhibition of 34.67 which was on par with emamectin benzoate (30.22%) and significantly differed from other two treatments. Spinosad and lufenuron recorded 25.78 percent and 23.56 percent inhibition of growth respectively which are statistically insignificant. (Table 1).

Effect of insecticides on sporulation of *M. rileyi* through spore suspension inoculation method

The spore production in compatibility of *M. rileyi* rice bran oil formulation with different insecticides through spore suspension inoculation method was studied and presented below. In *M. rileyi* rice bran oil formulation 1×10^8 spores ml^{-1} were recorded.

The observations indicated that considerably higher conidial yield was obtained in spinosad *i.e.* 0.81×10^8 spores ml^{-1} which was on par with novaluron of 0.71×10^8 spores ml^{-1}. Lufenuron and emamectin benzoate recorded 0.50×10^8spores ml^{-1} and 0.51×10^8 spores ml^{-1} which were on par with one another. In untreated control, the conidial yield of 0.97×10^8spores ml^{-1} was obtained which was on par with spinosad (Table 1).

In the results, as all the insecticides found to exhibit less than 35 percent inhibition of mycelial growth, all the tested four insecticides can be considered as compatible with *M. rileyi*. However, novaluron is found relatively more toxic for hyphal growth of *N. rileyi*. Lufenuron proved to be less toxic to *M. rileyi* indicating that it is more compatible among the four insecticides. Regarding sporulation among the four insecticides, in spinosad treatment highest conidial yield was recorded.

Table 1: Effect of four novel insecticides on growth of *M. rileyi* and sporulation through disc inoculation and spore suspension inoculation method

S. No.	Treatment	Dose of insecticides per 100 ml of SMAY medium	Disc inoculation method		Spore suspension inoculation method
			Growth of mycelium (mm)	Percent inhibition of mycelium growth	Spores per ml
1	Lufenuron 5.4% W/W EC	0.1 ml	68.80	23.56b (28.82)	0.50 × 108 a
2	Novaluron 10% EC	0.1 ml	58.80	34.67a (36.06)	0.71 × 108 bc
3	Emamectin benzoate 5% SG	0.05 g	62.80	30.22ab (33.33)	0.51 × 108 ab
4	Spinosad 45% SC	0.03 ml	66.80	25.78b (30.49)	0.81 × 108 cd
5	Untreated control		90.00	0.00c (0.00)	0.97 × 108 d
	F			Significance	
	Sig			0.000	

Values are the means of three replications; Values in parenthesis are arc sine transformed values

Values followed by same letter are not significantly different as per DMRT; Means are significantly different at P (0.01)

Lufenuron is a benzoylurea insecticide, an insect growth regulator. Intoxications with lufenuron to non-targets are very infrequent due to its low toxicity, high safety margin. Novaluron is an insect growth regulator, benzoylphenyl urea, reported to have posing low risk to the environment and non-target organisms. Spinosad is a novel mode of action insecticide derived from a family of natural products obtained by fermentation of *Saccharopolyspora spinosa*. It has low mammalian toxicity, good environmental profile, approved for use in organic agriculture by several national and international certifications. Emamectin benzoate is the fermentation product of soil actinomycete, *Streptomyces avermitilis*. It is considered as biological pesticide.

Neeraja and Manjula (2014) [6] studied the inhibitory effect of some commonly used insecticidies and fungicides on sporulation of *Nomuraea rileyi* invitro through poison food technique. Among the ten tested insecticides, at recommended concentrations, spinosad, fenvalerate recorded conidial yield of 0.50×10^8 and 0.45×10^8spores ml^{-1} however, their inhibition crossed 50 percent. Indoxacarb allowed 0.30×10^8spores ml^{-1}. Monocrotophos and dimethoate were at a par with each other in their effect to the fungus. Chlorpyriphos, profenophos, endosulfan, carbaryl, thiodicarb did not allow any sporulation (showing 100% inhibition) Sulphur treatment resulted in spore yield of 0.42×10^8 spores ml^{-1} (66.60%inhibition).

Chaudhari*et al.* (2017) [5] reported that in contrary to the present results, compatibility of widely used newer lufenuron 5EC was found to be highly detrimental to its growth (100%), followed by emamectin benzoate (77.76%), indoxacarb (75.58%), novaluron (75%), and spinosad (62.43%). In contrast, lambda-cyhalothrin 5EC was found less toxic at recommended dose. Combination of lambda-cyhalothrin at half of the recommended dose and *N. rileyi* showed synergism in toxicity against *S. litura*.

The results are in agreement with Babu (2012) [2] who conducted that the *In vitro* compatibility studies with the green muscardine fungus, *M. anisopliae* (Metch.) Sorokin and commonly used pesticides. They tested insecticides *viz*.,spinosad, indoxacarb, novaluron and cartap hydrochloride by spraying on mycelia of *M.anisopliae* and after 21 days, 6.42, 5.86, 5.74 and 5.30 cm radial growth of *M. anisopliae* were recorded respectively and all these treatments were on par with each other and with that of untreated 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 percent, 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.

The results are supported by (Barad*et al*. 2014) [3] who studied the laboratory compatibility of *N. rileyi* with different pesticides including 15 insecticides and 15 fungicides during 2009 - 10 at the Junagadh Agricultural University, Campus, Junagadh. The results showed that azadirachtin 0.0075 percent, spinosad 0.009 percent, fenvalerate 0.01 percent, endosulfan 0.07 percent, cypermethrin 0.009 percent, profenophos 0.05 percent and acephate 0.15 percent were found most compatible with *N. rileyi*.

Somar*et al*. (2016) [7] studied the invitro compatibility of *N. rileyi* with six commonly used pesticides in vegetable ecosystem by poison food technique at recommended dose (rd) and half of recommended dose (1/2RD). The results were expressed in percent mycelial growth inhibition of *N. rileyi* colony on the insecticide treated medium. At field recommended dose of chlorpyrifos 20% EC with highest inhibitory effect (79.21%) of *N. rileyi* was observed. This was followed by malathion 50 EC (77.88%) and azadirachtin 0.5% (61.33%) whereas bifenthrin 10% WP, imidaclorpid 17.8% SL and thiomethoxam 25% WG were found to be moderately toxic. Half of the recommended dose of malathion 50 EC, bifenthrin 10% WP and chlorpyrifos 20% EC were, however tolerated well by *N. rileyi* showing growth inhibition < 50%. Thiomethoxam 25% WG was found to be best in compatibility to *N. rileyi* with lowest percent of growth inhibition (20.78%).

Conclusion

The present investigations revealed that the compatibility studies indicated maximum mycelial growth of *N. rileyi* was recorded in Lufenuron treatment with 23.56 percent inhibition. The next safer insecticide among the four used was spinosad.

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