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Evaluation of Compatibility of Commercial Formulation of Entomopathogenic Fungi, *Metarhizium anisopliae* 1% WP (Kalichakra) with Different Concentrations of Insecticides under Laboratory Conditions

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ABSTRACT: The present investigation to evaluate the compatibility of entomopathogenic fungi, *Metarhizium anisopliae* with different concentrations of insecticides was conducted in Insecticides toxicology laboratory, Department of Entomology. Toxicity of five concentrations of two insecticides each, Spinetoram 11.7SC (Delegate) (0.0025, 0.005, 0.01, 0.02 and 0.04%) and Novaluron 5.25 + Indoxacarb 4.5SC (Plethora) (0.005, 0.01, 0.02, 0.04 and 0.06%) was tested against *M. anisopliae in vitro.* Observation revealed that spinetoram at 0.0025 and 0.005% concentration was found to be compatible and moderately toxic to the fungus as these concentrations recorded less than 50% inhibition and spore count was recorded 64.81 × 10^4 and 49.94 spores/ml, respectively. Concentration 0.01% was observed toxic and 0.02 and 0.04% each was found to be very toxic. All concentration of novaluron + indoxacarb despite of sufficient mycelium growth was observed very toxic to the fungus as it recorded low number of spore/ml.

Keywords: Entomopathogens, IPM, Insecticides, bioagents, *Metarhizium anisopliae.*

INTRODUCTION

Over the year effective insect pest management have been the key priority. Chemical pesticides have been the best choice for the farmers but there is also a rising concern about the reliance on pesticides and their detrimental impact. Chronic exposure of certain pesticides has been reported to cause various severe health issues, harmful impact on non-target organisms and residual buildup (Aktar *et al.,* 2009; Eskenazi *et al.,* 2007; Goulson, 2013; Grandjean and Landrigan 2014). Moreover, recent advancements have been notably achieved through the examination of organisms that target insects, encompassing bacteria, fungi, viruses, protozoans, and nematodes. Entomopathogenic fungi (EPF), plays a crucial role in insect pest control, representing a significant category of bio-agents found in various habitats such as freshwater, soil surfaces, and aerial environments. More than 700 species from 9 genera infecting the insect have been documented (Charnley, 1989) causing high level of epizootics in nature, making them the most adaptable and environmentally friendly biological control agents (Carruthers and Soper 1987). *Metarhizium anisopliae* has been reported to cause different diseases in insect pests and is capable to be used as an alternate of insecticides. The combination of specific strains of entomopathogenic fungi with carefully chosen insecticides can enhance effectiveness. This not only reduces the quantity of required insecticides but also mitigates the potential for environmental contamination and delays the development of insecticide resistance in pests (Ambethgar, 2009), hence, it is most suitable for integrated pest management (IPM). To ensure the effectiveness of IPM programs, it is crucial to understand the compatibility between entomopathogenic fungi and the pesticides employed in crop protection. The toxicity of pesticides could reduce the efficacy of EPF as their interaction may affect the growth, sporulation, germination and conidial viability (Beevi and Jacob 1988; Benz, 1987). The adverse effect of insecticides depends upon the fungi, dose of insecticides, its type and active ingredient present in the formulation. Numerous experiments have been conducted with the objective of identifying the adverse effects of pesticides on entomopathogenic fungi and providing valuable insights into the potential consequences of pesticide use on biocontrol agents targeting insects. Coming back to 60s, Urs *et al.* (1967) observed that Lindane (BHC) at 50% WP and malathion exhibited a complete inhibition of the vegetative growth of *M. anisopliae* across all tested concentrations. In contrast, Dimecron stimulated the growth of fungi at all concentrations. Whereas, now a days the insecticides which are very common in farmers such as fipronil, permethrin and imidacloprid showed compatibility with *M. anisopliae* except fipronil at higher concentration which was moderately toxic to fungus (Schumacher and Poehling 2012). Compatibility of these EPFs has also been recorded with Spinosad, deltamethrin (Derakhshan Shadmehri *et al.,* 2016); indoxacarb (Khun *et al.,* 2021). These studies are necessary to determine the effect of

insecticides on these entomopathogenic fungus which suggests the suitability of the combination for IPM program. The present study was therefore aimed to see the effect of commonly used insecticides on entomopathogenic fungus, *M. anisopliae.*

MATERIAL AND METHODS

Description of experimental area. The present study was conducted in Insecticides toxicology laboratory, Department of Entomology, College of Agriculture G.B. Pant University of Agriculture and Technology, Pantnagar, U.S. Nagar, Uttarakhand, India located at an altitude of 243.84 meter above mean sea level (MSL), 29 °N latitude and 79.3° E longitude.

Test insecticides. Two insecticides Spinetoram 11.7SC (Delegate) and Novaluron 5.25 + Indoxacarb 4.5SC (Plethora) with their five concentration each were taken for the experiment. Concentration 0.25X, 0.5X, X, 2X and 4X, and concentrations 0.25X, 0.5X, X, 2X and 3Xof Spinetoram and Novaluron + Indoxacarb, respectively were prepared and tested against the entomopathogenic fungi. Dilution of 30ml of each concentration was prepared inside Laminar Air Flow (LAF) using Pearson square method. These dilutions then were kept in 100 ml autoclaved conical flask.

Test Entomopathogenic fungi: Locally available commercial product of *M. anisopliae* sold as Kalichakra 1WP (1 × 10^8 CFU/gm) was used to conduct the experiment. To obtain pure culture of fungus, larvae of *Galleria mellonella* was exposed to the commercial product. After development of mycelium on the body of larvae it was inoculated to the petri plates filled with the most basic medium *viz.*, single standard Potato Dextrose Agar (ssPDA) for the fungal culture refinement under LAF and these petri plates was then incubated at 28±2°C. The fungus was further sub cultured for experiment purpose and fully colonized mycelium was taken for the same.

Preparation of Experiment: Poisoned food technique (Schmitz, 1930) was used to test the compatibility of EPF with different concentration of insecticides. The experiment contains 5 treatments of each insecticides including 1 control with 4 replications each. A double-strength (ds) PDA medium was prepared, in this medium, as it is considered to be best growing media for the maximum growth and Sporulation (Guroo *et al.*, 2021). All ingredients except water was doubled to that of the normal recommendation (Table 1). This dsPDA was fortified with different concentration of spinetoram and novaluron + indoxacarb by mixing both in 100 ml conical flask at 1:1 ratio (30ml DsPDA and 30 ml insecticide) and control's dsPDA was fortified with autoclaved distilled water. The fully colonized petri plate of *M. anisopliae* was used to cut the bits/discs of 5mm diameter by a cork borer and then were inoculated to the petri plates filled with fortified dsPDA. Petri plates were then incubated at 28±2°C. Observation on radial growth was recorded when petri plates in control were fully covered with mycelium. Percent inhibition was calculated using the following formula given by Agarwal *et al.* (2001).

$$I\% = \frac{(C-d)-(T-d)}{C-b} \times 100$$

Where, I is inhibition pe cent of mycelium, C is the radial growth of mycelium in control (mm), T represents radial growth of mycelium in treatment (mm) and d is the diameter of bit/disc taken for inoculation. A standard sample colony area in relation to all colony area was chosen for spore production quantification at 15 days after inoculation (DAI). Each disk was placed in a glass tube and the spores were suspended in 10 ml of water containing 0.02% Tween 20 and quantified using a Neubauer chamber. The determination of the compatibility between each concentration of insecticide and the EPF was assessed based on the T-value, a metric introduced by Alves *et al.* (1998). This evaluation involved considering parameters such as percent growth inhibition and the impact on the fungus's spore-forming ability, as outlined in the formula

$$T = \frac{20(CV) \times 80(ESP)}{100}$$

The T value signifies the adjusted value used for product classification, where CV stands for the percentage of vegetative growth in the treatment compared to the control, and ESP represents the percentage of sporulation in the treatment relative to the control. T values are categorized based on a predefined scale (Table 2).

Table 1: Composition of culture medium.

Sr. No.	Requirement	Quantity (g/ml)	
		ssPDA	dsPDA
1.	Peeled potatoes	200g	400g
2.	Agar	20g	40g
3.	Dextrose	20g	40g
4.	Distilled water	1000ml	1000ml

Table 2: Classification of compatibility according to T value.

T value	Classification
0-30	Very Toxic
21-45	Toxic
46-60	Moderately Toxic
61-90	Compatible
90-100	Very Compatible

Statistical analysis: Analyses of variance were performed using ANOVA, and a Duncan's Multiple Range test (DMRT) (Duncan, 1955) with a confidence level of 95% (p = 0.05) was used as Post hoc to compare the means using SPSS.

RESULTS AND DISCUSSION

Table 3 provides a comprehensive overview of the impact of five different spinetoram concentrations (ranging from 0.0025% to 0.04%) on the growth and development of *M. anisopliae*. Notably, the lowest concentration tested (0.0025%) resulted in a significant radial growth of *M. anisopliae* mycelium, measuring 52.00mm, accompanied by a notable 44.21% inhibition compared to the control. Moreover, the spore production at this concentration was recorded at 64.81 × 10^4 spores/ml. The response of *M. anisopliae* at the

0.0025% concentration suggests a high level of compatibility with spinetoram, as indicated by a T value of 70.65. However, as the concentration of spinetoram increased, different levels of toxicity were observed. Specifically, at 0.005% and 0.01%, spinetoram was classified as moderately toxic (T value = 56.11) and toxic (T value = 31.31) to *M. anisopliae*, respectively. At these concentrations, the radial growth of the entomopathogenic fungus was reduced to 47.50mm and 41.50mm, with corresponding spore productions of 49.94×10^4 and 31.31×10^4 spores/ml. The concentrations of 0.02% and 0.04% proved to be highly toxic to *M. anisopliae*, with recorded T values of 20.58 and 13.54, respectively. These concentrations led to a substantial decrease in radial growth, indicating a severe inhibitory effect on the fungus. These detailed results underscore the concentration-dependent nature of spinetoram's impact on *M. anisopliae*, with implications for its practical use in pest management strategies.

However, the investigation into the impact of different concentrations of novaluron + indoxacarb on *M. anisopliae* reveals intriguing insights into the inhibitory effects on the fungal mycelium and spore production. At the modest concentration of 0.005%, the application of novaluron + indoxacarb induced a radial growth of *M. anisopliae* mycelium measuring 48.00mm. This concentration demonstrated a significant inhibition of 48.94% compared to the control. The observed inhibitory effect at this concentration indicates a potent ability of the combination to restrict the growth of *M. anisopliae* mycelium. As the concentrations of novaluron + indoxacarb increased to 0.01%, 0.02%, 0.04%, and 0.06%, a notable trend emerged. These concentrations exhibited even higher levels of inhibition, surpassing more than 60% in radial mycelium growth compared to the control. This escalating inhibitory trend suggests a dose-dependent relationship, with higher concentrations intensifying the suppressive impact on *M. anisopliae* mycelial expansion. In addition to the inhibitory effects on radial growth, spore counts at these increased concentrations displayed a noteworthy reduction compared to the control. This reduction in spore production further supports the overall inhibitory nature of the novaluron + indoxacarb combination on the reproductive capacity of *M. anisopliae*. An important finding is the classification of all concentrations, including 0.005%, as highly toxic to *M. anisopliae*. This categorization underscores the potency of the novaluron + indoxacarb in adversely affecting the entomopathogenic fungus across various concentrations. The designation of "highly toxic" implies a substantial hindrance to the vitality and functionality of *M. anisopliae*, which has implications for its potential use in pest management strategies.

The results of the study indicate that spinetoram, especially at lower concentrations, exhibits compatibility with *M. anisopliae*. This observation aligns with findings from a study conducted by Khun *et al.* (2021), focused on evaluating the effects of spinetoram on the germination, mycelial growth, and sporulation of *M. anisopliae in vitro*. In their study, spinetoram showed positive effects on the fungus, suggesting a harmonious interaction between the insecticide and *M. anisopliae*. Interestingly, the response of *M. anisopliae* to indoxacarb was found to be encouraging in the study by, presenting a favorable outcome.

Table 3: Compatibility of *Metarhizium anisopliae* with different concentration of Spinetoram 11.7SC (Delegate) and Novaluron 5.25 + Indoxacarb 4.5SC (Plethora).

Insecticide	Concentration (%)	Radial Growth (mm)**	Inhibition (%)	Spores/ml (x 10^4) 15 DAI	T value	Classification***
Spinetoram 11.7SC (Delegate)	0.0025	52.00 ± 0.408^{e}	44.21	64.81 ± 1.569^{b}	70.65	C
	0.005	47.50 ± 0.645^{d}	49.55	49.94 ± 1.363^{c}	56.11	MT
	0.01*	41.50 ± 0.500^{c}	56.67	31.31 ± 1.067^{d}	37.80	T
	0.02	38.50 ± 0.500^{b}	60.24	13.13 ± 0.298^{e}	20.58	VT
	0.04	34.00 ± 0.707^{a}	65.57	6.50 ± 1.005^{f}	13.54	VT
	Control	89.25 ± 0.479^{f}	0.00	87.88 ± 3.244^{a}	-	-
	SEM±	1.208		1.687		
	CD @ 5%	1.633		5.052		
	CV	2.179		7.986		
Novaluron 5.25 + Indoxacarb 4.5SC (Plethora)	0.005	48.00 ± 0.408^{b}	48.94	5.31 ± 0.695^{b}	16.24	VT
	0.01	35.00 ± 0.408^{c}	64.38	3.36 ± 0.217^{bc}	11.31	VT
	0.02*	34.25 ± 0.250^{c}	65.27	2.5 ± 0.433^{bc}	10.26	VT
	0.04	23.25 ± 1.109^{d}	78.30	1.63 ± 0.582^{bc}	6.89	VT
	0.06	17.75 ± 0.479^{e}	84.87	0.38 ± 0.072^{c}	4.37	VT
	Control	89.25 ± 0.750^{a}	0.00	77.5 ± 2.953^{a}	-	
	SEM±	0.635		1.288		
	CD @ 5%	1.900		3.856		
	CV	3.077		16.820		

*Concentration based on the literature available (Spinetoram= 0.25X, 0.5X, X, 2X and 4X; Novaluron + Indoxacarb= 0.25X, 0.5X, X, 2X and 3X); DAI= days after inoculation; Means (± SEM) followed by the same letters are at par; **Observations on radial growth were recorded once control plate was fully covered with mycelium of *Metarhizium anisopliae*; *** Toxicity classification, C= Compatible, T= Toxic, MT= Moderately Toxic and VT= Very Toxic

However, in contrast to these findings, the current study reveals that novaluron+ indoxacarb at various concentrations, was observed very toxic to fungus. This discrepancy in results underscores the complex and context-specific nature of the interactions between different pesticides and biocontrol agents. The observed disparities between the current study and previous research outcomes could be attributed to several factors. One key factor is the inherent variability in fungal isolates under examination. Different strains of *M. anisopliae* may respond differently to various pesticides. Additionally, variations in the formulation types and concentrations of active ingredients used in each study can significantly influence the outcomes. The differences in experimental conditions and methodologies may also contribute to the divergent findings. Comparisons with the insecticide spinosad, which belongs to the same chemical group as spinetoram, offer valuable insights. In a study by Derakhshan Shadmehri *et al.* (2016), spinosad demonstrated favorable results, showcasing good mycelium growth and spore germination that were significantly similar to the control, even at double concentration. This implies that spinosad, exhibited compatibility with the fungus as spinetoram do.

CONCLUSIONS

Spinetoram was found to be less toxic to *M. anisopliae* at lower concentration and the toxicity increased with the increase in concentration as T value was decreasing. At lower concentrations not more than 50% of inhibition was found. However, another insecticide novaluron + indoxacarb despite of having a significant difference in radial growth of mycelium and less than 50% inhibition at lower concentration, was found to be very toxic at each concentration as it recorded significantly smaller number of spores.

FUTURE SCOPE

Use of insecticides alone may cause several impacts such as development of resistance, residual effect in soil and produce, harmful effect on environment etc. Mixing biological agents such as entomopathogenic fungi can reduce these problems as this practice reduce the dose of insecticide used, fungus can be the cause of epizootics and ultimately it adds the volume as well as value to the produce. Hence, the utilization of insecticides is feasible at specific low concentrations, concurrently applied with entomopathogenic fungus for an integrated strategy of insect-pests management. Ongoing investigations are being conducted to explore the impacts of these combinations on target organisms.

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Conflict of Interest. None.

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