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Compatibility of pesticides with *Metarhizium anisopliae*

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Abstract
Metarhizium anisopliae is the most considerable entomopathogenic fungal species. The entomopathogenic fungi are often reported as causing high levels of epizootics in nature and are the most versatile environmentally safe biological control agents. The present investigations were carried out at Biocontrol Laboratory, Agricultural Entomology Section, College of Agriculture, Dhule during Jan 2018 to Dec 2018. The pure culture of *Metarhizium anisopliae* was maintained at Agricultural Entomology Section, College of Agriculture, Dhule on PDA slants. The compatibility of nineteen pesticides were evaluated by employing 'Poisoned food technique' and their effect on growth characteristic of *Metarhizium anisopliae* was studied. All the insecticidal treatments were recorded reduction in vegetative growth, of *M. anisopliae.* Among all the tested insecticide, the treatment with azadirachtin recorded the highest vegetative growth of *M. anisopliae* followed by flonicamid, imidacloprid and flubendamid indicating their compatibility with *Metarhizium anisopliae.* Among the tested fungicidal carbendazim and propiconazole did not show any vegetative growth of *Metarhizium anisopliae*, while fungicide treatment with copper oxychloride showed least vegetative growth of *Metarhizium anisopliae*. The rest of the insecticidal treatments in descending order are Azadirachtin 3000 ppm -Flonocamid 50% WG - Spiromesifan 22.9%SC - Imidaclopride 17.8%SL - Flubendamide 39.35%SC - Clorantraniliprole 18.5% SL - Deltamethrin 2.8% EC - Spinosad 45% SC - Fipronil 5% SC - Emamectin benzoate 5% SG with *Metarhizium anisopliae*.

Keywords: *Metarhizium anisopliae*, compatibility

Introduction
Metarhizium anisopliae, formerly known as *Entomophthora anisopliae* (basionym), is a fungus that grows naturally in soils throughout the world and causes disease in various insects. It is a mitosporic fungus with asexual reproduction, which was formerly classified in the form class Hyphomycetes of the phylum Deuteromycota (also often called Fungi Imperfecti). Many entomopathogenic fungi especially *Metarhizium anisopliae* are used as biological control agents of insects including gregarious insect pests. But field application of fungi cannot give satisfactory results as pesticides due to many abiotic and biotic factors. Entomopathogenic fungi are important as natural control agents of many insects, including several pests [2]. In Integrated Pest Management (IPM), biological control with entomopathogens should be considered as an important reduction factor in pest population density. So, the conservation of entomopathogens is needed if they occur naturally, are applied or introduced with the objective of controlling pests. However, the use of incompatible pesticides may inhibit the development and reproduction of these pathogens, affecting IPM [1, 3 and 5]. On the other hand, the use of selective pesticides is an important strategy in IPM programs. In some cases, compatible products may be associated with entomopathogenic fungi, increasing the control efficiency, decreasing the amount of insecticides required and minimizing the risks of environmental contamination and pests resistance expression [6 and 9]. The Farmers and the officers working in the Department of Agriculture are always asking regarding the compatibility of *M. anisopliae* with the insecticides and fungicides available in the market. The use of entomopathogenic fungi in integrated pest management (IPM) cannot be ignored. A number of examples exist where application of different selective chemical insecticides and fungi when used in combination, provided satisfactory control against many agricultural insect pests.

Material and Methods

The study was conducted under laboratory condition at Agricultural Entomology Section, College of Agriculture, Dhule during January, 2018 to December, 2018 in completely randomized design with three replications. The pure cultures of *M. anisopliae* was collected from Bio-agent production laboratory of Agricultural Entomology Section, College of Agriculture, Dhule and maintained on PDA slants and Petri plates. The fungi was cultured on PDA medium autoclaved at 121^0C (15 Psi) for 15-20 min and poured in sterilized petri plates. Total sixteen insecticides and three fungicides were evaluated by employing 'Poisoned food technique' and their effects on growth characteristics of Metarhizium anisopliae were studied.

Statistical analysis was carried out by analyzing the available data in Completely Randomized Design (CRD) and the data was subjected to arcsin transformation, prior to analysis.

Results and Discussion

The data on effect of pesticides on mycelial growth and growth inhibition of *M. anisopliae* at 3, 5 and 7 days after inoculation revealed that all the treatments were showed significant difference for mycelial growth and per cent growth inhibition of the fungus. The untreated control i.e. culture plate without pesticides recorded significantly highest mycelial growth of 34.33, 59.50 and 80.00 mm at 3, 5 and 7 days after inoculation.

Among the pesticides tested for their compatibility with *M. anisopliae* at 3 days after inoculation, the highest (27.33mm) mycelial growth was observed in flubendamide 39.35% SC and the next superior treatment for their compatibility was spinosad 45% EC (25.16mm) and was at par with deltamethrin 2.8% EC (24.16mm) and flonicamid 50% WG (24.16mm).

At 5 days after inoculation among the various pesticides tested for their compatibility with *Metarhizium anisopliae* the highest (57.66mm) mycelial growth was observed in azadirachtin 3000 ppm and the next superior treatment for their compatibility was spinosad 45% EC (48mm) and was at par with flonicamid 50% WG (47.33mm) and chlorantraniliprole 18.5% SC (46.50mm).

7 day's after inoculation among the various pesticides tested for their compatibility with *Metarhizium anisopliae* the highest (79mm) mycelial growth was observed in azadirachtin 3000 ppm and was at par with flonicamid 50% WG (78mm) treatment.

The results on compatibility of pesticides with *M. anisopliae* clearly indicates that the pesticides having better compatibility with *M. anisopliae* as having maximum mycelial growth in descending order are azadirachtin 3000 ppm - flonicamid 50% WG - spiromesifen 22.9% SC - imidaclopride 17.8% SL - flubendamide 39.35% SC - clorantraniliprole 18.5% SL - deltamethrin 2.8% EC - spinosad 45% SC - fipronil 5% SC - emamectin benzoate 5% SG. While no mycelial growth was observed with fungicides carbendazim 50% WP and propiconazole 25% EC.

At 3 days after inoculation, data on effect of per cent growth inhibition of fungus *M. anisopliae* revealed the significant difference for growth inhibition. The treatment with flubendamide 39.35% SC recorded significantly minimum of 21.90% growth inhibition of *M. anisopliae* and showed its superiority over rest of the treatments, the next effective treatment for growth inhibition of *M. anisopliae* were spinosad 45% SC(21.11%), deltamethrin 2.8% EC (30.94%), flonicamid 50% (30.92%), imidacloprid 17.8% SL (33.31%), chlorantraniliprole 18.5% SC (34.24%) and spiromesifen 22.9% SC (36.20%).

At 5 days after inoculation, the growth inhibition was minimum in the insecticidal treatment azadirachtin 3000 ppm (3.07%) and was significantly superior over the remaining treatments which indicate more compatibility with *M. anisopliae.*

At 7 days after inoculation, considering per cent reduction in growth of the mycoagent by various insecticides over untreated control, azadirachtin 3000 ppm emerged as most compatible pesticide with *M. anisopliae* as it showed least (1.25%) average reduction in the growth and then flonicamid 50% WG (2.50%).

Among the insecticides and fungicides tested for the compatibility study, the data revealed that the fungicides viz., carbendazim 50%WP and propiconazole 25% EC recorded the highest growth inhibition of 93.75% at 7 days after inoculation. The mycelial growth of *M. anisopliae* in both the fungicides were not recorded at 3, 5and 7 days after inoculation. These results indicated the incompatibility of these fungicides with *M. anisopliae.*

The present finding of the vegetative growth rate of *M. anisopliae* are in confirmation with compatibility tests of insecticides and entomopathogenic fungi *viz*., *Beauveria bassiana* and imidachloprid under *in vitro* condition to find out safer insecticides [4]. Results revealed that among the insecticides tested, imidachloprid (0.005%) was highly safe and most compatible to these entomopathogenic fungi. In present findings also imidachloprid showed better compatibility with *M.anisopliae.*

The compatibility of the *Metarhizium anisopliae* (Metschnikoff) Sorokin isolate ICIPE 69, with insecticides (thiamethoxam and imidacloprid) used in French bean production [8]. Results revealed that the insecticide imidacloprid was highly compatible with *M. anisopliae* and thiamethoxam.

The effects of different concentrations of fipronil and imidacloprid as potential candidates for combined applications on two strains of the entomopathogenic fungus *Metarhizium anisopliae* (MA) [11]. According to a physiology parameter compatibility classification the results showed that both insecticides (fipronil and imidacloprid) were compatible with both tested MA strains and are in confirmation with present findings.

In vitro toxicity of insecticides on the vegetative growth of *Metarhizium anisopliae* (strain CG 168) [12]. The insecticides exhibited the least degree of toxicity to this fungal pathogen. The agrochemicals compatible with *M. anisopliae* were the insecticides thiamethoxam and lambda-cyhalothrin.

The compatibility of *Metarhizium anisopliae* tested with insecticides and fungicides. Three concentrations (0.1X, 0.5X and 1X) of each chemical were evaluated in the laboratory based on the recommended dose for field application by food poison technique. M19 and M48 isolates showed compatibility with imidacloprid at 0.5X and 0.1X while, M19 displayed an enhancement in the vegetative growth with imidacloprid (2%) [7].

The entomopathogenic fungi like *Beauveria, Metarhizium, Nomuraea and Fusarium* spp. are isolated from naturally infected rice leaf folder (LF) larvae (*Cnaphalocrocis medinalis* Guenee). As the insecticide imidacloprid is commonly used at 0.02% a.i. to control the LF and other insect pests of rice, their interactions with the pathogens were

assessed to reveal the compatible combinations. They reported that the growth of *Beauveria*, *Metarhizium* and *Fusarium* spp. were not affected by the recommended field (FR) doses of the pesticides [10]. Many newer insecticides have been introduced in the market and the sufficient literature is not available on these new compounds hence the efforts have been made to know the compatibility of these insecticides with *M. anisopliae*.

Table 1: Compatibility of pesticides with *M. anisopliae* at 3rd, 5th and 7th day of inoculation

Sr. No.	Treatment	Dose (gm/ml)/lit	Mycelial growth (mm) Mean			Growth Inhibition (%) Mean		
			3rd day	5thday	7thday	3rd day	5thday	7thday
1	Acetamiprid 20% SP	0.20	18.00	30.66	44	48.58 (44.18)*	48.43 (44.10)*	45.00 (42.12)*
2	Diafenthiuron 50% WP	1.20	18.83	26.00	32	46.16 (42.79)	56.54 (48.76)	60.10 (50.83)
3	Deltamethrin 2.8% EC	0.89	24.16	36.33	68	30.94 (33.79)	35.85 (36.72)	15.00 (22.78)
4	Thiamethoxam 25%WG	0.20	21.00	29.16	43	40.01 (39.23)	50.96 (45.55)	46.25 (42.84)
5	Imidacloprid 17.8% SL	0.23	23.33	36.16	72	33.31 (35.24)	39.20 (38.76)	10.00 (18.41)
6	Fipronil 5% SC	3.00	21.33	35.00	52	39.02 (38.62)	41.18 (39.91)	35.00 (36.26)
7	Spiromesifen 22.9% SC	0.84	22.33	37.50	75	36.20 (36.98)	36.96 (37.44)	6.25 (14.43)
8	Triazophos 40% EC	2.00	21.00	32.66	48	40.05 (39.22)	45.10 (42.18)	40.00 (39.22)
9	Flonicamid 50% WG	0.30	24.16	47.33	78	30.92 (33.75)	20.45 (26.84)	2.50 (8.89)
10	Lambda cyhalothrin 5% EC	1.00	16.83	30.33	45	51.88 (40.08)	49.01 (44.43)	43.75 (41.40)
11	Flupyridifurone 17.09% SL	0.20	17.55	33.16	45	49.97 (44.98)	44.24 (41.69)	43.75 (41.40)
12	Chlorantraniliprole 18.5% SC	0.32	23.00	46.50	68.66	34.24 (35.79)	21.84 (27.86)	14.25 (21.98)
13	Spinosad 45% SC	0.44	25.16	48.00	62	28.11 (32.00)	19.31 (26.04)	22.50 (28.30)
14	Flubendamide 39.35% SC	0.30	27.33	45.00	71	21.90 (27.89)	24.35 (29.52)	11.25 (19.54)
15	Emamectin benzoate 5% SG	0.40	19.83	33.66	51	43.28 (41.13)	43.41 (41.21)	36.25 (37.01)
16	Azadirachtin 3000 ppm	4.00	21.50	57.66	79	38.54 (38.37)	3.07 (9.84)	1.25 (6.41)
17	Carbendazim 50% WP	0.20	5	5	5	85.42 (67.55)	91.59 (73.14)	93.75 (75.52)
18	Copper oxychloride 50 WP	2.50	18.33	35.00	42	47.60 (43.62)	41.15 (39.88)	47.50 (43.56)
19	Propiconazole 25% EC	1.00	5	5	5	85.42 (67.55)	91.59 (73.14)	93.75 (75.52)
20	Untreated control	-	34.33	59.50	80	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)
	S.E.±		0.56	0.79	0.89	0.95	1.59	0.82
	CD at 5%		1.60	2.28	2.56	2.71	4.56	2.36

Note:* Figures in parenthesis are arc sin transformed values.

Conclusion

All the insecticidal treatments showed significant variation in vegetative growth of *Metarhizium anisopliae*. Among all the tested insecticide, the treatment with Azadirachtin recorded the highest vegetative growth of *Metarhizium anisopliae* followed by flonicamid, imidacloprid, and flubendamid. While insecticidal treatment with diafenthiuron showed the least vegetative growth of *Metarhizium anisopliae.* Among the tested fungicides carbendazim and propiconazole showed no vegetative growth of *Metarhizium anisopliae*, while fungicide treatment with copper oxychloride showed least vegetative growth of *Metarhizium anisopliae*. All the rest of insecticidal treatments showed moderate compatibility with *Metarhizium anisopliae.* Researchers should conduct the compatibility test for other commercial pesticides.

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