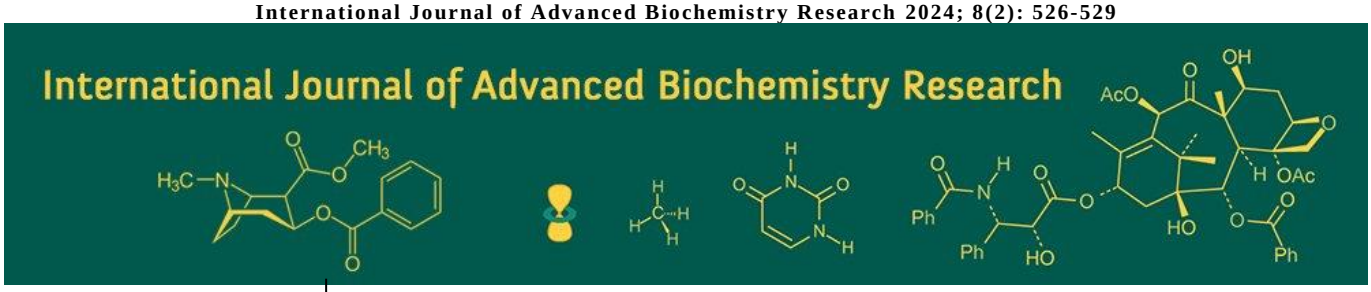

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Effect of chemical pesticides on growth characteristics of *Metarhizium anisopliae* (Metschnikoff) Sorokin

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Abstract
The outcomes on compatibility of different chemical pesticides with *M. anisopliae* clearly indicate that the highest mycelial growth (75.17 mm) was observed in flonicamid 50% WG followed by imidacloprid 17.8% SL (70.50 mm). The minimum (16.48 percent) growth inhibition of *M. anisopliae* was observed in pesticidal treatment with flonicamid 50% WG which indicates its better compatibility with *M. anisopliae*. Among chemical pesticides tested for their compatibility, the minimum percent growth inhibition was observed in flonicamid 50% WG (16.48 percent) followed by imidacloprid 17.8 SL (21.67 percent), deltamethrin 2.5% EC (26.30 percent) and chlorantraniliprole 18.5% SC (27.78 percent) indicating better compatibility with *M. anisopliae* than dimethoate 30% EC (69.07 percent), clothanidin 25% WG (54.81 percent) and difenthiuron 50% WP (51.30 percent) in which more than 50 percent growth inhibition was observed. Among fungicides, all the three fungicides propiconazole 25% EC (94.44 percent), difenoconazole 25% EC (77.22 percent) and copper oxychloride 50% WP (56.85 percent) indicating that they are not compatible with *M. anisopliae*.

Keywords: Compatibility, mycelial growth, pesticides, growth inhibition, fungicide etc

Introduction
In order to conserve the ecofriendly biological microorganisms, biological control agents in nature, particularly within agricultural and horticultural ecosystems, must be protected from a wide range of harmful pesticides. As a result, determining their compatibility and interaction with pesticides, which is a key component of IPM programmes, is critical. *In vitro* studies have shown that various pesticides have selective effect on entomofungal infections, according to several researchers (Alves, 1986; Silva *et al*., 1993) [2, 13]. To increase insect mortality, a compatible admixture of insecticides at sub-lethal concentrations and entomopathogenic fungus can operate synergistically. This is especially advantageous since it lowers the insecticide application dose, reduces environmental contamination and lowers the risk of resistance. To provide safe and effective control of insect pests, several compatible insecticides with entomopathogenic fungi and other biocontrol agents are utilized in various combinations (Asi *et al.*, 2010; Bitsadze *et al.*, 2013) [3, 4].
Insecticides have a negative impact on non-target predatory organisms in nature, including EPFs like *B. bassiana* and *M. anisopliae*, which are entomopathogenic fungi. However, there are interactions between insecticides and entomopathogens. Insecticides at low doses combined with an entomopathogenic fungus can work together to increase insect pest mortality. This combination is especially beneficial since it reduces the amount of insecticide used, reduces environmental contamination and reduces pest resistance (Abidin *et al*., 2017) [1].

Materials and Methods
The investigation on compatibility of *Metarhizium anisopliae* with botanical, biorational and chemical pesticides were carried out in Biocontrol laboratory, Department of Agriculture Entomology, Post Graduate Institute, MPKV, Rahuri, during 2020-2021.

Poison food technique
Standard poison food technique was followed to assay the effect of botanical, biorational and chemical pesticides on *M. anisopliae*.

Quantity of pesticide required

The amount of toxicant (i.e. actual ingredient) in required quantity of PDA was calculated with the help of following formula,

$$\text{Amount of pesticide required} = \frac{\text{Percent of solution desired} \times \text{Quantity of solution required}}{\text{Strength of the formulation available}}$$

The insecticide doses were calculated for field application rate based on 500 litres/ha or with high volume sprayers. The pesticides were evaluated by poisoned food technique. Requisite quantity of chemical pesticides (Table 1) were added to the PDA medium in flask before solidification (medium temperature 46-48 °C) to get desired concentration and mixed thoroughly. Then poured equally into the three petriplates and kept in laminar air flow. The medium was aseptically allowed to solidify under laminar air flow cabinet. After complete cooling of the PDA medium in petriplates the culture of *M. anisopliae* were inoculated under aseptic condition. Mycelial mat was cut with sterile cork borer (5 mm diameter) from 10 days old culture of *M. anisopliae* and placed aseptically in the centre of petriplates containing the poisoned media. Suitable check without poison was kept for comparison under same condition. Fungal colony diameter was measured at 3rd, 5th and 7th days after inoculation and compared with standard check to measure the degree of toxicity of different pesticides used in study. Inhibition of colony growth over untreated check was worked out for respective pesticides.

Vegetative growth of *M. anisopliae*

Radial mycelial growth of the fungus was measured after 3rd, 5th and 7th days after inoculation and compared with untreated control. The percent reduction in radial growth was calculated by using formula,

$$R = C\text{-}T/C \times 100$$

Where,
R - Percent reduction of radial growth
C - Radial growth of fungi grown on untreated medium
T - Radial growth of fungi grown on pesticide treated medium

The experiments were carried out under laboratory condition in completely randomized design during the year 2020-21 with 3 replications & 11 treatments of chemical pesticides. The data so obtained was analyses by standard statistical procedures.

Table 1: Details of the chemical pesticides used to study compatibility of *M. anisopliae*

Sr. No	Active Ingredient	Dose/liter	Dose (ml/g)/ha	
			a.i.(g)	g/ml
T_1	Dimethoate 30% EC	2.0 ml/l	300	1000
T_2	Imidacloprid 17.8% SL	0.25 ml/l	22.25	125
T_3	Deltamethrin 2.5% SC	1.5 ml/l	18.75	750
T_4	Difenthiuron 50% WP	1.2 g/l	300	600
T_5	Flonicamid 50% WG	0.2 g/l	50	100
T_6	Clothanidin 25% WDG	0.1 g/l	12.50	50
T_7	Chlorantraniliprole 18.5% SC	0.3 ml/l	27.75	150
T_8	Copper Oxychloride 50% WP	2.5 g/l	625	1250
T_9	Propiconazole 25% EC	1.5 ml/l	187.5	750
T_{10}	Difenoconazole 25% EC	1.0 ml/l	125	500
T_{11}	Untreated check	-	-	-

Result and Discussion

At 3rd day after inoculation, the data on the impact of chemical pesticides on mean mycelial growth and percent growth inhibition of *M. anisopliae* revealed that the untreated control had the maximum (52.00 mm) mycelial growth and was considerably superior to the other treatments. Among the various pesticides tested for their compatibility with *M. anisopliae* the highest (33.33 mm) mycelial growth was observed in imidacloprid 17.8% SL and was significantly superior over rest of the pesticides. The next superior treatment for their compatibility was flonicamid 50% WG with mean mycelial growth 30.83 mm.

The fungicide propiconazole 25% EC was not found compatible with *M. anisopliae*, as no mycelial growth was observed at 3rd day after inoculation and it shows maximum 90.38% growth inhibition of *M. anisopliae*. The minimum (35.83%) growth inhibition of *M. anisopliae* was observed in pesticide treatment with imidacloprid 17.8% SL and was significantly superior over rest of the pesticides which indicates its better compatibility with *M. anisopliae*. The next superior treatment in percent growth inhibition was flonicamid 50% WG (40.67%) (Table 2).

The Data on effect of pesticides on mycelial growth and growth inhibition of *M. anisopliae* at 5th day after inoculation revealed that the highest (74.50 mm) mycelial growth was observed in untreated check and was significantly superior over the rest of the pesticidal treatments. Among the various pesticides tested for their compatibility with *M. anisopliae* the highest (42.33 mm) mycelial growth was observed in flonicamid 50% WG and was at par with imidacloprid 17.8% SL (41.50 mm). The fungicide propiconazole 25% EC was not found compatible with *M. anisopliae*, as no mycelial growth was observed at 5th day after inoculation and it shows maximum 93.29% growth inhibition of *M. anisopliae*. The minimum (43.14%) growth inhibition of *M. anisopliae* was observed in pesticide treatment with flonicamid 50% WG and was at par with imidacloprid 17.8% SL (44.26%) and chlorantraniliprole 18.5 SC (46.95%) which indicates their better compatibility with *M. anisopliae* (Table 2).

Table 2: Effect of various pesticides on vegetative growth and growth inhibition of *M. anisopliae*

Treatment	Dose		3 DAI		5 DAI		7 DAI	
	(gm/ml)/ ha	(gm/ml)/ Lit.	Mean mycelial growth (mm)	Mean growth inhibition (%)	Mean mycelial growth (mm)	Mean growth inhibition (%)	Mean mycelial growth (mm)	Mean growth inhibition (%)
Dimethoate 30% EC	1000	2.0	20.00 (4.53)*	61.54 (51.67)**	23.67 (4.92)*	68.24 (55.69)**	27.83 (5.32)*	69.07 (56.21)**
Imidacloprid 17.8% SL	125	0.25	33.33 (5.82)	35.83 (36.74)	41.50 (6.48)	44.26 (41.70)	70.50 (8.42)	21.67 (27.70)
Deltamethrin 2.5% SC	750	1.5	23.67 (4.92)	54.49 (47.58)	34.67 (5.93)	53.47 (46.99)	66.33 (8.17)	26.30 (30.82)
Difenthiuron 50% WP	600	1.2	25.83 (5.13)	50.27 (45.16)	32.17 (5.71)	56.77 (48.89)	43.83 (6.66)	51.30 (45.74)
Flonicamid 50% WG	100	0.2	30.83 (5.60)	40.67 (39.62)	42.33 (6.54)	43.14 (41.05)	75.17 (8.70)	16.48 (23.94)
Clothanidin 25% WDG	50	0.1	17.17 (4.20)	67.02 (54.95)	30.33 (5.55)	59.30 (50.36)	40.67 (6.42)	54.81 (47.76)
Chlorantraniliprole 18.5% SC	150	0.3	26.83 (5.23)	48.39 (44.08)	39.50 (6.32)	46.95 (43.25)	65.00 (8.09)	27.78 (31.78)
Copper Oxychloride 50% WP	1250	2.5	16.00 (4.06)	69.25 (56.33)	31.67 (5.67)	57.42 (49.28)	38.83 (6.27)	56.85 (48.94)
Propiconazole 25% EC	750	1.5	5.00 (2.35)	90.38 (71.93)	5.00 (2.35)	93.29 (74.98)	5.00 (2.35)	94.44 (76.37)
Difenoconazole 25% EC	500	1.0	9.17 (3.11)	82.39 (65.19)	15.83 (4.04)	78.74 (62.54)	20.50 (4.58)	77.22 (61.50)
Untreated check	--	-	52.00 (7.25)	0.00 (0.00)	74.50 (8.66)	0.00 (0.00)	90.00 (9.51)	0.00 (0.00)
SE±			0.06	0.65	0.07	0.73	0.07	0.77
CD at 5%			0.17	1.92	0.20	2.16	0.21	2.28

*Figures in parenthesis are √x+0.5 transformed values. **Figures in parenthesis are arc sin transformed values.

The Data on effect of pesticides on mycelial growth and growth inhibition of *M. anisopliae* at 7th day after inoculation revealed that the highest (90.00 mm) mean mycelial growth was observed in untreated check and was significantly superior over the rest of the pesticidal treatments. Among the various pesticides tested for their compatibility with *M. anisopliae* the highest (75.17 mm) mycelial growth was observed in flonicamid 50% WG followed by imidacloprid 17.8% SL (70.50 mm).The fungicide propiconazole 25% EC was not found compatible with *M. anisopliae* as no mycelial growth was observed even at 7th day after inoculation and it shows maximum 94.44% growth inhibition of *M. anisopliae*. The minimum (16.48%) growth inhibition of *M. anisopliae* was observed in insecticidal treatment with flonicamid 50% WG and was significantly superior over rest of the pesticides which indicates its better compatibility with *M. anisopliae*. The next superior treatment in percent growth inhibition was imidacloprid 17.8% SL (21.67%). The results on compatibility of chemical pesticides with *M. anisopliae* clearly indicates that the pesticides having better compatibility with *M. anisopliae* as having maximum mean mycelial growth in descending order are flonicamid 50% WG > imidacloprid 17.8% SL > deltamethrin 2.5% SC > chlorantraniliprole 18.5% SC > difenthiuron 50% WP > clothanidin 25% WDG > copper oxychloride 50% WP > dimethoate 30% EC > difenoconazole 25% EC > propiconazole 25% EC at 7th day after inoculation (Table 2).

The results clearly indicates that among chemical pesticides the minimum percent growth inhibition was observed in flonicamid 50% WG (16.48%) followed by imidacloprid 17.8 SL (21.67%), deltamethrin 2.5% EC (26.30%) and chlorantraniliprole 18.5% SC (27.78%) indicating better compatibility with *M. anisopliae* then dimethoate 30% EC (69.07%), clothanidin 25% WG (54.81%) and difenthiuron 50% WP (51.30%) in which more than 50% growth inhibition was observed. Among fungicides, all the three fungicides propiconazole 25% EC (94.44%), difenoconazole 25% EC (77.22%) and copper oxychloride 50% WP (56.85%) indicating that they are not compatible with *M. anisopliae* (Table 2)

The present finding on compatibility of *M. anisopliae* with chemical pesticides are in corroboration with Sain *et al.* (2019) [12] who studied compatibility with the EPF in comparison to other chemical pesticides such as imidacloprid, fipronil, profenophos and triazophos, *M. anisopliae* with insecticides such as spiromesifen, difenthiuron, buprofezin, pyriproxyfen, and flonicamid were more compatible with EPFs at half doses. The present finding of the vegetative growth rate of *M. anisopliae* are in agreement with Khan *et al.* (2012) [7] who reported that imidacloprid (0.005%) were highly safe and most compatible to these *M. anisopliae*. In present findings also imidacloprid had shown better compatibility with *M. anisopliae*. Niassy *et al.* (2012) [9] who studied the compatibility of the *Metarhizium anisopliae* (Metschnikoff) Sorokin isolate ICIPE 69, with insecticides, *viz*., thiamethoxam and imidacloprid. Results revealed that the insecticide imidacloprid was highly compatible with *Metarhizium anisopliae* are in confirmation with present findings. Filho *et al.* (2001) [5] who studied compatibility of entomopathogenic fungus, *M. anisopliae* with thiamethoxam and imidacloprid. Statistical analysis showed that the reproductive and vegetative growth of *M. anisopliae* was not affected by thiamethoxam and imidacloprid. In present findings also imidacloprid had shown better compatibility with *Metarhizium anisopliae*. In conformation with the findings of Joshi *et al.* (2018) [6] who studied that in-vitro toxicity of four fungicides, *viz*., mancozeb 75% WP, carbendazim 50% WP, propiconazole 25% EC and hexaconazole 5% EC at different concentration for their effect on growth, inhibitory or synergistic effects and spore

germination of *Beauveria bassiana* and *Metarhizium anisopliae* by growing them on insecticides and fungicides treated media. Among all fungicides tested only mancozeb 75% WP proved safe up to some extent at lower concentrations (0.5 and 0.25%) to test fungi with average amount of spore germination, whereas carbendazim 50% WP, hexaconazole 5% EC and propiconazole 25% EC were completely inhibitory in its action at all the concentrations. In present findings all the three fungicides, *viz*., propiconazole 25% EC, difenoconazole 25% EC and copper oxychloride 50% WP completely inhibiting the mycelial growth of *M. anisopliae* revealing that they are not compatible with *M. anisopliae*. Present findings are in corroboration with Khan *et al.* (2012) [7] who mentioned that members of triazole group including tebuconazole, hexaconazole, propiconazole and difenoconazole were not compatible with *B. bassiana* and *M. anisopliae* and caused complete or strong vegetative growth inhibition and spore germination. Present finding are not in agreement with Reddy *et al.* (2018) [11] reported that difenoconazole and tricyclazole fungicides were less toxic to *Metarhizium anisopliae*. The concentration of difenoconazole showed a negative correlation with growth of *M. anisopliae* during 10 days of incubation. A significant negative correlation between the growth of *M. anisopliae* and concentration of tebuconazole had an effect after 10 days of incubation, but it was stronger at 100 and 1000 ppm than difenoconazole, tricyclazole, hexaconazole, propiconazole and myclobutanil. Propiconazole fungicide was less toxic to *Metarhizium anisopliae* but was highly toxic to *Beauveria bassiana* and *Lecanicillium lecanii* @ 1000 and 10,000 ppm treatments implying that triazole fungicides are ineffective against entomopathogenic fungi tested @ 1000 ppm and 10000 ppm. Rachappa *et al.* (2007) [10] who reported that imidacloprid and spinosad were found safe to the fungus by inhibiting only 11.10 and 5.10 percent growth, respectively. Dimethoate was found comparatively less detrimental (30.77 to 33.77% inhibition) to the fungal growth. In general, significantly lesser growth inhibition was noticed in deltamethrin (36.7%). Imidacloprid and spinosad can be mixed with the fungus to get enhanced effect. In present finding, dimethoate 30% EC recorded 69.07% growth inhibition and imidacloprid 17.8% SL was found safe to the *M. anisopliae*. Present findings are not in agreement with Kotwal *et al.* (2012) [8] who observed that *Metarhizium anisopliae* and copper oxychloride found to be most compatible and recorded less percent growth inhibition 28.66, 33.00 and 23.00 percent at three different concentrations.

Conclusion

Flonicamid 50% WG was found to be highly compatible with *M. anisopliae* followed by imidacloprid 17.8% SL.

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