Indian Journal of Agricultural Sciences **80** (3): 265–8, March 2010

Effect of different insecticides, fungicides and herbicides on growth, sporulation and germination of fungi (*Metarhizium anisopliae*)*

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Received: 15 July 2009; Accepted: 29 December 2009

Key words: Fungicide, Germination, Herbicide, Insecticide, *Metarhizium,* Sporulation

Entomopathogenic fungi, like *Beauveria* and *Metarhizium* constitute a suitable component in recent integrated pest management (IPM) strategies which are more or less persistent in every soil type like clay, sand etc. (Vanninen *et al*. 2000). However, pesticides (insecticides, fungicides and herbicides) which are regularly used to manage different harmful pests influence them by affecting their growth, sporulation and germination (Beevi and Jacob 1987). Batista *et al*. (2001) reported that the action of the pesticides on growth and sporulation of the microorganisms varied depending on chemical nature of the products, its concentration and the microbial species. In view of above fact, a laboratory experiment was carried out to test the effect of different pesticides (fungicides, herbicides and insecticides) on growth, sporulation and germination of *M. anisopliae* (Metschnikoff) Sorokin.

An experiment was carried out at the Department of Entomology of the University, Pantnagar to study the effect of different pesticides on growth, sporulation and germination of *M. anisopliae*. Under the pesticidal treatments, some fungicides (wanis, propiconazole, kitazin, validamycin, triadimefan and bitertenol), insecticides (dimethoate, quinalphos, monocrotophos, phosphamidon and malathion) and herbicides (diquat, paraquat, atrazine, butachlor and 2, 4-D) were used for the study. There were 8 concentrations, viz 5 000, 2 500, 1 000, 500, 300, 200, 150 and 100 ppm out of which 500 ppm was recommended dose. A control was kept and each concentration was replicated thrice for each experiment.

The fungus (*M. anisopliae*) was grown on sabouraud dextrose agar (SDA) medium at 25±2°C and 95±5% relative humidity for experimental use. The poison food technique method was used to observe linear growth and conidial germination. Double strength of different pesticides solutions was prepared for further study. Simultaneously double strength of SDA medium was also prepared for each concentration of pesticides (fungicides, insecticides and herbicides). Previously prepared double strength pesticide solutions were mixed in medium cooled to 30–40°C separately, under aseptic condition in laminar flow. About 25 ml of poisoned medium was poured in Petri-dishes and kept for solidification. The solidified medium was inoculated with 5 mm disc of 10-days-old actively growing culture of *M. anisopliae*. The Petri-dishes were incubated at 25±2°C and 95±5% relative humidity for growth of inoculated fungus. The Sabouraud dextrose agar (SDA) medium without pesticides was also poured in Petri-dishes and seeded with 10-days-old actively growing culture of *M. anisopliae* as control for comparison. Observations of each concentration of pesticides were recorded by measuring the radial growth of fungus up to 18 days from the date of inoculation. The mean diameter was calculated of each concentration of pesticides and compared with control to find out per cent inhibition.

The poison food technique method was again used to observe the effect of pesticides on sporulation. Conidia of *M. anisopliae* grown on different pesticide treated medium were harvested separately after 18 days from the date of inoculation. The upper surface of media bearing mycelial growth was washed in laminar flow under aseptic condition from the conical flask using 100 ml of sterilized distilled water having 0.02% Tween 80 as wetting agent. The surface was gently scrubbed with a sterilized infection-loop (Rombach *et al.* 1986). The conidial counts were made with the help of Improved Nuebauer Weber, England haemocytometer (Jones 1962).

For observations of conidial germination, the fungal suspension of 0.1 ml was poured in Petri-dishes having very thin layer of pesticides-treated medium, under aseptic

*Short note

Based on the complete information of MSc thesis submitted to GBPUAT, Pantnagar during 2002

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Table 1 Effect of different insecticides on linear growth, sporulation and germination of *M. anisopliae*

Treatment	Average linear growth (LG) (in mm) spore production (SP) (× 10^5 conidia/ml) and per cent spore germination (SG) at concentration																	
	2 500	1 000		700			500			250			150			100		
	SG	LG	SG	LG	SP	SG	LG	SP	SG	LG	SP	SG	LG	SP	SG	LG	SP	SG
Dimethoate	30.00	0.00	49.67	0.00		63.00	11.67	1.33	72.33	12.00	8.33	78.33	16.00	11.67	80.66	19.67	16.67	88.00
Quinalphos	28.33	0.00	40.67	7.33		44.33	8.66	0.00	48.33	13.33	2.33	71.00	14.33	8.67	79.66	16.33	11.00	85.00
Monocrotophos	37.33	8.33	47.00	9.00		55.00	13.33	1.33	63.00	15.00	5.33	72.67	17.00	13.00	83.00	23.33	25.00	86.67
Phosphamidon	40.33	0.00	56.33	8.00		61.67	10.33	0.00	65.67	13.00	0.67	75.00	14.67	2.67	85.33	20.00	9.00	91.33
Malathion	41.00	0.00	47.00	6.33		56.00	9.00	0.00	61.00	12.33	1.67	69.67	13.00	5.67	81.00	18.67	9.00	87.33
Control	95.67	36.3	95.67	36.3		95.67	36.3	367.0	95.67	36.3	367.0	95.67	36.3	367.0	95.67	36.3	367.0	95.67
CD (*P*=0.05)	1.40	3.93	4.72	4.15		5.13	4.37	0.66	3.93	4.39	1.05	4.25	4.33	3.92	4.61	4.92	4.90	4.49
SEM±	1.20	1.27	1.53	1.34		1.67	1.42	0.21	1.27	1.42	0.33	1.38	1.40	1.24	1.49	1.60	1.55	1.45

Table 2 Effect of different fungicides on linear growth, sporulation and germination of *M. anisopliae*

Treatment	Average linear growth (LG) (in mm), spore production (SP) (× 10^5 conidia/ml) and per cent spore germination (SG) at concentration														
	700 ppm			500 ppm			250 ppm			150 ppm			100 ppm		
	LG	SP	SG	LG	SP	SG	LG	SP	SG	LG	SP	SG	LG	SP	SG
Wanis	6.00			8.67		0.00	10.00	0.00	7.33	13.00	6.33	16.67	15.00	8.67	19.67
Propiconazole	0.00			0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Kitazin	0.00			6.33		0.00	7.62	0.00	7.00	9.33	1.67	13.00	11.00	7.00	24.33
Validamycin	0.00			6.33		4.67	8.67	3.33	11.00	13.33	9.67	16.33	18.00	14.67	24.0
Triadimefan	0.00			6.33		0.00	7.33	0.00	0.00	11.00	0.00	0.00	13.67	0.00	0.00
Bitertenol	0.00			0.00		0.00	8.00	0.00	10.33	8.33	4.67	14.00	10.00	11.33	17.33
Control	38.00			38.00		96.33	38.00	302.0	96.33	38.00	302.0	96.33	38.00	302.0	96.33
CD (*P*=0.05)	1.32			1.52		1.08	1.62	1.10	2.29	2.09	2.54	3.10	1.91	2.71	3.82
SEM±	0.43			0.50		0.36	0.53	0.36	0.76	0.69	0.92	1.02	0.62	0.88	1.25

condition. The Petri-dishes were incubated at 25±2°C and 95±5% relative humidity. Observations were taken after 24 hr as suggested by Walstad *et al*. (1970).

It was found that different pesticides affected the growth, sporulation and germination of test fungus according to their concentration. Higher concentration, i e 5 000 and 2 500 of all the test insecticides completely hampered the mycelial growth of the *M. anisopliae* (Table 1), but the sporulation was recorded at 500 ppm concentration in all the test insecticides. At lowest concentration, i e at 100 ppm concentration, highest growth and sporulation was recorded in monocrotophos-treated medium (25.0×10^5 conidial/ml). However, highest spore germination was recorded in phosphamidon (91.0%)-treated medium which was at par with dimethoate (88.0%) and malathion where spore germination was 88.0 and 87.3%, respectively. Lowest spore germination was recorded in quinalphos-treated medium which produced 85% less germination over the control where germination was 95.6%. Gupta *et al.* (2002) reported that monocrotophos and chlorpyriphos were found moderately tolerant to *M. anisopliae* which showed higher tolerance over *B. bassiana* at 1 000 and 2 000 ppm concentration. However, Rachappa *et al*. (2007) reported that chlorinated hydrocarbons (endosulfan and dicofol) were highly detrimental to the fungus (60.69% inhibition) compared to organophosphates (46.66% inhibition) carbamates (45.45% inhibition). Among the organophosphates, chlorpyriphos found more detrimental by retarding its growth significantly (69.23%) over others, followed by dichlorvos (59.82%) and malathion. Conversely, dimethoate, phosphamidon, profenophos and monocrotophos were found comparatively less detrimental (30.77 to 33.77% inhibition) to the fungal growth. Pachamuthu *et al.* (1999) reported that chlorpyriphos did not affect conidial germination but did adversely affect the growth and sporulation of *M. anisopliae*. Similarly, the colonies cultured on sabouraud dextrose agar media amended with 50 ppm of chlorpyriphos had significantly reduced sporulation compared with the control and no sporulation was observed in colonies cultured on media amended with 500 ppm of chlorpyriphos. Similarly, Mohamed *et al.* (2004) observed that chlorpyriphos was the most toxic organophosphate to mycelial growth and sporulation at all concentrations and malathion and quinalphos were highly toxic to sporulation and germination, respectively.

Table 3 Effect of different herbicides on linear growth, sporulation and germination of *M. anisopliae*

Treatment	Average linear growth (LG) (in mm), spore production (SP) (× 10^5 conidia/ml) and per cent spore germination (SG) at concentration														
	700 ppm			500 ppm			250 ppm			150 ppm			100 ppm		
	LG	SP	SG	LG	SP	SG	LG	SP	SG	LG	SP	SG	LG	SP	SG
Atrazine	0.00			0.00	0.00	0.00	11.33	0.00	7.33	14.00	9.37	16.00	19.33	12.33	25.67
Diquat	0.00			8.00	8.00	0.00	11.33	11.67	10.33	14.00	12.67	17.33	18.33	16.00	28.33
Paraquat	0.00			9.67	0.00	4.33	12.33	10.00	10.33	12.66	11.67	23.00	14.30	15.67	43.00
Butachlor	8.67			9.67	5.00	0.00	10.33	10.33	14.00	12.33	15.00	22.67	14.00	17.00	35.33
2, 4-D	0.00			8.00	6.00	0.00	10.00	12.00	20.00	11.33	15.67	31.00	16.67	17.67	42.67
Control	40.00			40.00	350.0	96.66	40.00	350.0	96.66	40.00	350.0	96.66	40.00	350.0	96.66
CD (*P*=0.05)	1.65			2.81	1.90	1.56	2.61	2.27	1.93	2.41	1.48	1.55	2.97	2.47	2.02
SEM±	1.18			1.23	1.23	0.51	1.49	1.35	0.95	1.34	1.10	1.15	1.61	1.42	1.30

All the test fungicides influenced the growth, sporulation and germination of conidia according to the concentration. There mycilial growth of *M. anisopliae* was recorded at 750 ppm but there was no sporulation and germination at this concentration. The sporulation and germination was recorded at and below the 500 ppm concentration which increased with the decreasing in concentration of fungicides in media. At lowest, 100 ppm, concentration highest growth and sporulation was recoded in validamycin-treated medium (18.0 mm and 14.67×10^5 conidia/ml) which was significantly higher than all the test fungicides while lowest growth and sporulation (11.0 mm and 7.0×10^5 conidia/ml) with highest germination was recorded in kitazin-treated medium (Table 2). No sporulation was recorded in propiconazole and triadimefan-treated medium at all the test concentration. Finding is in support of Rachappa *et. al.* (2007) who has reported that carbendazim, propiconazole, chlorothalonil and hexaconazole were found to be highly detrimental to fungus by retarding the growth, sporulation and germination, totally. Li and Holdom (1994) found complete growth inhibition of *M. anisopliae* in medium treated with field recommended dosage of propiconazole. Present finding is in favour of Gopalkrishnan and Mohan (2000) who have reported that captan, wettable sulphur and triadimefan were comparatively safe to the *M. anisopliae* under laboratory studies.

The growth, germination and sporulation of fungus influenced by the particular herbicide and their concentration. All the test herbicides above 700 ppm concentration inhibited the growth and sporulation of fungus except in butachlors treated medium which had 8.67 mm linear growth. However, germination of spore was recorded below the 500 ppm concentration in all the test herbicides except in paraquat-treated medium where germination was 4.33% at 500 ppm concentration. At lowest concentration (100 ppm) highest growth, sporulation and germination was recorded in atrazine, 2, 4-D and paraquat-treated medium, respectively (Table 3). The spore germination recorded in paraquat-treated medium was significantly higher than atrazine (25.6%), diquat (28.3%) and butachlor (35.3%). Su (1988) reported that paraquat was found to be least toxic to entomogenous fungi, *B. bassiana*. It was also noticed that germination and virulence was not affected by paraquat at 1 200 ppm (Calderon *et al.* 1991). On the basis of spore germination, paraquat was found to be a safe herbicide, for *M. anisopliae*, followed by 2, 4-D. Tang and Hou (1998) reported that there was no significant variation in conidial germination between atrazine, nitrofen, glyphosate and pendimethalin (23.01%), but the toxicity was more than two times of 2, 4-D and butachlor which inhibited 10.26 and 12.39% conidial germination, respectively.

On the basis of present study on growth, sporulation and germination, monocrotophos (insecticide) validamycin (fungicide) and 2, 4-D (herbicide) was found to be safe pesticides at recommended dose (500 ppm) as compared to rest of the test pesticides for *M. anisoplae*.

SUMMARY

An experiment was carried out to study the effect of different pesticides, ie insecticides (dimethoate, quinalphos, monocrotophos, phosphamidon and malathion), fungicides (wanis, propiconazole, kitazin, validamycin, triadimefan and bitertenol) and herbicides (diquat, paraquat, atrazine, butachlor and 2, 4-D) on growth, sporulation and germination of *Metarhizium anisopliae*. There were 8 concentrations, i e 5 000, 2 500, 1 000, 500, 300, 200, 150 and 100 ppm and each with 3 replications. During the study it was found that all the test pesticides influenced the growth, sporulation and germination of fungus according to the concentrations. On the basis of growth, sporulation and germination, monocrotophos (insecticide), validamycin (fungicide) and 2, 4-D (herbicide) was found to be safe herbicides as compared to rest of the test pesticides for *M. anisopliae*.

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