https://doi.org/10.33307/entomon.v45i2.523
ENTOMON 45(2): 143-148 (2020)
Article No. ent. 45208

Tolerance of *Metarhizium anisopliae* Sorokin isolates to selected insecticides and fungicides

Jancy Merlin Johnson, K. B. Deepthy and Mani Chellappan

Department of Agricultural Entomology, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur 680656, Kerala, India. Email: janmerjoh.jmj@gmail.com

ABSTRACT: An experiment was conducted to assess the compatibility of the popular insecticides like spinosad, cypermethrin, imidacloprid and chlorantraniliprole as well as fungicides copper oxychloride, carbendazim and hexaconazole with native isolates of *M. anisopliae* (MC 2, MC 4, MC 7). Among the isolates, MC 2, MC 7 and MC 4 were found compatible with insecticides spinosad, imidacloprid and chlorantraniliprole as well as fungicide copper oxychloride. Isolates MC 2 and MC 7 exhibited highest growth with only 3.70 and 5.18 per cent inhibition in the PDA medium amended with highest dose of copper oxychloride (0.30 g/ l) when compared to MC 4 (7.03 % inhibition). Among the three isolates tested, the isolate MC 7 was more compatible with highest growth at all higher doses of chlorantraniliprole (0.35 ml/L), spinosad (0.38ml/ l) and imidacloprid (0.15g/ l) by recording least per cent growth inhibition (11.00, 11.41 and 14.44 per cent inhibition respectively). The insecticide cypermethrin was slightly toxic to all the isolates of *M. anisopliae* and fungicides, carbendazim and hexaconazole were not compatible with the *M. anisopliae* isolates.
© 2020 Association for Advancement of Entomology

KEY WORDS: Entomopathogenic fungi *Metarhizium anisopliae*, compatibility, insecticides and fungicides

INTRODUCTION

Entomopathogenic fungi (EPF) are fungal microorganisms that are pathogenic to pests. *Metarhizium anisopliae* is one among them and is effective against several species of insects including beetles, termites, leafhoppers, mosquitoes and lepidopterans. It has been recovered from a variety of crop ecosystem, rendering it an ideal candidate for exploration on stress tolerant isolates. Incompatibility of insecticides and fungicides with fungi is one among the abiotic stresses. Combined use of mycoinsecticides and chemical insecticides is a promising pest control option for minimizing adverse chemical effects and also reduces frequency of application of mycoinsecticide. Several studies have reported that *M. anisopliae* is a dominant species in intensively cultivated arable lands and it was thought to be due to the ability of *M. anisopliae* to tolerate agricutltural chemicals and mechanical disturbances (Vanninen and Hokkanen, 1988; Vanninen, 1995). More than that, many researches have been implemented for exploring the compatibility of entomopathogens with insecticides (Silva *et al*., 2013; Kassab *et al*., 2014; Sain *et al*., 2019).

* Author for correspondence

© 2020 Association for Advancement of Entomology

Combined use of incompatible insecticides and fungicides might inhibit the entire functioning of entomopathogenic fungi which adversely affect the system of integrated pest management. Therefore, identification of the fungal isolates which are compatible to pesticides will retain biocontrol potential and will be effective in managing pests.

MATERIALS AND METHODS

Experiments on compatibility of different insecticides and fungicides with *M. anisopliae* native isolates were conducted at College of Horticulture, Vellanikkara, Thrissur, Kerala during 2018 under laboratory conditions. Native isolates of *Metarhizium anisopliae* namely MC 2, MC 4, MC 7 isolated from Moncompu in Alappuzha district were tested as per the protocol of Grover and Moore (1962), following the method of poisoned food technique. Four insecticides *viz*., spinosad, cypermethrin, imidacloprid and chlorantraniliprole as well as three fungicides namely copper oxychloride, carbendazim and hexaconazole were used in this experiment (Table 1). Compatibility of the isolates was evaluated by exposing them to different doses *i.e*., lower dose, recommended dose and higher dose (recommended dose as per package of practices, KAU). Desired quantity of chemical was measured out and mixed thoroughly in the sterilized molten potato dextrose agar medium and poured onto the Petri plates. Control plates without the addition of fungicides and insecticides were also maintained. A five millimeter mycelial disc of each isolate was placed at center of the medium and kept for incubation at 26 ± 2°C for 14 days. Radial growth was measured and the percent reduction of growth (Vincent, 1927) compared to control was calculated using the formula,

Percent inhibition = (C-T) × 100/C where,

C = radial growth of isolate in PDA plate (cm)

T = radial growth of the salt amended PDA plate (cm)

Sporulation of the isolates was also observed and visually analysed. Depending upon the growth inhibition values, the pesticides are again classified on a 1-4 index where one denotes harmless (< 50 % reduction in growth), two is slightly harmful (50-79 %), three designates moderately harmful (80-90 %) and four implies harmful (> 90%) according to Hassan's classification scheme (Hassan, 1989). Data was accorded to Analysis of Variance (ANOVA) employing Web Agri Stat Package (WASP 2.0). Multiple comparisons between the treatment means were done with Duncan's Multiple Range Test (DMRT) and appropriate transformations were considered according to the method elucidated by Gomez and Gomez (1984).

Table 1. Details of insecticides and fungicides used in the study

Chemical name	Trade name and formulation	Doses used (ml or g/ l)		
		Lower	Recommended	Higher
Spinosad	Taffin, 45 SC	0.28	0.33	0.38
Cypermethrin	Cyperguard, 25 EC	0.35	0.40	0.45
Imidacloprid	Admire, 70 WG	0.05	0.10	0.15
Chlorantraniliprole	Coragen, 18.5 SC	0.25	0.30	0.35
Copper oxychloride	Fytolan, 50 WP	0.20	0.25	0.30
Carbendazim	Bavistin, 50 WP	0.50	1.00	1.50
Hexaconazole	Contaf, 5 EC	1.50	2.00	2.50

RESULTS AND DISCUSSIONS

In general, growth of all isolates reduced at higher doses of insecticides and fungicides. A considerable decrease in the growth and sporulation was noticed in the PDA amended with fungicides when compared to insecticides (Table 2). In the case of PDA amended with spinosad at different doses, all the isolates showed a growth inhibition of less than 19.50 per cent. Isolate MC 7 was superior among the isolates with least inhibition from 8.11 to 11.41 per cent at higher doses of spinosad. The extent of inhibition increased as the dose of spinosad increased in the medium. Sporulation was adversely affected only at higher doses of insecticide (Table 3). Isolate MC 7 produced medium sporulation even

Table 2. Effect of insecticides and fungicides on the growth of Metarhizium anisopliae isolates

Sl. No.	Insecticides and fungicides	Growth inhibition over control (%) *			Standard error	Grade
		MC 2	MC 4	MC 7		
1.	Spinosad					
	@ 0.28 ml/l	4.61(2.97)[a]	4.42(2.54)[ab]	3.62(2.12)[b]	0.332	1
	@ 0.33 ml/l	16.64(9.59)[a]	10.71(6.16)[b]	8.11(4.67)[c]	0.313	1
	@ 0.38 ml/l	17.40(10.02)[b]	19.23(11.10)[a]	11.41(6.59)[c]	0.359	1
2.	Cypermethrin					
	@ 0.35 ml/l	53.00(32.22)[a]	51.51(30.75)[b]	53.74(33.00)[a]	0.336	2
	@ 0.40 ml/l	56.23(33.72)[c]	61.90(38.48)[a]	59.20(36.03)[b]	0.335	2
	@ 0.45 ml/l	60.71(36.90)[b]	64.04(40.11)[a]	62.92(38.40)[a]	0.370	2
3.	Imidacloprid					
	@ 0.05 g/l	13.64(7.87)[a]	10.30(5.95)[b]	7.10(4.03)[c]	0.363	1
	@ 0.10 g/l	19.20(11.10)[a]	16.70(9.59)[b]	11.93(6.80)[c]	0.385	1
	@ 0.15 g/l	21.50(12.41)[a]	17.43(10.02)[b]	14.44(8.30)[c]	0.339	1
4.	Chlorantraniliprole					
	@ 0.25 ml/l	0.00(0.00)[b]	1.85(1.06)[a]	0.00(0.00)[b]	0.210	1
	@ 0.30 ml/l	2.96(1.69)[b]	4.81(2.75)[a]	0.00(0.00)[c]	0.287	1
	@ 0.35 ml/l	8.88(4.88)[a]	7.77(4.45)[a]	1.11(0.84)[b]	1.117	1
5.	Copper oxychloride					
	@ 0.20 g/l	1.11 (0.63)[c]	4.81(1.69)[b]	2.96(2.75)[a]	0.257	1
	@ 0.25 g/l	2.59(1.48)[c]	5.92(3.39)[a]	4.07(2.33)[b]	0.314	1
	@ 0.30 g/l	3.70(2.12)[c]	7.03(4.03)[a]	5.18(2.97)[b]	0.314	1
6.	Carbendazim					
	@ 0.50 g/l	86.33 (59.61)[b]	93.40 (68.38)[a]	58.51 (35.77)[c]	0.336	4
	@ 1.00 g/l	100	100	100	-	4
	@ 1.50 g/l	100	100	100	-	4
7.	Hexaconazole					
	@ 1.50 ml/l	100	100	100	-	4
	@ 2.00 ml/l	100	100	100	-	4
	@ 2.50 ml/l	100	100	100	-	4

Values given in the parentheses are arcsine transformed values

Table 3. Effect of insecticides and fungicides on the sporulation of *Metarhizium anisopliae* isolates

Sl. No.	Insecticides and fungicides	Sporulation of the isolates		
		MC 2	MC 4	MC 7
1.	Spinosad			
	@ 0.28 ml/l	+++	+++	+++
	@ 0.33 ml/l	++	+	++
	@ 0.38 ml/l	-	-	++
2.	Cypermethrin			
	@ 0.35 ml/l	+	++	++
	@ 0.40 ml/l	-	+	+
	@ 0.45 ml/l	-	-	++
3.	Imidacloprid			
	@ 0.05 g/l	+	+	++
	@ 0.10 g/l	-	+	+++
	@ 0.15 g/l	-	-	+++
4.	Chlorantraniliprole			
	@ 0.25 ml/l	+++	+++	+++
	@ 0.30 ml/l	++	+++	+++
	@ 0.35 ml/l	+	+	++
5.	Copper oxychloride			
	@ 0.20 g/l	-	+++	+++
	@ 0.25 g/l	++	++	+++
	@ 0.30 g/l	+	+	++
6.	Carbendazim			
	@ 0.50 g/l	++	-	+++
	@ 1.00 g/l	-	-	-
	@ 1.50 g/l	-	-	-
7.	Hexaconazole			
	@ 1.50 ml/l	-	-	-
	@ 2.00 ml/l	-	-	-
	@ 2.50 ml/l	-	-	-

+++ : high sporulation, ++ : medium sporulation, + : sparse sporulation, - : no sporulation (visual observation)

at highest dose of spinosad. Cypermethrin on the other hand, caused inhibition of more than 50 per cent in all isolates even at the lowest dose (Table 2).

All the isolates were equally incompatible with cypermethrin, suggesting that combined application of cypermethrin and *M. anisopliae* should not be advisable. The isolates had less than 22 per cent growth inhibition at all doses of imidacloprid, with growth inhibition of isolates ranging between 7.10 and 21.50 per cent. The isolate MC 7 was consistently superior to other isolates in terms of growth and sporulation. The inhibition was dose dependent for all isolates, with highest degree of inhibition being at the highest dose of the insecticide (0.15 g/ l). The three isolates were also found to be compatible with chlorantraniliprole at all doses used in this study. The growth inhibition caused was less than nine per cent for all isolates at all doses. Significantly superior radial growth along with high sporulation at higher doses was exhibited by the isolate MC 7 when compared to other two isolates (Table 2).

Screening of isolates for fungicide compatibility was also carried out. Less than eight per cent growth inhibition was observed for all isolates at different doses of the copper oxychloride (COC), proving its compatibility with *M. anisopliae*. Among the three isolates tested, MC 2 recorded least growth inhibition of 1.11 to 3.70 per cent at different doses of copper oxychloride (Table 2). However, high sporulation was exhibited by the isolate MC 7 with medium sporulation even at the highest dose of COC (0.30 g/ l) [Table 3]. Growth of all isolates was considerably inhibited even at the lowest dose of carbendazim (0.50 g/ l). Total growth inhibition was observed in all isolates at recommended dose of 1 g/ l and above (Table 2). At 0.50 g/ l, the inhibition in the growth of all isolates was ranged between 58.51 and 86.33 per cent. MC 2 and MC 7 registered medium and high sporulation respectively at the lowest dose of fungicide. No sporulation was observed at higher doses [Table 3]. All isolates of *M. anisopliae* resulted in 100 per cent growth inhibition at all doses of hexaconazole (1.50, 2, 2.50 ml/ l) depicting that the isolates obtained in the present study were incompatible with fungicide hexaconazole. Based on Hassan's classification scheme, insecticides spinosad, imidacloprid, chlorantraniliprole and fungicides copper oxychloride were categorized to index 1, insecticide cypermethrin to 2 and fungicides carbendazim and hexaconazole to index 4.

Combined application of pesticide and entomopathogenic fungi provides satisfactory control against many agricultural pest. But use of incompatible pesticides in soil could hamper the growth and development of beneficial fungi. In this context, experiment was conducted in order to screen the native isolates of *M. anisopliae* for tolerance to different insecticides and fungicides. Compatibility of four isolates of *M. anisopliae* from Punjab and Pakistan with a number of pesticides had been reported by Akbar *et al*. (2012). The isolate M70 recorded highest radial growth of 6.81 cm and a spore yield of 1.26 × 10^8/ml in PDA amended with recommended dose of spinosad whereas imidacloprid, indoxacarb, cypermethrin, acetamiprid supported only moderate conidial germination. The study concluded that insecticides like spinosad, imidacloprid and acetamiprid were more compatible with *M. anisopliae* than other insecticides tested. The insecticide spinosad and imidacloprid were more compatible than cypermethrin with *M. anisopliae* isolates in the present study and was in tune with the findings of Akbar *et al*. (2012).

Mochi *et al*. (2005) reported that imidacloprid had no effect on the survival and growth of *M. anisopliae*. Imidacloprid had been found as compatible with *M. anisopliae* by several authors. Quintela and McCoy (1998) reported that combined application of *M. anisopliae* and imidacloprid resulted in higher mortality of root weevil grub, *Diaprepes* sp. in soil. Moreover in the study of Neves *et al*. (2001) also confirmed compatibility of imidacloprid with *M. anisopliae*. But imidacloprid was found moderately toxic at maximum dose and incompatible at minimum dose with entomopathogens as stated by Filho *et al*. (2001). Study of Joshi *et al*. (2018) found complete inhibitory action of fungicides carbendazim and hexaconazole on the growth of *M. anisopliae*. According to Mochi *et al*. (2005), CO_2 production by *M. anisopliae* was suppressed in soil for 4-6 days when co- applied with fungicides (COC, tebuconazole *etc*), but after that there is no significant difference between the respiratory activity of *M. anisopliae* in fungicide treated and untreated soil. The tested acaricides, herbicides and insecticides had only less impact on respiratory activity of fungi and hence suggested for the combined application with fungi. The insecticides used in the present study were more compatible with *M. anisopliae* isolates than the fungicides used and were in line with the reports of Mochi *et al*. (2006) who studied the effects of insecticides and fungicides in the growth of *M. anisopliae*. Most of the fungicides were incompatible with the entomopathogens while there was a greater compatibility between insecticides and *M. anisopliae*. Laboratory bioassays alone doesn't determine the effective compatibility of entomopathogens with pesticides hence additional field or greenhouse studies are required to confirm the compatibility or incompatibility of pesticides with biocontrol agents before they recommend in crop management strategies..

ACKNOWLEDGEMENT

Authors greatly thank Kerala Agricultural University for providing funds and facilities for the study.

REFERENCES

Akbar S., Freed S., Hameed A., Gul H. T., Akmal M., Malik M.N., Naeem M. and Khan M.B. (2012) Compatibility of *Metarhizium anisopliae* with different insecticides and fungicides. African Journal of Microbiological Research 6(17): 3956-3962.

Filho A., Almeida J. E. and Lamas C. (2001) Effect of thiamethoxam on entomopathogenic microorganisms. Neotropical Entomology 30(3): 437-447.

Gomez K. A. and Gomez A. A. (1984) Statistical Procedures for Agricultural Research. John Wiley and Sons, Australia. 639 pp.

Grover R.K. and Moore J.D. (1962) Toximetric studies of fungicides against brown rot organism *Sclerotina fruticola*. Phytopathology 52: 876-880.

Hassan S.A. (1989) Testing methodology and the concept of the IOBC/WPRS working group. In: Pesticides and Non-Target Invertebrates (P.C.Jepson, ed.), Intercept, Wimborne, Dorset. 1-8 PP.

Joshi M., Gaur N. and Pandey R. (2018) Compatibility of entomopathogenic fungi *Beauvaria bassiana* and *Metarhizium anisopliae* with selective pesticides. Journal of Entomological and Zoological Studies 6(4): 867-872.

Kassab S.O., Loureiro E.D.S., Rossoni C., Pereira F.F., Barbosa R.H., Costa D.P. and Zanuncio J.C. (2014) Combinations of *Metarhizium anisopliae* with chemical insecticides and their effectiveness in *Mahanarva fimbriolata* (Hemiptera: Cercopidae) control on sugarcane. Florida Entomologist 97(1): 146-154.

Mochi D.A., Monteiro A.C. and Barbosa J.C. (2005) Action of pesticides to *Metarhizium anisopliae* in soil. Neotropical Entomology 34: 961-971.

Mochi D.A., Monteiro A.C., Bortoli S.A., Doria H.O.S. and Barbosa J.C. (2006) Pathogenicity of *Metarhizium anisopliae* for *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) in soil with different pesticides. Neotropical Entomology 35(3): 382-389.

Neves P.M., Hirose E., Tchujo P.T. and Moino J.R. and A.L.C.I.D.E.S. (2001) Compatibility of entomopathogenic fungi with neonicotinoid insecticides. Neotropical Entomology 30(2): 263-268.

Quintela E.D. and McCoy C.W. (1998) Synergistic effect of imidacloprid and two entomopathogenic fungi on the behaviour and survival of larvae of *Diaprepes abbreviates* (Coleoptera: Curculionidae) in soil. Journal of Economic Entomology 91(1): 110-122.

Sain S.K., Monga D., Kumar R., Nagrale D.T., Hiremani N.S. and Kranthi S. (2019) Compatibility of entomopathogenic fungus with insecticides and their efficacy for IPM of *Bemisia tabaci* in Cotton. Journal of Pesticide Science 44(2): 97-105.

Silva R.A.D., Quintela E.D., Mascarin G.M., Barrigossi J.A.F. and Liao L.M. (2013) Compatibility of conventional agrochemicals used in rice crops with entomopathogenic fungus *Metarhizium anisopliae*. Scientia Agricola 70(3): 152-160.

Vanninen I. (1995) Distribution and occurrence of four entomopathogenic fungi in Finland: effect of geographical location, habitat type and soil type. Mycological Research 100: 93-101.

Vanninen I. and Hokkanen H. (1988) Effects of pesticides on four species of entomopathogenic fungi. Annales Agriculturae Fenniae 27: 345-353.

Vincent J.M. (1927) Distortion of fungal hyphae in the presence of certain inhibitors. Nature 59: 850.

(Received March 08, 2020; revised ms accepted June 16, 2020; printed June 30, 2020)