Short scientific report *Journal of Plantation Crops, 2011, 39 (1): 196-200*

Compatibility of *Metarhizium anisopliae* (Metsch.) Sorokin with some chemical and botanical pesticides used in coconut pest management

(Manuscript Received: 20-12-10, Revised: 24-02-11, Accepted: 11-04-11)

Keywords: Coconut, compatibility, insecticide, *Metarhizium anisopliae*, microbial control

The green muscardine fungus *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hypomycetes) is one of the most effective and successful biocontrol agent of rhinoceros beetle *(Oryctes rhinoceros* Linn.), a major pest of coconut and oil palm. The adult rhinoceros beetles damage palms by boring through the unopened spindle and inflorescence and breed in dead and decaying organic debris like cattle dung, compost, coir dust *etc.* (Nirula *et al.*, 1955; Mohan and Pillai, 1982).

Several systemic and contact pesticides such as chlorpyriphos, monocrotophos, carbaryl *etc.* are recommended for the control of coconut pests (Nair *et al.*, 1997; Rajan *et al.*, 2009). The weed plant, *Clerodendron infortunatum*, was found to elicit insect growth regulatory activity in *O. rhinoceros* and incorporation of this plant in the breeding sites of the beetle is recommended for pest management (Chandrika Mohan *et al.*, 2001). The present investigation was aimed to understand the *in vitro* effects of some chemical and botanical pesticides on the growth and sporulation of *M. anisopliae* in order to develop adequate techniques that can permit the compatible use of this entomopathogen with these pesticides. The study was carried out at the Biological Control Laboratory of the Central Plantation Crops Research Institute (CPCRI), Kayamkulam, Kerala and the strain of *M. anisopliae* var. *major* used in this study was obtained from stock culture maintained at CPCRI.

Commonly recommended pesticides used for pest management in coconut cultivation *viz.*, Carbaryl, Chlorpyriphos and Monocrotophos (chemical pesticides), commercial formulation of Azadirachtin (botanical pesticide) and extract of the weed plant *Clerodendron infortunatum* Linn. (Verbenaceae) were tested in the laboratory (Table 1) using standard poison food technique (Nene and Thapliyal, 1997). Required quantities of the pesticides were added to sterilized Potato Dextrose Agar medium (Table1). Leaves of *C. infortunatum* were collected from Kayamkulam, Kerala during March 2009 and the leaf extract was prepared as per method described by Borgio *et al.*(2008). Each pesticide was evaluated at four different concentrations and compared with control plates grown on pure PDA maintaining ten replicates in each treatment.

1000 ml of Potato Dextrose Agar (PDA) medium was prepared and sterilized. From the prepared media, 250 ml was taken in separate sterilized conical flask and the pesticides/ botanical/ plant extract at the required concentration were added to it before solidification at a temperature of approximately 45°C and stirred well. 25 ml of the respective media was poured into Petri dishes (90 mm) maintaining ten dishes per each pesticide treatment as replicates. *M. anisopliae* culture disc of 1 cm diameter was cut and inoculated on the centre of the respective media after solidification. The plates were then sealed with Parafilm and incubated at 30±2^{0}C and 80±5 % RH. Colony diameter was measured daily from the fourth day onwards and continued for 30 days. From a 30 day old Petri dish culture of *M. anisopliae*, spore count was taken using a haemocytometer. Data was analysed statistically using ANOVA.

The data were standardized by the classification of Alves *et al*. (1998) based on the mean values as percent sporulation and vegetative growth of the fungal colonies, using the formula:

$$T = \frac{20\,[VG] + 80\,[SP]}{100}$$

where T is the corrected value of vegetative and reproductive growth for product classification, *VG* is percent vegetative growth compared to control, and *SP* is percent sporulation compared to control. The T values

Table. 1. Pesticides used in the study

Commercial name	Active ingredient and content	Field recommended conc. in coconut pest management	Conc. tested in medium (%)	Qnty. of pesticide mixed in 250 ml of medium
Sevin	Carbaryl 50% W.P.	Red palm weevil (1%)	0.01	0.05g
		Rhinoceros beetle (0.02%)	0.02	0.1g
		Coreid bug (0.1%)	0.05	0.25g
		slug caterpillar (0.15%)	0.1	0.5g
Classic-20	Chlorpyriphos 20% EC	White grub (0.05%)	0.0125	0.156ml
		Termites (0.05%)	0.025	0.313ml
			0.05	0.625ml
			0.1	1.25ml
Phoskill	Monocrotophos 36% WSC	Mealy bugs (0.05%)	0.0125	0.087ml
		Red palm weevil	0.025	0.174ml
		(10ml+10ml water)	0.05	0.347ml
			0.1	0.694ml
Eco neem plus	Azadirachtin -10,000 ppm	Coconut eriophyid mite (0.004%)	0.001	0.25ml
			0.002	0.5ml
			0.004	1ml
			0.008	2ml
Aqueous extract of *Clerodendron infortunatum*	*C. infortunatum* 100 %	Rhinoceros beetle (10%)	5%	12.5ml
			10%	25ml
			15%	37.5ml
			20%	50ml

for the classification of the effect of chemical products on the fungi are as follow: 0 to 30 (very toxic), 31 to 45 (toxic), 46 to 60 (moderately toxic) and >60 (compatible).

For studying the effect of pesticides on micromorphometry of *M. anisopliae*, the fungus was grown by slide culture method (Aneja, 2004) in poisoned PDA media with the respective pesticides for all the treatments and control. After 30 days the slides were stained with lactpophenol blue and examined under microscope for various micro morphological characters

Results on radial growth of the fungus at 10th, 20th and 30th day of inoculation are presented in Table 2. There is a significant difference among the treatments and also a doze induced suppression in radial growth of the fungus in the pesticide fortified media.

Table. 2. Radial Growth and spore count of *M. anisopliae in* various pesticide concentrations treated media

Pesticide	Radial growth (cm)			Spore count (spores/cm^3)
	10th day	20th day	30th day	
Carbaryl 0.01%	0.87± 0.04	1.57± 0.05	2.42 ± 0.07	248 x 104
Carbaryl 0.02%	0.22 ±0.03	0.47 ±0.04	1.05 ± 0.1	244 x 104
Carbaryl 0.05%	0.00	0.00	0.00	144 x 104
Carbaryl 0.1%	0.00	0.00	0.00	26 x 104
Monocrotophos 0.0125%	0.13 ±0.01	0.38± 0.02	0.79 ±0.02	74 x 104
Monocrotophos 0.025%	0.06 ±0.01	0.21± 0.01	0.66 ±0.01	51 x 104
Monocrotophos 0.05%	0.00	0.00	0.00	31 x 104
Monocrotophos 0.1%	0.00	0.00	0.00	28 x 104
Chlorpyriphos 0.0125%	0.81 ± 0.02	1.38 ±0.01	1.50 ± 0.02	10 x 104
Chlorpyriphos 0.025%	0.46 ± 0.03	1.17 ±0.02	1.40 ± 0.03	6 x 104
Chlorpyriphos 0.05%	0.06 ±0.01	0.37 ± 0.01	0.41 ± 0.01	0.0
Chlorpyriphos 0.1%	0.00	0.00	0.00	0.0
Azadirachtin 0.001%	0.24 ± 0.06	0.55 ±0.05	0.95 ± 0.1	71 x 104
Azadirachtin 0.002 %	0.17 ±0.02	0.35 ±0.05	0.75 ± 0.08	50 x 104
Azadirachtin 0.004%	0.00	0.00	0.00	23 x 104
Azadirachtin 0.008%	0.00	0.00	0.00	20 x 104
C.infortunatum 5%	0.80 ±0.08	2.15 ± 0.16	3.15 ± 0.5	175 x 104
C.infortunatum 10%	0.83 ± 0..3	2.25 ±0.30	3.04 ±0.20	225 x 104
C.infortunatum 15%	0.74± 0.03	2.08 ±0.18	2.78 ±0.50	270 x 104
C.infortunatum 20%	1.09 ± 0.08	2.37 ±0.40	3.13 ±0.35	305 x 104
Control	1.19 ± 0.21	1.68 ± 0.32	2.63 ±0.51	373 x 104
CD (P=0.05)	0.15	0.19	0.20	7.75

Carbaryl at higher concentrations (0.05 and 0.1 %) showed total inhibition of fungal growth. However, carbaryl concentrations of 0.01 % though showed slow growth on 10th day (0.87 cm) gained comparable growth with that of control plates and is on par with control from 20th (1.57 cm) and 30th (2.42 cm) day of inoculation. The pesticide monocrotophos showed significantly lower growth in all the concentrations compared to control. Chlorpyriphos completely suppressed the growth of *M. anisopliae* at 0.1 % concentration and at lower concentrations also the pesticide showed significantly slower growth than control. The botanical pesticide azadirachtin at 0.004 and 0.008 % completely suppressed the growth of *M. anisopliae*. At lower concentrations, *viz*., 0.001 % and 0.002 % the growth was significantly lower than that of control. Aqueous extract of the weed plant *C.infortunatum* showed high synergistic interaction on the growth of *M. anisopliae*. There was an added effect on growth of the fungus with increasing concentrations of *C.infortunatum* which was significantly higher (2.08 to 2.37 cm) than the fungus grown on pure PDA medium (1.68 cm) after 20th days of inoculation.

There is significant difference in spore production by *M. anisopliae* when various pesticides were added to the growth media (Table 2). *M. anisopliae* grown on pure PDA showed maximum spore count ($373x10^4$ spores/cm^3) followed by *C. infortunatum* at 20 % concentration with spore count of 305 x 10^4 spores/cm^3. In chemical and botanical pesticide treated media there is a dose induced suppression of spore production. Among the pesticides, chlorpyriphos was found to be very toxic with maximum suppression of spore production (97.3 to 100 % in various treatments) followed by monocrotophos (80-92 % reduction) and azadirachtin (80 to 94 % reduction). *C. infortunatum* showed a dose induced increase with the sporulation of *M. anisopliae*. The spore count increased from 175 $x10^4$ spores/cm^3 in 0.001 % concentration to $305x10^4$ spores/cm^3 in 0.004 % concentration.

No significant difference in size of spore (conidia) was observed in various treatments (Fig.1). The conidia showed an average size of 11.91 ± 0.43µm long and 2.44 ± 0.23 µm width. There was no further growth of hyphae in most of the higher concentrations of pesticides, hence the observations on hyphal micrometry was recorded from the lowest concentrations of treatments. The hyphae showed an average width of 2.00 ± 0.13µm.

The pesticides were classified based on "*T* value" and the compatibility of *M. anisopliae* with tested pesticides is presented in Table 3. Among the chemical

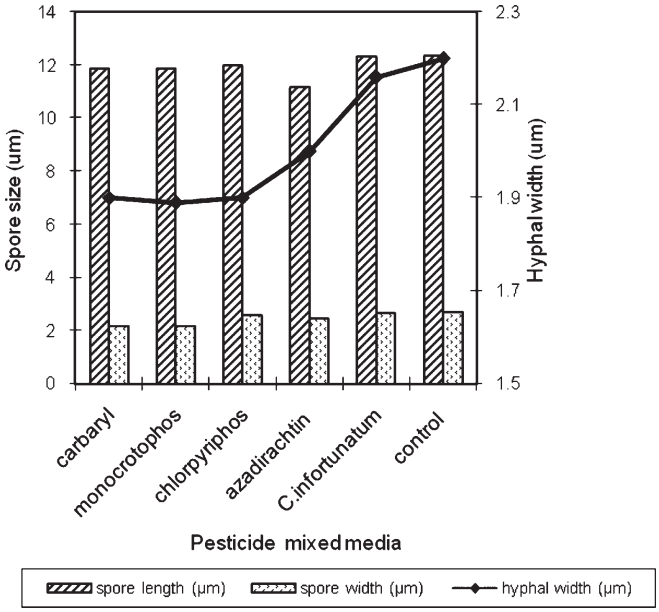

Fig. 1. Spore and hyphal size of *M. anisopliae* grown on various pesticide mixed media

pesticides, carbaryl at the lowest concentration of 0.01 % was on par with control regarding radial growth of fungus after 20 days of inoculation and it also showed 66.5 % sporulation compared to control. This concentration was graded as "C" (compatible) with *M. anisopliae* with T value of 71.59. Carbaryl 0.02 % was graded as moderately toxic as the T value was 59.17. There was 75 to 100 % inhibition of fungal growth in various other pesticide treatments. *M. anisopliae* failed to germinate in medium treated with chlorpyriphos/monocrotophos /carbaryl at 0.1 % concentration and also on azadirachtin mixed media at 0.004 and 0.008 % concentration. These pesticides were graded as 'VT' (very toxic) with *M. anisopliae*. The extract of the weed plant *C. infortunatum* favoured growth of *M anisopliae* and showed synergistic interaction (5-19 % more growth) with pure PDA and was graded as 'C'. Hence this plant parts can be incorporated in the breeding sites in combination with *M. anisopliae* in coconut ecosystem for suppression of immature stages of *O. rhinoceros.* The reason or mechanism of enhanced efficiency of *M anisopliae* in combination with *C. infortunatum* is yet to be unravelled.

The use of incompatible plant extract may inhibit the development and reproduction of the pathogen effecting pest control (Anderson and Roberts, 1983) and the present study also revealed the antagonism between azadirachtin and *M. anisopliae*. On the other hand, the use of selective products is an important strategy in IPM. In some cases, compatible products may be associated with entomopathogenic fungi, increasing control

Table. 3. Compatibility of various pesticides with *M. anisopliae*

Pesticides	% VG	%SP	T value#	classification**
Carbaryl 0.01%	92.01	66.48	71.59	C
Carbaryl 0.02%	34.22	65.41	59.17	MT
Carbaryl 0.05%	0	38.60	30.88	T
Carbaryl 0.1%	0	6.97	5.57	VT
Monocrotophos 0.0125%	30.03	19.83	21.87	VT
Monocrotophos 0.025%	25.09	13.67	15.95	VT
Monocrotophos 0.05%	0.0	8.31	6.64	VT
Monocrotophos 0.1%	0.0	7.50	6.00	VT
Chlorpyriphos 0.0125%	57.03	2.68	13.55	VT
Chlorpyrphos 0.025%	53.23	1.61	11.93	VT
Chlorpyriphos 0.05%	15.58	0.0	3.11	VT
Chlorpyriphos 0.1%	0.0	0.0	0.0	VT
Azadirachtin 0.001%	36.23	19.03	22.47	VT
Azadirachtin 0.002%	28.55	13.40	16.43	VT
Azadirachtin 0.004%	0.0	6.16	4.93	VT
Azadirachtin 0.008%	0.0	5.36	4.28	VT
C. infortunatum 5%	119.77	46.91	61.48	C
C. infortunatum 10%	115.58	60.32	71.37	C
C. infortunatum 15%	105.70	72.38	79.04	C
C. infortunatum 20%	119.01	81.76	89.21	C
Control	100	100	100	

VG= Percentage vegetative growth compared to control, SP = Percentage sporulation compared to control,
# T value (as per Alves *et al*., 1998)
** C- Compatible, MT -Moderately toxic, T-Toxic, VT- Very Toxic

efficiency (Quintela and McCoy, 1998). Synergistic interaction with aqueous extract of *C. infortunatum* revealed in the present study agrees with this finding.

Li and Holdom (1995) observed that chlorinated hydrocarbon insecticides are more deleterious than other insecticides groups to the entomopathogen. They observed extremely detrimental effect of chlorpyriphos, tempephos and malathion to mycelial growth and sporulation of *M anisopliae*, while carbamate insecticides like carbosulfan, methonyl and oxymyl were moderately toxic. Rachappa *et al.* (2007) reported 69.2 % inhibition in growth of *M anisopliae* by chlorpyriphos, 38.5 % inhibition by monocrotophos and 50.9 % inhibition by carbaryl. Mohammed *et al*. (1987) reported that chlorpyriphos as most toxic organophosphate to mycelial growth and sporulation at all concentrations. The present study also revealed inhibitory activity of chlorpyriphos and monocrotophos on *M. anisopliae*

Spore yield of fungus in insecticides mixed media depend upon its inhibiting action on colony growth and it varied from 7x 10^4 to 249x 10^4 spores/cm^3 in pesticide treatments and 175 x 10^4 to 305 x 10^4 spores/cm^3 in *C. infortunatum* in various concentrations. Obviously conidial count was less in chlorpyriphos and monocrotophos treated plates due to strong inhibitory properties of the chemicals disallowing fungal sporulation. On the contrary in *C. infortunatum* treated plates at the rate of 20 % showed comparable spore load as that of control.

The result of the present study indicates that carbaryl at 0.01%, which is the recommended dose for treatment of breeding sites of *O. rhinoceros* for pest control or *C. infortunatum* at 5 to 20 % can be used in combination with *M anisopliae* in coconut agro ecosystem without compromising the activity of the green muscardine fungus. For other pesticides screened, precaution might be taken to avoid mixing of pesticide in *M. anisopliae* treated sites or safe interval between insecticide application and entomopathogen inoculation might be sought for supplementary use of this entomopathogen for control of crop pests.

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