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Compatibility of selected pesticides with three entomopathogenic fungi of sugarcane pests

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ABSTRACT: **Compatibility of selected insecticides, fungicides and weedicides, commonly used in sugarcane, with *Beauveria bassiana* (Balsamo-Criv.) Vuill. *Beauveria brongniartii* (Sacc.) Petch and *Metarhizium anisopliae* (Metschnikoff) Sorokin, the entomopathogenic fungi that occur naturally on several pests of the crop, was evaluated in *in vitro* assays. Radial growth, biomass and spore production used as parameters did not show consistent trend for the five insecticides tested. On the basis of per cent reduction in spore production, however, chlorpyriphos (0.04%) was most toxic to all three fungi (100%); lindane (0.04%) was most toxic to *B. brongniartii* (100%) but least toxic to *B. bassiana* (26.3%) and *M. anisopliae* (17.1%); monocrotophos (0.036%) was moderately toxic to *B. bassiana* (43.0%) and *M. anisopliae* (35.2%), and least toxic to *B. brongniartii* (13.4%); malathion (0.10%) was most toxic to *M. anisopliae* (88.2%) and *B. brongniartii* (69.1%), and moderately toxic to *B. bassiana* (43.0%); endosulfan (0.035%) was moderately toxic to all three species (49.5 – 58.1%). Carbendazim (0.05%) was completely toxic to all three fungi (100%); mancozeb 0.08% was also equally toxic to all three fungi (69.5 – 100.0%). Glyphosate (0.205%) was most toxic (88.1%) to *B. bassiana* and moderately toxic to *B. brongniartii* (39.3%) and *M. anisopliae* (58.2%); atrazine (0.35%) was moderately toxic (40.5 – 55.7%) to all three fungi; 2,4-D (0.20%) was moderately toxic to *B. bassiana* (45.9%) and *B. brongniartii* (63.3%), and least toxic (17.7%) to *M. anisopliae*. The implications of the results in sugarcane pest management involving entomopathogenic fungi are discussed.**

KEY WORDS: *Beauveria bassiana*, *Beauveria brongniartii*, compatibility, entomopathogenic fungi, *Metarhizium anisopliae*, pesticides, sugarcane

INTRODUCTION

Sugarcane, the second most important commercial crop after cotton, is attacked by a large number of pests in India, which include borers, sucking pests, termites and white grubs (David and Nandagopal, 1986). Several of these pests play host to entomopathogenic fungi such as *Beauveria bassiana* (Balsamo-Criv.) Vuill., *Beauveria brongniartii* (Sacc.) Petch and *Metarhizium anisopliae* (Metschnikoff) Sorokin. Among these, *B. bassiana* occurred naturally on shootborer *Chilo infuscatellus* Snellen and root borer *Emmalocera depressella* Swinhoe whereas *M. anisopliae* was isolated from internode borer *Chilo sachariphagus indicus* (Kapur); both were pathogenic to their hosts in the laboratory (Easwaramoorthy and Santhalakshmi, 1987 & 1993; Easwaramoorthy *et al.*, 2001). *Beauveria brongniartii* was mass multiplied on commercial scale using a low-cost

molasses based method (Easwaramoorthy *et al.*, 2002); the fungus caused significant infection levels in the white grub *Holotrichia serrata* Fabricius in laboratory, pot-culture and field experiments (Easwaramoorthy *et al.*, 2004).

Several systemic and contact insecticides such as phorate, dimethoate, malathion, quinalphos, monocrotophos, chlorpyriphos and carbofuran are recommended for the control of sugarcane pests (Ananthanarayana and David, 1986). Similarly, fungicides and weedicides are used to fight diseases and weeds, respectively (Sundara, 1998). Despite the fact that sugarcane canopy restricts the use of pesticides either to initial stages of crop growth or to combat problem pests in the later stages, there is a need to assess their compatibility with entomopathogenic fungi of current and future importance to maximize their combined efficacy. Although several studies on compatibility of pesticides with the above three species of fungi are reported in the world literature (Gupta *et al.*, 2002), Indian studies have been a few (Vyas *et al.*, 1990; Sharma and Gupta, 1998) and these have not included weedicides. In the present study, selected insecticides, fungicides and weedicides recommended and commonly used in sugarcane crop system were evaluated for compatibility with the three species of entomopathogenic fungi.

MATERIALS AND METHODS

Maintenance of fungal cultures

Pure cultures of *B. bassiana* (root borer isolate), *B. brongniartii* (white grub isolate) and *M. anisopliae* (white grub isolate) maintained on potato dextrose agar (PDA) in slants were sub-cultured at monthly interval on the same medium in Petri-plates to obtain sufficient quantities of inoculums.

Pesticides evaluated

Three groups of pesticides comprising insecticides (5), fungicides (2) and weedicides (3) at field recommended dosages/concentrations were evaluated for compatibility with the three species of fungi selected (Table 1). The actual concentrations (ppm) and the quantities of formulations (ml or g/100 ml medium) used for insecticides and fungicides were derived from recommended field concentrations (%). For weedicides, field recommended dosages (kg a.i./ ha) were first converted to field concentrations (%) using 500 liters/ha as the quantity of spray fluid. These field concentrations were used for deriving actual concentrations (ppm) and quantities of formulations (ml or g/100 ml medium) as in the case of insecticides and fungicides. Glyphosate, atrazine and 2, 4-D were evaluated at the normal or recommended field dosages of 1.03, 1.75 and 1.00 kg a. i./ ha, respectively and higher (1.5 times) dosages.

Evaluation protocol

Radial growth, biomass and spore production were the parameters used to assess the impact of pesticides on the fungi. For examining radial growth, calculated quantities of pesticides (Table 1) were added aseptically to 100 ml sterilized potato dextrose agar in 250 ml conical flasks when the medium was cool. The thoroughly mixed pesticide-medium was poured into 9 cm diameter Petri-plates at 25 ml per plate and allowed to solidify. These plates were inoculated with 10 mm disc of each fungus previously grown on PDA in Petri-plates and incubated in the laboratory for 15 days to allow sufficient conidial production. Diameter (cm) of the growing colony was recorded 15 days after inoculation. Each treatment was replicated thrice and a control without pesticide was maintained.

To study biomass and spore production, 100 ml potato dextrose broth sterilized in 250 ml culture flask was mixed with pesticides and inoculated with 10 mm fungal discs in two separate sets of flasks. For biomass observations, the fungal mat was removed from the flask after 20 days of incubation, the excess broth filtered through Whatman no.1 filter paper, dried to a constant weight at 45-50°C and dry weight recorded. For spore production, fungal mat was collected after 20 days of incubation, ground in a blender, filtered through muslin and the filtrate was made up to one-liter volume. This suspension was diluted serially and the spore

Table 1. Pesticides and concentrations used in compatibility studies

Pesticide	Field recommended concentration (%)	Concentrationin medium (ppm)
A. Insecticides		
1. Chlorpyriphos 20 EC	0.040	400
2. Lindane 20 EC	0.040	400
3. Monocrotophos 36 EC	0.036	360
4. Malathion 50 EC	0.100	1000
5. Endosulfan 35 EC	0.035	350
B. Fungicides		
1. Carbendazim 50 WP	0.050	500
2. Mancozeb 80 WP	0.080	800
C. Weedicides		
1. Glyphosate 41 SL (Normal)	0.205	2050
Glyphosate 41 SL (Higher)	0.3075	3075
2. Atrazine 50 WDP (Normal)	0.350	3500
Atrazine 50 WDP (Higher)	0.525	5250
3. 2,4-D 80 WP (Normal)	0.200	2000
2,4-D 80 WP (Higher)	0.300	3000

concentration was assessed using a haemocytometer. The spore production was expressed as number/100 ml broth. Each treatment was replicated thrice with suitable control without pesticides.

Data analysis

The data from radial growth, biomass and spore production tests were analyzed statistically using Analysis of Variance (ANOVA) technique with suitable transformations, wherever needed. The means were compared using Duncan's Multiple Range Test (DMRT) as per Gomez and Gomez (1984).

RESULTS AND DISCUSSION

A. Compatibility of insecticides

The insecticides tested significantly reduced growth parameters of *B. bassiana* in comparison with control (Table 2). Monocrotophos 0.036 per cent least affected radial growth (25.0%) while chlorpyriphos 0.04 per cent, lindane 0.04 per cent, malathion 0.10 per cent and endosulfan 0.035 per cent showed greater (46.8 – 51.8%) inhibitory effect. Chlorpyriphos reduced biomass production most (100%) while endosulfan reduced it least (9.8%), the latter being on par with control; monocrotophos lindane and malathion showed intermediate (19.5 –

Table 2. Effect of insecticides on radial growth, biomass and spore production of three entomopathogenic fungi

Insecticide / Concentration (%)	*Beauveria bassiana*			*Beauveria brongniartii*			*Metarhizium anisopliae*		
	Radial growth (cm)	Biomass (g)	Spore production (x 10^{10}/100 ml broth)	Radial growth (cm)	Biomass (g)	Spore production (x 10^{10}/100 ml broth)	Radial growth (cm)	Biomass (g)	Spore production (x 10^{10}/100 ml broth)
Chlorpyriphos (0.04)	2.13a	0.00 (0.71)[$]a	0.00 (0.71)a	2.63b	0.00 (0.71)a	0.00 (0.71)a	2.00a	0.00 (0.71)a	0.00 (0.71)a
Lindane (0.04)	2.00a	0.66 (1.08)c	3.00 (1.88)d	2.33ab	0.00 (0.71)a	0.00 (0.71)a	2.07ab	0.82 (1.15)c	2.66 (1.78)e
Monocrotophos (0.036)	3.00b	0.55 (1.02)b	2.32 (1.68)c	4.20d	0.51 (1.00)c	3.37 (1.97)d	2.23abc	0.84 (1.16)c	2.08 (1.61)d
Malathion (0.10)	1.93a	0.66 (1.08)c	2.32 (1.68)c	2.23a	0.58 (1.04)c	1.20 (1.30)b	2.33bc	0.00 (0.71)a	0.38 (0.94)b
Endosulfan (0.035)	2.03a	0.74 (1.11)cd	1.77 (1.51)b	3.47c	0.36 (0.92)b	1.63 (1.46)c	2.37c	0.76 (1.12)b	1.62 (1.46)c
Control	4.00c	0.82 (1.15)d	4.07 (2.14)e	6.73e	0.81 (1.14)d	3.89 (2.09)e	4.70d	0.97 (1.21)d	3.21 (1.93)f
SEM ±	0.091	0.012	0.014	0.124	0.024	0.011	0.089	0.006	0.015

[$] Means followed by the same letter do not differ significantly ($P>0.05$) by DMRT.[5] Figures in parentheses are $\sqrt{x+0.5}$ transformed values.

32.9%) effect. Chlorpyriphos completely inhibited spore production while lindane was least (26.3%) inhibitory; endosulfan, malathion and monocrotophos displayed intermediate (43.0 – 56.5%) effect.

Malathion, lindane and chlorpyriphos at the tested concentratins, affected radial growth of *B. brongniartii* to the tune of 60.9 – 66.9 per cent while endosulfan was less inhibitory (48.4%) (Table 2); monocrotophos showed least effect (37.6%) which, too, was significantly different from control. Chlorpyriphos and lindane produced no biomass; endosulfan, monocrotophos and malathion reduced biomass by 28.4 – 55.6 per cent. Chlorpyriphos and lindane completely inhibited spore production; endosulfan and malathion reduced spore production to a considerable extent (58.1 – 69.2%); monocrotophos showed least effect (13.4%) on spore production.

All insecticides at the concentrations tested, significantly affected radial growth of *M. anisopliae* more or less uniformly (49.6 – 57.4%) with only minor overlapping differences among themselves (Table 2). Chlorpyriphos and malathion produced undetectable biomass; monocrotophos, lindane and endosulfan reduced it by 13.4 – 21.6 per cent. Chlorpyriphos failed to produce any spores closely followed by malathion, which reduced spore production by 88.2per cent; lindane, monocrotophos and endosulfan reduced it by 17.1 – 49.5 per cent.

Insecticides differentially affected growth and sporulation of the three species of fungi in the present study. For example, both chlorpyriphos and lindane supported radial growth of *B. bassiana* but the latter alone produced spores normally. Similarly, no relationship was noticed between biomass and spores produced by *B. brongniartii* for different insecticides. Three neonicotinoid insecticides not only showed such differential effect on conidia germination, vegetative growth and conidiogenesis of *B. bassiana*, *M. anisopliae* and *Paecilomyces* sp. but also enhanced some parameters (Neves *et al.*, 2001). When different *in vitro* techniques were evaluated, *B. bassiana* produced variable response in parameters emphasizing the need to standardize protocols for compatibility tests (Silva and Neves, 2005). Besides underlining the importance of technique, these studies also highlighted the interplay of pesticide, concentration or rate and fungal species. Nevertheless, in view of the pronounced effects on vegetative growth and sporulation in some cases when pesticide-fungus mixture was incorporated in to the culture medium (Silva and Neves, 2005) and the importance of sporulation and spore survival in soil or plant surface for facultative entomopathogenic fungi, it is reasonable to regard spore output as a better indicator of pesticide toxicity.

When grouped on the basis of reduction in spore production as most toxic (70-100%), moderately toxic (30-70%) and least toxic (0-30%), insecticides differed in their toxicity to the three species of fungi: chlorpyriphos was most toxic (100%) to all three fungi; lindane was most toxic to *B. brongniartii* (100%) but least toxic to *B. bassiana* (26.3%) and *M. anisopliae* (17.1%); monocrotophos was moderately toxic to *B. bassiana* (43.0%) and *M. anisopliae* (35.2%), and least toxic to *B. brongniartii* (13.4%); malathion was most toxic to *M. anisopliae* (88.2%) and *B. brongniartii* (69.1%), and moderately toxic to *B. bassiana* (43.0%); endosulfan was moderately toxic to all three species (49.5 – 58.1%). Earlier studies that indicated chlorpyriphos and monocrotophos as inhibitory to the three fungi by up to 50 per cent at a higher concentration of 1000 ppm (Sharma and Gupta, 1998; Gupta *et al.*, 2002), though based on colony diameter that seemed to be less dependable in our studies, broadly agreed with the pattern in our studies by both radial growth and spore production. On the other hand, the inhibitory effect of linadane on *B. brongniartii* (Vyas *et al.*, 1990) based on sporulation, besides growth, was comparable to the method and effect observed in our study.

B. Compatibility of fungicides

The two fungicides significantly reduced growth parameters of the three fungi (Table 3). Carbendazim (0.05%) completely inhibited radial

growth of *B. bassiana* while mancozeb (0.08%) reduced it by 55.8 per cent. In a similar trend, carbendazim produced no biomass and mancozeb significantly reduced it (76.3%). Consequently, carbendazim failed to produce spores and mancozeb drastically reduced (85.7%) it. Carbendazim reduced radial growth of *B. brongniartii* by 33.1per cent while mancozeb reduced it by 65.2 per cent. Both fungicides at the respective concentrations completely affected biomass and spore production. Carbendazim completely inhibited radial growth, biomass and spore production of *M. anisopliae*. Mancozeb significantly reduced radial growth (30.8%), biomass (45.2%) and spore production (69.5%).

Using the same categorization of spore production followed for insecticides, carbendazim was most toxic to all three species of fungi with 100 per cent suppression of spore production, despite some radial growth in *B. brongniartii*. Mancozeb was also equally toxic to the three fungi with a slightly lower range of spore suppression (69.5 – 100.0%), notwithstanding the moderate levels of radial growth and biomass. Mancozeb reduced growth of *B. brongniartii* by 50 per cent at 1000 ppm (Sharma and Gupta, 1998) whereas it completely inhibited growth of *B. bassiana* at a lower 100 ppm; carbendazim at a lower concentration (100 ppm) was completely inhibitory to *B. bassiana* and *M. anisopliae* (Gupta *et al.*, 2002). With minor differences, these studies endorsed the relative toxicity of carbendazim and mancozeb observed in our studies.

C. Compatibility of weedicides

Weedicides at recommended and higher dosages significantly reduced growth and sporulation of *B. bassiana* (Table 4). Glyphosate at 0.205 and 0.3075 per cent inhibited radial growth least (7.7 – 16.3%); atrazine at 0.350 and 0.525 per cent moderately affected fungal growth (29.5 – 33.3%); 2,4-D at 0.200 and 0.300 per cent completely inhibited radial growth. Biomass showed a similar trend with dosage dependent suppression rates for glyphosate (19.1 – 25.5%), atrazine (38.3 – 44.7%) and 2,4-D (85.1 – 93.6%). Glyphosate at both

Table 3. Effect of fungicides on radial growth, biomass and spore production of three entomopathogenic fungi

Fungicide / Concentration (%)	*Beauveria bassiana*			*Beauveria brongniartii*			*Metarhizium anisopliae*		
	Radial growth (cm)	Biomass (g)	Spore production (× 10^{10}/100 ml broth)	Radial growth (cm)	Biomass (g)	Spore production (× 10^{10}/100 ml broth)	Radial growth (cm)	Biomass (g)	Spore production (× 10^{10}/100 ml broth)
Carbendazim (0.05)	0.00 (0.71)a	0.00 (0.71)a	0.00 (0.71)a	3.90a	0.00 (0.71)a	0.00 (0.71)a	0.00 (0.71)a	0.00 (0.71)a	0.00(0.71)a
Mancozeb (0.08)	1.77 (1.51)b	0.14 (0.80)b	0.58 (1.04)b	2.03b	0.00 (0.71)a	0.00 (0.71)a	3.37(1.97)b	0.23 (0.85)b	0.98(1.22)b
Control	4.00 (2.12)c	0.59 (1.04)c	4.07 (2.14)c	5.83c	0.44 (0.97)b	3.89 (2.09)b	4.87(2.32)c	0.42 (0.96)c	3.21(1.93)c
SEM ±	0.019	0.006	0.019	0.148	0.002	0.003	0.024	0.003	0.019

Figures in parentheses are $\sqrt{x+0.5}$ transformed values.

Means followed by the same letter do not differ significantly ($P>0.05$) by DMRT.

Table 4. Effect of weedicides on radial growth, biomass and spore production of three entomopathogenic fungi

Weedicide/ Concentration (%)	*Beauveria bassiana*			*Beauveria brongniartii*			*Metarhizium anisopliae*		
	Radial growth (cm)	Biomass (g)	Spore production (x 10^{10}/100 ml broth)	Radial growth (cm	Biomass (g)	Spore production (x 10^{10}/100 ml broth)	Radial growth (cm)	Biomass (g)	Spore production (x 10^{10}/100 ml broth)
Glyphosate (0.205)	3.60(2.02)*c	0.38c	0.44a	3.90b	0.51c	3.27c	3.43c	0.42b	1.37c
Glyphosate (0.3075)	3.97(2.11)d	0.35c	0.27a	4.57c	0.56f	2.45d	3.83c	0.33a	0.33a
Atrazine (0.35)	3.03(1.88)b	0.29b	2.20c	5.00d	0.42c	2.39d	4.63d	0.76d	1.58c
Atrazine (0.525)	2.87(1.83)b	0.26b	3.14d	4.40c	0.46d	1.54b	4.67d	0.66c	0.82b
2,4-D (0.20)	0.00(0.71)a	0.07a	2.00c	1.53a	0.07b	1.98c	2.97b	0.40b	2.70d
2,4-D (0.300)	0.00(0.71)a	0.03a	1.32b	1.23a	0.03a	0.58a	1.90a	0.31a	0.34a
Control	4.30(2.19)e	0.47d	3.70e	5.30d	0.605g	5.39f	5.13e	0.86e	3.28e
SEM ±	0.021	0.026	0.076	0.123	0.006	0.119	0.135	0.013	0.069

* Figures in parentheses are $\sqrt{x+0.5}$ transformed values. Means followed by the same letter do not differ significantly ($P>0.05$) by DMRT.

dosages reduced spore production most (88.1 – 92.7%) despite the lowest effect on radial growth and biomass; 2,4-D followed next with inhibition rates of 45.9 – 64.3 per cent while atrazine affected it least (15.1 – 40.5%).

Atrazine at both the tested concentrations affected radial growth of *B. brongniartii* least (5.7 – 17.0%) while 2,4-D at 0.200 and 0.300 per cent inhibited it most (71.1 – 76.8%); glyphosate at 0.205 and 0.3075 per cent showed intermediate effect (13.8 – 26.4%) (Table 4). Glyphosate affected biomass least (7.4 – 15.7%) while 2,4-D inhibited it most (88.4 – 95.0%); atrazine occupied intermediate position (24.0 – 30.6%). In a similar trend, the three weedicides inhibited spore production in a dosage-dependent manner in the increasing order as glyphosate (39.3 – 54.5%), atrazine (55.7 – 71.4%) and 2,4-D (63.3 – 89.2%).

Atrazine at both the concentrations affected radial growth of *M. anisopliae* least (9.0 – 9.7%) followed by glyphosate at 0.205 and 0.3075 per cent with inhibition rates (25.3 – 33.1%) not differing between concentrations; 2,4-D at 0.20 and 0.30 per cent affected radial growth most (42.0 – 62.9%) with significant differences between concentrations (Table 4). Atrazine inhibited biomass least (11.6 – 23.3%) while glyphosate (51.2 – 61.6%) and 2,4-D (53.5 – 64.0%) were significantly more inhibitory. Spore production was significantly reduced by glyphosate (58.2 – 89.9%), atrazine (51.8 – 75.0%) and 2,4-D (17.7 – 89.6%) with higher dosage showing greater inhibitory effect.

A comparison of the response of the three fungi on the basis of inhibition of spore production revealed that glyphosate at the recommended concentration (0.205%) was most toxic to *B. bassiana* (88.1%), and moderately toxic to *B. brongniartii* (39.3%) and *M. anisopliae* (58.2%). Atrazine at the recommended concentration (0.350%) was moderately toxic (40.5 – 55.7%) to all three fungi, while 2,4-D (0.20%) was moderately toxic to *B. bassiana* (45.9%) and *B. brongniartii* (63.3%), and least toxic (17.7%) to *M. anisopliae*. At the higher concentration, weedicides showed enhanced toxicity ratings.

Compatibility information from the present *in vitro* studies may be used to decide the right combination and time of application of pesticides and entomopathogenic fungi in sugarcane, particularly *B. brongniartii* which was found to be effective against white grubs as pressmud or lignite formulation applied to soil (Easwaramoorthy *et al.*, 2002 & 2004). The severe inhibitory effect of chlorpyriphos and lindane, the former recommended for the control of shoot borer and termites in sugarcane and the latter currently exempted for use against termites, on the fungus calls for asynchrony in application to harmonize their combined use. Similarly, carbendazim recommended as sett treatment and mancozeb applied as foliar spray warrant greater care since both were equally toxic to the fungus. Despite the moderate toxicity of glyphosate, atrazine and 2,4-D to the fungus in the increasing order at recommended concentrations, their use requires prudence since a greater proportion of these weedicides generally applied to soil or ground vegetation is likely to reach the resident entomopathogenic fungi in the soil. Although the current use of *B. bassiana* and *M. anisopliae* that naturally attack different borers in sugarcane is far from being extensive, pesticide compatibility will be an important consideration in their future exploitation. However, minimal pesticide usage in sugarcane (David, 1987), the possible differential reaction of pesticides with entomopathogenic fungi in the laboratory and field (Mietkiewski *et al.*, 1997) and planned application schedules enable their combined use.

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