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Original Article

COMPATIBILITY OF *BEAUVERIA BASSIANA* (BALS.) VUILL ISOLATES WITH SELECTED INSECTICIDES AND FUNGICIDES AT AGRICULTURE SPRAY TANK DOSE

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ABSTRACT

The effects of six commonly used chemical pesticides in which three are insecticides namely quinolphos, monocrotophos, cypermethrin and three fungicides carbendazim, cooper oxychloride and mancozeb on germination, mycelial growth of thirty isolates of Beauveria bassiana was studied. Except mancozeb all the insecticides and fungicides doesn't show a significant inhibition effect on the germination of conidia. There was significant inhibition of mycelia growth in few isolates with insecticides as well as fungicides except copper oxychloride. Copper oxychloride promoted the mycelia growth in many isolates where as mancozeb inhibited the mycelia growth of all the isolates tested in the present study. This work suggests that the most appropriate insecticide and fungicides for use in Integrated Pest Management Programs in combination with Beauveria bassiana isolates. It is important to test the compatibility of isolates of entomopathogenic fungi with the pesticides commonly used in IPM programmes.

Keywords: entomopathogenic fungi; *Beauveria bassiana*; Integrated Pest Management; fungicides.

INTRODUCTION

The introduction of artificial fungal inoculum should remain within the agricultural context for which they are developed. That is they should be integrated into other agrochemical measures for a given crop. An evaluation of effects of chemical pesticides on *B. bassiana* was taken up to identify tolerant isolates. *B. bassiana* isolates have been screened for tolerance to the chemical insecticides and fungicides Field efficiency of these fungi is majorly influenced by compatibility with other chemical protection strategies employed. Evaluation of effect of several fungicides on different parameters like germination, growth and virulence of commercially available entomopathogenic fungi are done (Shah *et al.* 2009). Sensitivity of entomopathogenic fungus *Isaria fumosorosa* to several commercial grade fungicides under *in vitro* and *in vivo* has been reported (Alessandro *et al.* 2011). Study on compatibility of *Metarhizium anisopliae* and *Pacilomyces fumosorosus* with selective insecticides has been reported (Ramzan asi *et al.* 2010). Compatability of *Beauveria bassiana* isolates with several pesticides by studying the effect on conidial germination, vegetative growth and sporulation. (Alizadeh *et al.* 2007). Studies on effect of mancozeb and copper oxychloride on growth and sporulation of *Metarhizium anisopliae* are proved to be highly sensitive to these agrochemicals. Effect of fungicides on *Nomuraea rileyi* were reported (Sosa- Gomez *et al.* 2003).

In the current investigation therefore, evaluated the effects of selected chemical pesticides (three insecticides and three fungicides) which are commonly used in agriculture fields on the germination and mycelial growth of *B. bassiana* isolates from locally field infected insects and isolates collected from national and international organizations.

MATERIALS AND METHODS

Chemical pesticides tested

The commercial formulations were used for the compatibility studies instead of the pure active ingredient. Instances in which the adjuvants and carriers in the formulation did affect the entomopathogenic fungus are known (Inglis *et al.* 2001). Sensitivity was tested at the field recommended concentration of the chemicals. The following insecticides were used in the present study such as organophosphate based formulations quinalphos(0.3%), monocrotophos(0.15%) and synthetic pyrethroid, cypermethrin (0.12%). The commercial formulation of following fungicides are used in the investigation are carbendazim(0.05%), copper oxychloride(0.3%) and mancozeb(0.3%). The conidial germination and mycelia growth bioassays were set up in quadruplicate.

Compatibility tests

The commercial products, active ingredients, recommended dosage and agricultural spray tank dose of each pesticide used were tested for compatibility with 30 isolates of *Beauveria bassiana* (Tables1 and 2) collected from various national, international collection centers and few isolates from locally infected insect pests.

Germination bioassay

Glass slides were sterilized by autoclaving. They were coated with 0.2 ml of molten SDAY medium in a Laminar air flow cabinet and allowed to air dry in the cabinet for 30 min. For each isolate two slides were used one as the control and the other as the test. On the control slide 100µl of conidial suspension (10^5 ml^{-1}) was deposited with a micropipette (Gilson, Germany). For the test, the test chemical was added to 10 ml of conidial suspension to a concentration at field recommended dose and mixed thoroughly on a vortex mixer. On the test slide 100µl of this suspension was dispensed. The slides were placed in moist chambers (Petri dishes (150 mm diameter) lined with moist filter paper). The slides were supported on glass rods in the Petri dish to prevent direct contact with the moist blotting paper and incubated at 25 ± 2°C for 24h. After incubation, the Petri dish covers were removed and the water droplets formed on the slides were allowed to air dry in a Laminar flow unit. The slides were then stained with lacto phenol cotton blue and examined with an inverted microscope. At least 200 conidia were examined for germination counts on each slide. (Fransen,1995). Conidia producing clearly visible germ tube were considered as germinated. Percent germination of conidia was calculated from these counts (Tables1 and 2).

Growth bioassay

The effect of the test chemicals on fungal growth was assessed by comparison of growth (as measured from dry mass) of the fungus in

a liquid medium with and without the test chemical. The growth bioassays were set up in 100 ml Erlenmeyer flasks. They were autoclaved with 15 ml of SDY medium. For each isolate, two cultures control and the test were set up. To the test flask, filter sterilized pesticide formulation was added in volumes required to achieve appropriate concentration (spray tank dose). A 0.1 ml of conidial suspension of 10^7conidia/ml concentration was inoculated into each flask and incubated in a rotary incubator shaker with 150 rpm at 25 °C for 10 days. The flasks were observed everyday to observe the initiation and progress of growth. (Storey and Gardner, 1986). At the end of 10 days the fungal mass in each flask was filtered, using previously weighed filter paper. The filter papers with the filtrates were oven dried to a constant weight and weighed (Table 1 and 2).

Statistical analysis

The germination and growth response in all the isolates to the field recommended dose of pesticides were analysed separately for each pesticide by one-way analysis of variance procedures utilizing the general linear models of StatSoft, Inc. (1995). Treatment means were separated by the Student-Newman-Keuls (SNK) test (Newman, 1939). Percentage inhibition of conidial germination and mycelial growth in test compared to the control was calculated.

RESULTS

Conidial germination was by and far not inhibited in most of the pesticide formulations (Tables 1). Germination of conidia was delayed by 10 to 12 hours in presence of pesticide formulation. Significant inhibition of mycelial growth was observed in most of the *B. bassiana* isolates in presence of pesticide formulations at field recommended dose (Tables 1). The fungicide mancozeb completely inhibited both conidial germination and mycelial growth in all the thirty *B. bassiana* isolates at agricultural spray tank

Table 1. Results of Percent inhibition of conidial germination mycelia and growth bioassay of thirty isolates of *Beauveria bassiana* in liquid medium (SDY) in presence of quinalphos formulation (0.15% v/v), monocrotophos formulation (0.15% v/v) cypermethrin (pyrethroid) (0.12% v/v) at agricultural spray tank dose of respective formulation for compatibility survey.

Isolate Number	% Inhibition of conidial Germination of *B. bassiana* isolates[a] (Mean ± S.E)[b]			% Inhibition of growth bioassay of *B. bassiana* isolates[c] (Mean ± S.E)[d]		
	Quinalphos	Monocrotophos	Cypermethrin	Quinolphos	Monocrotophos	Cypermethrin
NRRL 3108	12.06 ± 4.61^{ab}	17.28 ± 6.95^{b}	1.05 ± 2.08^{c}	9.39 ± 5.47	100	100
NRRL 22864	100 ± 0.00^{a}	36.54 ± 4.88^{a}	100 ± 0.00^{a}	100	100	100
NRRL 22865	100 ± 0.00^{a}	36.83 ± 6.36^{a}	2.41 ± 1.36^{c}	100	100	42.21 ± 2.54
NRRL 22866	1.26 ± 4.96^{c}	10.97 ± 2.86^{ab}	8.59 ± 3.50^{b}	100	100	100
NRRL 20698	2.08 ± 1.39^{e}	5.06 ± 1.73^{ab}	7.14 ± 3.89^{b}	51.27 ± 3.40	19.91 ± 2.08	100
NRRL 20699	100 ± 0.00^{a}	11.82 ± 5.86^{ab}	2.73 ± 1.67^{c}	100	8.08 ± 2.85	27.37 ± 3.43
NRRL 20700	2.65 ± 1.08^{c}	1.71 ± 3.48^{c}	1.30 ± 0.49^{c}	100	100	100
ARSEF 326	3.28 ± 3.71^{c}	3.59 ± 1.67^{c}	6.04 ± 4.18^{c}	100	100	100
ARSEF 739	1.69 ± 2.79^{c}	1.39 ± 0.85^{c}	4.30 ± 2.43^{c}	100	100	100
ARSEF 1149	0.66 ± 3.28^{c}	2.21 ± 0.85^{c}	1.05 ± 0.79^{c}	54.72 ± 1.51	100	100
ARSEF 1166	1.60 ± 1.17^{c}	2.13 ± 1.45^{c}	13.58 ± 4.71^{b}	100	22.63 ± 3.73	100
ARSEF 1169	2.31 ± 3.01^{c}	4.59 ± 5.40^{ab}	9.30 ± 6.24^{b}	100	100	100
ARSEF 1314	9.81 ± 5.10^{ab}	5.16 ± 2.95^{ab}	1.07 ± 0.29^{c}	5.10 ± 2.73	100	100
ARSEF 1315	0.76 ± 4.09^{c}	2.42 ± 1.84^{c}	3.17 ± 1.87^{c}	18.84 ± 5.86	100	100
ARSEF 1316	5.51 ± 3.35^{c}	0.59 ± 1.25^{c}	2.75 ± 2.37^{c}	100	100	100
ARSEF 1512	1.47 ± 3.00^{c}	3.10 ± 1.02^{c}	15.29 ± 2.84^{b}	100	100	100
ARSEF 1788	0.50 ± 3.42^{c}	10.26 ± 3.01^{ab}	4.47 ± 1.27^{c}	100	100	100
ARSEF 2860	1.33 ± 0.39^{c}	0.48 ± 1.92^{c}	1.18 ± 0.92^{c}	100	100	100
ARSEF 3041	1.83 ± 4.54^{c}	2.34 ± 0.61^{c}	1.24 ± 1.39^{c}	100	100	100
ARSEF 3120	3.95 ± 5.01^{c}	14.66 ± 7.61^{b}	5.92 ± 3.02^{c}	100	100	100
ARSEF 3286	100 ± 0.00^{a}	4.68 ± 1.02^{ab}	100 ± 0.00^{a}	100	100	100
ARSEF 3387	100 ± 0.00^{a}	9.33 ± 1.61^{ab}	2.54 ± 1.32^{c}	100	100	55.81 ± 2.30
ITCC 913	2.41 ± 1.61^{c}	1.42 ± 0.55^{c}	0.83 ± 0.71^{c}	32.61 ± 1.21	29.10 ± 2.73	29.35 ± 1.16
ITCC 1253	1.18 ± 2.65^{c}	1.12 ± 1.50^{c}	1.19 ± 2.20^{c}	100	13.73 ± 7.03	100
ITCC 4521	20.75 ± 3.50^{b}	4.17 ± 1.52^{ab}	2.81 ± 1.52^{c}	100	100	100
ITCC 4644	1.88 ± 2.26^{c}	1.60 ± 0.82^{c}	1.98 ± 1.72^{c}	100	100	100
ITCC 4688	1.91 ± 2.10^{c}	0.82 ± 0.38^{c}	1.31 ± 0.53^{c}	100	100	0.39 ± 0.25
BB2	0.83 ± 1.78^{c}	1.13 ± 0.72^{c}	2.97 ± 1.93^{c}	100	100	100
BB3	16.64 ± 5.73^{ab}	4.50 ± 2.22^{ab}	9.52 ± 2.79^{b}	100	100	100
BB4	2.96 ± 1.96^{c}	0.80 ± 0.53^{c}	3.97 ± 1.53^{c}	100	100	100

[a]Percent inhibition was calculated relative to the percent conidial germination in the control treatment: 100 – [(percent conidial germination in experimental treatment ÷ percent conidial germination in the control treatment) × 100].
[b]Mean (± SE) was calculated from four replicates.
Means in same column followed by same letter are not significantly different ($P \geq 0.05$, SNK test).

[c]Percent inhibition was calculated relative to the mycelial growth (in mg) in the control treatment: 100 – [(mycelial growth (in mg) in experimental treatment ÷ mycelial growth (in mg) in the control treatment) × 100].
[d]Means were calculated from four replicates (in space).
Rep. = Replicates, 1 to 6 were replication of the experiment (in time).

dose. At the spray tank dose quinalphos completely inhibited the conidial germination in the four isolates of *B. bassiana* where as in twenty six isolates no significant inhibition was observed (P=0.05) (Table 1). In twenty four isolates mycelial growth was completely inhibited and in six isolates partial inhibition of mycelial growth was observed in presence of spray tank dose of quinalphos (Table 1). Conidial germination was not much affected by monocrotophos formulations at agricultural spray tank dose, in seven isolates there was more than 10% of inhibition (Table 1). However, mycelial growth was completely inhibited in twenty five isolates (Table 1).

Cypermethrin has no significant effect on conidial germination of all thirty isolates at agricultural spray tank dose. However in twenty five isolates there was a complete inhibition of mycelial growth (Table 1). Among the five isolates in which mycelial growth was observed in presence of cypermethrin in one isolates ITCC4688 there was no significant inhibition. Bavistin completely inhibited (100%) the conidial germination in two *B. bassiana* isolates (NRRL 22864 and NRRL 22865) while a significant inhibition was observed in five isolates (Table 2). No significant inhibition of conidial germination was observed in the presence of copper oxychloride in all except two isolates. Complete inhibition of conidial germination was observed in one isolate (ARSEF-3041) and a significant inhibition in other isolate (ITCC 4688) (Table 2).

In growth bioassays, response ranging from complete inhibition to a significant increase in the mycelial growth was observed (Table 1 and 2). Exposure of conidia to the fungicide mancozeb at agricultural field spray tank dose was found detrimental to all the tested *B. bassiana* isolates. This fungicide completely inhibited the conidial germination and mycelial growth in all the tested *B. bassiana* isolates at agricultural spray tank dose.

Table 2. Percent inhibition of conidial germination and mycelial growth (in liquid medium) of *Beauveria bassiana* isolates by two fungicide suspensions at agricultural spray tank dose in compatibility survey.

Isolate number	% Inhibition of conidial Germination of *B. bassiana* isolates* (Mean ± S.E)		% Inhibition of mycelial growth of *B. bassiana* isolates* (Mean ± S.E)	
	Carbendazim	Copper oxychloride	Carbendazim	Copper oxychloride
NRRL 3108	2.16 ± 0.75^{c}	0.44 ± 1.07^{c}	100 ± 0.00^{a}	23.15 ± 4.00^{b}
NRRL 22864	100 ± 0.00^{a}	-4.36 ± 1.07^{c}	100 ± 0.00^{a}	-25.78± 3.44^{d}
NRRL 22865	100 ± 0.00^{a}	3.52 ± 1.31^{c}	100 ± 0.00^{a}	100 ± 0.00^{a}
NRRL 22866	24.33 ± 4.49^{b}	-1.30 ± 1.89^{c}	100 ± 0.00^{a}	-3.44 ± 2.66^{c}
NRRL 20698	0.84 ± 0.85^{c}	3.27 ± 1.13^{c}	100 ± 0.00^{a}	100 ± 0.00^{a}
NRRL 20699	39.33 ± 3.92^{ab}	-0.90 ± 1.17^{c}	100 ± 0.00^{a}	-57.23± 3.01^{ef}
NRRL 20700	54.20 ± 2.47^{ab}	1.96 ± 0.80^{c}	100 ± 0.00^{a}	34.93 ± 3.87^{ab}
ARSEF 326	36.82 ± 3.20^{ab}	0.47 ± 0.77^{c}	100 ± 0.00^{a}	100 ± 0.00^{a}
ARSEF 739	2.26 ± 1.74^{c}	0.97 ± 0.92^{c}	100 ± 0.00^{a}	47.11 ± 1.68^{ab}
ARSEF 1149	2.33 ± 1.49^{c}	3.06 ± 1.77^{c}	100 ± 0.00^{a}	27.01 ± 3.28^{b}
ARSEF 1166	3.51 ± 1.84^{c}	-1.14 ± 0.83^{c}	100 ± 0.00^{a}	-16.16± 5.82^{c}
ARSEF 1169	1.10 ± 0.34^{c}	1.86 ± 0.96^{c}	100 ± 0.00^{a}	100 ± 0.00^{a}
ARSEF 1314	2.15 ± 1.71^{c}	-1.14 ± 3.06^{c}	100 ± 0.00^{a}	-15.48 ± 2.04^{c}
ARSEF 1315	0.61 ± 0.55^{c}	1.06 ± 0.97^{c}	5.32 ± 0.86^{ab}	100 ± 0.00^{a}
ARSEF 1316	0.65 ± 0.65^{c}	-1.08 ± 0.64^{c}	3.27 ± 0.31^{b}	-28.92 ± 5.35^{cd}
ARSEF 1512	4.41 ± 0.84^{c}	-1.46 ± 1.47^{c}	100 ± 0.00^{a}	-15.04 ± 3.17^{c}
ARSEF 1788	14.04 ± 4.51^{bc}	2.04 ± 0.86^{c}	100 ± 0.00^{a}	100 ± 0.00^{a}
ARSEF 2860	10.28 ± 1.66^{bc}	1.79 ± 0.35^{c}	100 ± 0.00^{a}	100 ± 0.00^{a}
ARSEF 3041	14.45 ± 6.41^{bc}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}
ARSEF 3120	46.99 ± 2.08^{ab}	4.12 ± 2.98^{c}	100 ± 0.00^{a}	-28.45 ± 2.45^{cd}
ARSEF 3286	13.80 ± 5.44^{bc}	2.93 ± 0.16^{c}	100 ± 0.00^{a}	-25.39 ± 3.82^{cd}
ARSEF 3387	2.50 ± 1.38^{c}	0.95 ± 0.64^{c}	100 ± 0.00^{a}	41.75 ± 2.23^{ab}
ITCC 913	1.09 ± 1.41^{c}	1.04 ± 0.39^{c}	4.58 ± 0.57^{b}	11.70 ± 1.68^{b}
ITCC 1253	5.35 ± 3.79^{c}	-3.27 ± 1.98^{c}	100 ± 0.00^{a}	-50.23 ± 3.52^{ef}
ITCC 4521	53.38 ± 3.03^{ab}	17.44 ± 2.27^{b}	100 ± 0.00^{a}	30.77 ± 2.08^{ab}
ITCC 4644	1.47 ± 0.88^{c}	-0.02 ± 0.45^{c}	100 ± 0.00^{a}	-57.31 ± 2.51^{ef}
ITCC 4688	4.92 ± 1.70^{c}	32.47 ± 4.51^{ab}	100 ± 0.00^{a}	46.13 ± 2.46^{ab}
BB2	1.44 ± 0.64^{c}	-2.72 ± 0.14^{c}	100 ± 0.00^{a}	-59.57 ± 2.34^{ef}
BB3	2.28 ± 2.10^{c}	-3.61 ± 1.42^{c}	100 ± 0.00^{a}	-86.25 ± 8.77^{e}
BB4	2.47 ± 1.80^{c}	-1.34 ± 1.79^{c}	100 ± 0.00^{a}	-28.92 ± 5.02^{cd}

[1]Percent inhibition was calculated relative to the percent conidial germination/mycelial growth in the control treatment: 100 – [(percent conidial germination/mycelial growth in experimental treatment ÷ percent conidial germination/mycelial growth in the control treatment) × 100].

[2]Mean (± SE) was calculated from four replicates.

Means in same column followed by same letter are not significantly different ($P \geq 0.05$, SNK test).

DISCUSSION

In the present study, three chemical insecticides and three fungicides tested for their compatibility with thirty *Beauveria bassiana* isolates by germination and mycelial growth bioassay. Germination of conidia was not significantly inhibited by pesticide formulations except fungicide mancozeb which inhibited germination in all the isolates. Whereas mycelial growth was significantly inhibited in most of the isolates tested.

Quinolphos has inhibited mycelia growth of *B. bassiana* in 24 of 30 isolates but no significant inhibition of conidial germination in 26 isolates. Among thirty isolates of *B. bassiana* five isolates showed tolerance with monocrotophos in mycelial growth assay whereas no significant inhibition of conidial germination in all isolates. In association with our results Uma devi *et al*. (2004) reported that few isolates of *B. bassiana* showed tolerance towards organophosphates. Also they explained the mechanism underlying the tolerance to organophosphates. Ambethgar *et al*. (2008) reported that quinolphos and monocrotophos showed a moderate fungistatic effect on *B. bassiana* isolates at field recommended dose. Cypermethrin a synthetic pyrethroid had no significant effect on conidial germination in 28 isolates, whereas in 25 isolates inhibited the mycelial growth. In contrast to our results Barci *et al*. (2009) reported that cypermethrin and *B. bassiana* found compatible. In association with our results, Cazorla and Morales (2010) reported that cypermethrin was not found compatible with field recommended dose by inhibiting conidial germination. Insecticide permethrin is found synergistic with *B. bassiana* in controlling West African insecticide-resistant *Anopheles gambiae* mosquitoes (Farenhorst, 2013).

The three fungicides tested for their compatibility with *B. bassiana* isolates exhibited varying results. Mancozeb was found detrimental to all the isolates of *B. bassiana* tested in the present study. It totally inhibited conidial germination. In fact, mancozeb is used for preventing *B. bassiana* infection in silkworm rearing (Kiran *et al.* 2011). Bavistin is a conversion product of benomyl which is used in germination bioassays to slow down the growth of germ tubes (Goettel *et al.* 2000). Counts of germinated conidia can be made more accurately when the germ tubes of nearby conidia do not anastomose. The germination pegs in most of the *B. bassiana* isolates showed an abnormal bent appearance with bursting at the tip in the presence of bavistin. Only three isolates (ARSEF 1315, ARSEF 1316 and ITCC 913) showed normal germ tube morphology in the presence of bavistin and showed growth in the growth bioassay. Thus three bavistin tolerant isolates were identified in the present study when 30 isolates were screened. The basis for decreased sensitivity to MBC in the isolates of *B. bassiana* was reported by Butters *et al.* (2003). The *B. bassiana* isolates differed in their response to copper oxychloride fungicide. While germination was completely inhibited in only one of the thirty isolates, growth was completely inhibited in 8 isolates. In some isolates growth was stimulated in the presence of this fungicide while in others, growth was inhibited - inhibition ranging from slight to significantly less than the control. Martins *et al.* (2012) reported that copper oxychloride inhibited the growth of *B. bassiana* but found less toxic when compared to other copper based systems. In fact copper is used in the medium for selective growth of entomogenous fungi (Akello *et al.* 2007). Though some highly sensitive to copper oxychloride, *B. bassiana* isolates have been found in the present study, the majority of the isolates (~ 2/3rds of the 30 tested) were found tolerant to copper oxychloride.From results of this work indicates that copperoxy chloride is the best fungicide formulation in combination with entomopathoenic fungi *B. bassiana* to use in integrated pest management program. Further field trails under green house should be done to confirm the synergistic performance of *B. bassiana* and fungicides or insecticides with integrated pest management approach.

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