Biocontrol Science and Technology, 2007; 17(10): 1059–1069

Taylor & Francis
Taylor & Francis Group

Growth and insect assays of *Beauveria bassiana* with neem to test their compatibility and synergism

MURALI C. MOHAN[1], NARASIMHA P. REDDY[2], UMA K. DEVI[2], RAMESH KONGARA[3], & HARI C. SHARMA[4]

[1]*Department of Biotechnology, Gandhi Institute of Technology and Management, Visakhapatnam, India,* [2]*Department of Botany, Andhra University, Visakhapatnam, India,* [3]*Navaneetha Evergreens, Tarluvada, Visakhapatnam District, India, and* [4]*Entomology Division, ICRISAT, Patancheru, India*

(Received 26 July 2007; returned 11 September 2007; accepted 27 September 2007)

Abstract
Beauveria bassiana is being used as a biopesticide for many insect pests. Neem oil (azadirachtin) is an eco-safe popular botanical pesticide. A biopesticde with a neem compatible isolate of *B. bassiana* will enable their simultaneous use in pest management. A sample of 30 isolates of *B. bassiana* from culture collections was screened for compatibility with a commercial formulation of neem oil (Margoside®) at the field recommended dose (0.3%, v/v). Compatibility was tested *in vitro* through germination and growth assays. In all isolates, conidial germination was delayed but not significantly decreased by neem. In the growth assays, 23 isolates were found compatible with neem. In the neem sensitive isolates, growth was decreased but not totally inhibited. The effect of combined treatment with *B. bassiana* and neem in comparison to single treatments with either of them on *Spodoptera litura* Fabricius was tested in laboratory bioassays. The combined treatment was found to have synergistic effect on insect mortality when a *B. bassiana* isolate compatible with neem was used, while, with an isolate sensitive to neem, an antagonistic effect was observed.

Keywords: *Azadirachtin,* Beauveria bassiana*, compatibility, germination and growth assays,* Spodoptera litura*, combination treatment*

Introduction

The integration of microbial pesticides with chemical pest management practices requires detailed compatibility studies. Data from such studies would enable farmers to select appropriate compounds and schedule microbial and chemical pesticide treatments such that benefits from compatible sets can be accrued and, with non-compatible pairs, the deleterious effect of the chemical on the microbe in the biopesticide can be minimized (Butt et al. 2001; Inglis et al. 2001; Lacey et al. 2001). A microbial pesticide compatible with a commonly used chemical pesticide can be used simultaneously or sequentially with it. A sub-lethal dose of chemical pesticide can act as a physiological stressor or behavioural modifier of the insects thereby

Correspondence: K. Uma Devi, Department of Botany, Andhra University, Visakhapatnam, 530 003, A.P., India. Tel: +91 891 2525582. Fax: +91 891 2755547. E-mail: umadevikoduru@gmail.com

ISSN 0958-3157 print/ISSN 1360-0478 online
DOI: 10.1080/09583150701714551

predisposing them to the action of the microbe in the biopesticide and also potentially expanding its host range (Inglis et al. 2001).

Beauveria bassiana (Balsamo) Vuillemin (Hypocreales; Clavicipitaceae) with a broad host range of ~700 insect species is a registered biopesticide used for management of several crop insect pests (Butt et al. 2001). Oil extracted from the seeds of neem (*Azadirachta indica* (A.) Juss) is being widely applied as a broad-spectrum pesticide against fungal, viral, nematode, and insect pests of crops (John 1999). Neem oil has been reported to be effective on more than 400 insect species (Atwal & Dhaliwal 1997). The most touted biologically active constituent of neem oil is the highly oxygenated azadirachtin and some of its natural analogues and derivatives (Varma & Dubey 1999). This botanical pesticide is considered ecologically benign because it lacks poisonous chloramine, phosphorous or nitrogen atoms present in chemical pesticides. Azadirachtin formulations have been found to act as ovipositional deterrents for several insects (Atwal & Dhaliwal 1997; Singh 2003). They are also reported to affect metamorphosis of insects leading to a decrease in their population (Mathur & Nigam 1993; John 1999; Gahukar 2000; Filotas et al. 2005). The compatibility of isolates of *B. bassiana* with azadirachtin formulations has been investigated previously (Rodríguez et al. 1997; Bajan et al. 1998; Gupta et al. 1999; Depieri et al. 2005). However, few isolates were tested and contradictory results have been reported. For example, neem oil was found compatible with *B. bassiana* by Rodríguez et al. (1997) but was reported to be inhibitory by Bajan et al. (1998) and Depieri et al. (2005). Compatibility tests with a large sample of isolates would indicate the general response of this fungus to neem. Therefore, we screened a large sample of 30 *B. bassiana* isolates from international culture collections and few local (Indian) isolates for compatibility with neem oil.

Oils are reported to facilitate adhesion of fungal conidia (Prior et al. 1988) and substantially reduce their effective lethal concentration (as measured from LC_{50}) (Prior et al. 1988; Bateman et al. 1993; Akbar et al. 2005). It has also been hypothesized that the prolongation of the intermoult period of insect larvae by the growth regulating action of azadirachtin may give time for the establishment and penetration of fungal conidia through the insect cuticle (Akbar et al. 2005; Filotas et al. 2005). The effect of treatment with neem oil and *B. bassiana* together in comparison to treatment with either of them alone was tested in insect bioassays.

Materials and methods

Compatibility tests

A sample of 30 isolates of *B. bassiana* from disparate insect hosts and geographic regions was screened for compatibility with neem oil (Table I). A commercial formulation of neem oil, Margoside® CK 20 EC (with 0.15% active ingredient azadirachtin; M/s Monofix Agroproducts Ltd, Hubli, India), was used. The adjuvant and carrier in insecticide formulations have been reported to affect growth of entomopathogenic fungi (Inglis et al. 2001). Therefore, a formulation of neem oil rather than the pure form of the active ingredient (azadirachtin) was used in the assays. Compatibility was tested at the field recommended concentration of 0.3% (v/v).

Table I. Effect of Margoside® (a commercial formulation of neem oil with 0.15% azadirachtin) on germination and mycelial growth of a sample of 30 isolates of the entomopathogenic fungus *Beauveria bassiana*.

Isolate Accession No*	Host insect	Geographic origin	Germination assay		Growth assay	
			Inhibition/Enhancement†‡	Delay (h)	Inhibition/Enhancement†	*t* value (*P*)‡
NRRL 3180	*Ostrinia nubilalis*	Unknown	1.18±0.79 [a]	3.5±1.5	19.44±3.8 [d]	18.58 (<0.001)[hs]
NRRL 22864	*Glichrochilus quadrisignatus*	Illinois, USA	1.12±0.80 [a]	1.0±1.0	20.44±1.76 [d]	18.58 (<0.001)[hs]
NRRL 22865	Unknown	Iowa, USA	0.19±0.04 [b]	1.0±1.0	21.24±1.20 [d]	18.58 (<0.001)[hs]
NRRL 22866	*Pachnaeus litus*	Florida, USA	1.16±0.14 [a]	0.5±0.5	5.91±1.26 [c]	0.23 (0.82)[ns]
NRRL 20698	*Dysdercus koenigii*	Lima, Peru	0.05±0.73 [c]	4.0±1.0	11.54±0.95 [cd]	1.64 (0.11)[ns]
NRRL 20699	Unknown	Illinois, USA	0.17±0.38 [b]	4.0±1.0	22.47±3.09 [d]	18.58 (<0.001)[hs]
NRRL 20700	*Popillia japonica*	Unknown	1.11±0.45 [a]	4.0±1.0	19.34±3.03 [d]	18.58 (<0.001)[hs]
ARSEF 326	*Chilo plejadellus*	Queensland, Australia	0.14±1.84 [b]	5.0±1.0	0.15±0.76 [ab]	2.69 (0.01)[s]
ARSEF 739	*Diabrotica paranoense*	Goias, Brazil	0.10±0.09 [b]	7.0±1.0	14.35±3.10 [cd]	1.64 (0.11)[ns]
ARSEF 1149	*Helicoverpa armigera*	Cordoba, Spain	0.06±0.87 [c]	1.0±1.0	18.30±3.99 [d]	18.58 (<0.001)[hs]
ARSEF 1166	*Helicoverpa armigera*	Cordoba, Spain	0.13±0.07 [b]	1.0±1.0	5.59±3.81 [c]	0.23 (0.82)[ns]
ARSEF 1169	*Sitona lineatus*	Senneville, France	0.11±0.01 [b]	1.0±1.0	5.62±2.77 [c]	0.23 (0.82)[ns]
ARSEF 1314	*Helicoverpa virescens*	La Miniére, France	0.16±0.01 [b]	4.0±1.0	0.12±0.36 [ab]	2.69 (0.01)[s]
ARSEF 1315	*Helicoverpa virescens*	La Miniére, France	0.15±0.08 [b]	4.0±1.0	18.74±4.08 [d]	18.58 (<0.001)[hs]
ARSEF 1316	*Helicoverpa virescens*	La Miniére, France	0.18±0.11 [b]	0.5±0.5	48.05±4.57 [b]	13.39 (<0.001)[hs]
ARSEF 1512	*Spodoptera littoralis*	La Miniére, France	1.12±0.5 [a]	3.0±1.0	5.05±3.83 [c]	0.23 (0.82)[ns]
ARSEF 1788	*Helicoverpa virescens*	Spain	0.14±0.12 [b]	1.0±1.0	5.61±3.03 [c]	0.23 (0.82)[ns]
ARSEF 2860	*Schizaphis graminum*	Idaho, USA	0.14±0.16 [b]	3.0±1.0	3.55±2.54 [c]	0.23 (0.82)[ns]
ARSEF 3041	*Reticulitermis flavipes*	Toronto, Canada	1.13±0.75 [a]	7.0±1.0	0.15±0.4 [ab]	2.69 (0.01)[s]
ARSEF 3120	*Senecio* sp.	Yvelines France	0.13±0.23 [b]	1.0±1.0	4.36±2.7 [c]	0.23 (0.82)[ns]
ARSEF 3286	*Spodoptera littoralis*	Montpellier, France	1.27±0.05 [a]	1.0±1.0	0.48±0.75 [a]	13.70 (<0.001)[hs]
ARSEF 3387	*Myzus persicae*	Washington, USA	1.06±4.08 [a]	1.0±1.0	6.40±3.71 [c]	0.23 (0.82)[ns]
ITCC 913	Unknown	The Netherlands	0.13±0.13 [b]	3.0±1.0	5.98±2.97 [c]	0.23 (0.82)[ns]
ITCC 1253	*Musca domestica*	Mumbai, north India	0.12±0.18 [b]	9.0±1.0	0.45±0.28 [a]	13.70 (<0.001)[hs]
ITCC 4521	*Diatraea saccharalis*	Karnal, north India	1.13±0.32 [a]	3.0±1.0	9.71±2.51 [cd]	1.64 (0.11)[ns]
ITCC 4644	*Deanolis albizonalis*	Ambajipeta, south India	0.16±0.02 [b]	0.5±0.5	0.11±0.56 [ab]	0.23 (0.82)[ns]
ITCC 4688	*Helicoverpa armigera*	Hyderabad, south India	0.18±0.36 [b]	5.0±2.0	6.85±2.2 [c]	0.23 (0.82)[ns]

Table I (*Continued*)

Isolate Accession No⋆	Host insect	Geographic origin	Germination assay		Growth assay	
			Inhibition/Enhancement[†‡]	Delay (h)	Inhibition/Enhancement[†]	*t* value (*P*)[‡]
BB2	*Spodoptera litura*	Bangalore, south India	1.14±0.06 [a]	1.0±1.0	0.51±0.18 [a]	13.70 (<0.001)[hs]
BB1	Soil	Bangalore, south India	0.17±0.12 [b]	4.0±2.0	5.88±3.79 [c]	0.23 (0.82)[ns]
BB4	*Helicoverpa armigera*	Warangal, south India	1.19±0.04 [a]	1.0±1.0	18.81±3.01[d]	18.58 (<0.001)[hs]

⋆ARSEF isolates are from the USDA ARS collection of entomopathogenic fungal cultures, Ithaca, NY, USA; NRRL isolates are from the entomopathogenic fungal culture collection, Peoria, IL, USA; ITCC isolates are from the Indian type culture collection, IARI, Delhi, India; BB isolates were isolated from insects collected in local fields and are yet to be accessioned. [†]Conidial germination/mycelial growth in test ÷ conidial germination/mycelial growth in control; values 1 and above indicate enhancement and below 1 indicate inhibition. Values followed by the same superscript letter have a similar response (SNK test). [‡]'*t*' value computed to test the significance of difference between test and control among isolates with similar response (with similar superscript letters); [hs]highly significant ($P<0.001$); [s]significant ($P<0.05$); [ns]not significant (>0.05). The '*t*' value computed for germination test for values with superscript [a, b] is 0.22 and 0.18, respectively, both of which are not significant.

The fungal cultures were established on Sabouraud dextrose agar medium with yeast extract (SDAY) (1% neopeptone, 4% dextrose, 1.5% agar and 1.0% yeast extract, pH 5.6 ± 0.2) from conidia stored in glycerol at -20°C. Conidia from 15-day-old culture slants were scraped with a spatula. Aqueous suspensions of the conidia were made with 0.01% Tween 80 (Polyethylene glycol sorbitan monooleate; Sigma-Aldrich, India). Conidial concentration in the suspension was estimated with a haemocytometer and then adjusted to required concentrations with water.

The viability of conidia was checked by germination assay on solid medium as described by Varela and Morales (1996). Autoclaved glass slides were each coated thinly with 200 μL of molten SDAY medium amended with benomyl (0.005%) (Benlate®, Du-Pont, India), penicillin (0.04%) (Sigma-Aldrich) and streptomycin (0.1%) (Sigma-Aldrich). Aqueous conidial suspension (100 μL with 10^5 conidia) was inoculated on the medium coated glass slide and incubated at $25 \pm 2^{\circ}$C for 24 h. Germination percentage was estimated by counting in ~ 10 different regions across the area under the coverslip with an inverted microscope (IX51, Olympus, India). Conidia with germ tubes at least as long as their diameter were considered as germinated. Cultures with more than 95% viable conidia were used in the experiments.

Germination and growth assays

Germination and growth assays were done as described by Fransen (1995). Conidial germination was tested on glass slides coated with SDAY medium. The control slides were coated with SDAY, and the test slides, with SDAY containing 0.3% Margoside. Conidial germination was computed as described above. The slides were observed at hourly intervals starting from 8 h after inoculation. The germination time was considered as the time by which $>50\%$ conidia germinated. The total number of conidia that germinated was counted 1 h post germination time. The effect of Margoside on fungal growth was assessed by comparison of growth (as measured from dry mass) of the fungus in liquid medium (SDY) with and without Margoside (0.3%, v/v). Cultures were initiated in 250 mL conical flasks containing 100 mL of SDY medium by inoculation of 1 mL of conidial suspension with $\sim 10^8$ conidia. The cultures were incubated at $25 \pm 1^{\circ}$C in an orbital incubator shaker at 100 rpm for 10 days. On the 11th day, each culture was filtered onto a Whatman No. 1 filter paper. The mycelial mass on the filter paper was washed with sterile distilled water, blotted on a blotting paper, placed in a Petri dish lined with a blotting paper and dried to a constant weight in an oven at 80°C. Four replicates were set up for each isolate in both germination and growth assays, and the experiment was repeated four times.

Insect bioassays

Second generation larvae from laboratory-reared *Spodoptera litura* (Lepidoptera; Noctuidae), at ICRISAT (International Crop Research Institute for Semi Arid Tropics, Patancheru, India) established from field-collected insects were bioassayed. Treatments were done on second instar larvae. Three *B. bassiana* isolates: ITCC 4688, ARSEF 1314 and BB1 which were found in the growth assays to be compatible, mildly sensitive and highly sensitive to Margoside®, respectively, were tested.

A detailed bioassay was done with the neem compatible isolate ITCC 4688. In field sprays, most of the inoculum falls on the canopy rather than directly on the insects.

Therefore, the insects are potentially infected from inoculum picked up from the leaf surface directly by contact. The bioassay was designed to examine insect mortality when inoculum was applied directly to the larva or to the leaf (which is used as a feed). Three types of treatments were done: (1) Margoside at field recommended concentration of 0.3% (v/v), (2) aqueous conidial suspension of *B. bassiana* (with 0.01% Tween 80) at a range of conidial concentrations: 10^5 to 10^7 viable conidia/mL, and (3) an aqueous mixture of 0.3% (v/v) Margoside and different (10^5 to 10^7 viable conidia/mL) concentrations of *B. bassiana* (with 0.01% Tween 80). When insects were directly treated, 100 μL of the inoculum was applied to each larva using a micropipette, taking care to completely spread it on the entire surface of the insect. In leaf treatments, 100 μL of inoculum was dispensed with a micropipette on a castor (*Ricinus communis*) leaf disc of 3.75 cm diameter spreading it with a pipette tip on the entire surface of the leaf disc.

With the Margoside sensitive isolates ARSEF 1314 and BB1, only larvae were treated and one *B. bassiana* concentration (10^7 viable conidia/mL) was tested.

Larvae treated with 0.01% Tween 80 in water served as controls. Each treatment batch consisted of 30 larvae. The larvae were placed individually in perforated round plastic boxes (3.75 × 7.50 cm) with lids and provided three castor leaf discs per day. In leaf treatments, the larvae were provided with inoculum treated leaf discs for three consecutive days followed by untreated leaf discs. The insect boxes were arranged in a completely randomized block design with three replicates per treatment (Goettel & Inglis 1997) in an environmental chamber maintained at 25 ± 1°C, 90% humidity, and 16/8 h (light/dark) cycle. Mortality of the larvae was recorded daily till the larvae in the treatment batch either died or pupated. Dead larvae were placed individually in Petri dishes lined with moist filter paper to facilitate mycosis. The bioassays were repeated twice.

Data analysis

In the compatibility assays, for each isolate, the ratio of conidial germination or mycelial growth in the test (0.3% Margoside) to the control (with no Margoside) was calculated for each replicate experiment and the mean ± SE computed. A value of 1 and above indicates compatibility; >1 denotes enhancing effect and <1, an inhibitory effect of neem. To identify isolates which showed similar response, the mean values of the ratios were separated by Student–Newman–Keuls (SNK) test (Newman 1939). For each group of isolates that showed similar response, the level of significance of their response (difference between test and control) was determined by a Student's *t*-test

In the insect bioassays, mortality in treatments was corrected for control mortality (Abbott 1925). The number of insects that showed mycosis was calculated as a proportion of the total number of dead insects. Mortality and mycosis values were arcsine percent square root transformed to normalize the distribution and the mean ± SE in each treatment was back transformed (Gomez & Gomez 1984). The median lethal time was calculated from the cumulative mortality data on each day of observation using survival analysis with a Weibull distribution (Lee 1992). Median lethal concentration (LC_{50}) was calculated through probit analysis.

For treatments with the neem-compatible isolate ITCC 4688, the significance of differences in mortality and mycosis caused in different modes of exposure (leaf/larva)

and types of exposure (single/combined) was assessed through a two-way ANOVA. The nature of the interaction between *B. bassiana* and neem in combination treatments was determined by probabilistic assessment (UNSCEAR report 1982). The interaction factor ω ($P_1 + P_2$) was calculated as: $\omega = \Delta P_{obs}/\Delta P_{exp}$, where ΔP_{obs} and ΔP_{exp} are the observed and expected probability of mortality in simultaneous treatment. ΔP_{exp} is computed as $\Delta P_{exp} = P_1 + P_2 - (P_1 \times P_2/1 - P_0)$ where P_1, P_2 and P_0 are the probability of mortality in *B. bassiana*, Margoside treatments and control, respectively. A value of $\omega = 1$ corresponds to additivity, $\omega > 1$ to synergism and $\omega < 1$ to antagonism (UNSCEAR report 1982). Statistical analysis was done with SPSS and Statistica software packages (Version 7.5, SPSS Windows user's guide, Chicago, 1996; Version 6.0, Statistica for windows 1995).

Results

Germination and growth assays

No significant effect on percentage conidial germination was found in the presence of neem in any of the *B. bassiana* isolates (Table I). However, in all isolates germination was delayed in the presence of neem (Table I). The time delay ranged from 0.5 to 9 h. However, this initial time delay in germination was not reflected in all isolates in subsequent growth rate. Mycelial growth in the presence of neem was inhibited in seven isolates, enhanced in nine isolates while in the remaining 14 isolates, growth was similar to controls. Thus, from growth assays, 23 of the 30 isolates tested were inferred as neem compatible (Table I). No relationship between the original host insect or geographical origin of the fungal isolate and its sensitivity to neem was observed (Table I).

Insect bioassays

In the bioassay with the Margoside-compatible isolate ITCC 4688, significant differences were observed in mortality between the different modes (leaf vs. larva: $F = 10.98$, df = 6, $P = 0.0002$) and types (Margoside, *B. bassiana* and a combination of the two $F = 3.69$, df = 12, $P = 0.01$) of treatment. Direct application on the insect was more effective than treatment on the leaf (Table II). Combination treatment with Margoside and ITCC 4688 resulted in higher mortality and lower LT_{50} and LC_{50} values than single treatments with either of them alone (Tables II and III). Differences in mycosis on insect cadavers in different modes and types of treatment were however not significant (leaf vs. larva: $F = 2.33$, df = 5, $P = 0.11$; *B. bassiana* alone vs. *B. bassiana* + Margoside: $F = 0.71$, df = 10, $P = 0.7$).

In the bioassays with ARSEF 1314, an isolate mildly sensitive to neem, the combination treatment did not result in enhanced effect – mortality was slightly lower and LT_{50} value was higher in combination treatment compared to treatment with the fungus alone (Table II). In combination treatment with the isolate BB1, which was found highly sensitive to neem, there was a reduced effectiveness in comparison to treatment with the fungus alone – insect mortality and mycosis decreased and LT_{50} increased (Table II).

The overall interaction of *B. bassiana* and neem in combination treatment (as assessed from insect mortality) was synergistic with the neem tolerant isolate but antagonistic with neem sensitive isolates (Table II).

Table II. A comparison of response of second instar larvae of *Spodoptera litura* to treatment with the entomopathogenic fungus *Beauveria bassiana*, 0.3% (v/v) Margoside® (a commercial formulation of neem oil with 0.15% azadirachtin) and a combination of the two.

		% Mortality		% Mycosis		LT_{50} in days[†] (95% CI)	
Isolate*	Treatment	Larva	Leaf	Larva	Leaf	Larva	Leaf
ITCC 4688 (NS)	10^5 conidia/mL	41.5±0.3	37.7±1.7	86.7±2.2	83.2±3	8.01 (6.8–11)	8.1 (7.2–9.3)
	10^6 conidia/mL	66.7±1.2	53.3±1.1	90.4±2.7	88±3	5.7 (5.2–6.2)	6.5 (5.6–7.9)
	10^7 conidia/mL	79.0±1.3	68.0±1.2	90.8±3.4	93.8±1.6	4.82 (4.3–5.3)	4.85 (4.4–5.2)
ITCC 4688 (NS)	10^5 conidia/mL+0.3% Margoside	52.2±1.3	52.2±1.7	89.6±1.9	84.8±3.7	6.8 (6.0–9.9)	7.29 (6.6–8.1)
	10^6 conidia/mL+0.3% Margoside	73.4±1.2	68.9±0.7	88.6±3.7	88.8±1.3	5.42 (5.1–5.7)	5.32 (4.7–5.9)
	10^7 conidia/mL+0.3% Margoside	95.7±1.5 1.25[‡]	81.3±4.2	97.7±4.5	89.6±1.6	3.93 (3.6–4.2)	4.50 (4.2–4.7)
ARSEF 1314 (S)	10^7 conidia/mL	89.0±0.3	–	71.6±0.03	–	4.65 (4.1–5.01)	–
	10^7 conidia/mL+0.3% Margoside	88.8±0.1 0.96[‡]	–	76±0.03	–	4.84 (3.7–5.7)	–
BB 1 (HS)	10^7 conidia/mL	98.7±0.1	–	80.9±0.04	–	3.69 (3.11–4.15)	–
	10^7 conidia/mL+0.3% Margoside	90.2±4.2 0.90[‡]	–	62.9±0.01	–	5.37 (4.41–6.23)	–
–	0.3% Margoside	38.9±1.3	42.2±0.6	–	–	–	–

*NS, S and HS; not sensitive, sensitive and highly sensitive, respectively, to 0.3% Margoside. [†]CI, confidence interval. [‡]ω value: a value of 1 corresponds to additivity, >1 to synergism and <1 to antagonism between the components in combination treatment.

Table III. The effective lethal dose (LC_{50}) of *Beauveria bassiana* isolate ITCC 4688 on *Spodoptera litura* when applied alone and in combination with 0.3% Margoside® a commercial formulation of neem oil with 0.15% azadirachtin).

Treatment					
On	Single/combined	LC_{50}	95% CI	χ^2(df = 2)	*P*
Leaf	*B. bassiana*	3.6×10^5	1.9×10^5–6.7×10^5	2.51	0.29
	B. bassiana + Margoside®	0.4×10^5	0.1×10^5–1.1×10^5	0.48	0.79
Larva	*B. bassiana*	2.1×10^5	1.1×10^5–4.0×10^5	1.27	0.53
	B. bassiana + Margoside®	0.5×10^5	0.3×10^5–2.2×10^5	5.67	0.59

CI, confidence interval; df, degrees of freedom; *P*, probability.

Discussion

A majority of the *B. bassiana* isolates, most of them accessioned and available in culture collections, were found compatible with the commercial azadirachtin formulation. In the presence of neem, conidial germination slowed down in all isolates with a similar response continuing during subsequent growth phase of the mycelium in only seven of the 30 isolates tested. In other isolates, growth was comparable or even enhanced compared to the controls. Conidial germination and hyphal growth are temporally separated, physiologically different stages. Thus, neem can affect these two events in a different way. A growth enhancing effect of neem on *B. bassiana* has been reported earlier (Gupta et al. 1999). Anderson et al. (1989) noted that the enhancing effect of some pesticide formulations on growth is due to the adjuvants in the formulation. Adjuvants act as mild abrasives and break up conidial agglomerations, which increase number of propagules, thereby promoting better growth.

In the bioassays on *S. litura*, combination treatment with neem compatible *B. bassiana* isolate and neem was found to have synergistic effect on mortality. A similar observation was reported in *Tribolium castaneum* and aphids (Akbar et al. 2005; Filotas et al. 2005). In the present study, combination treatment resulted not only in the increase of mortality, but there was a quicker onset of death and the proportion of larvae that showed mycosis also increased compared to treatment with *B. bassiana* alone. Conidia from infected insects serve as secondary inoculum for the spread of fungal infection in insect populations. Combination treatment of neem and another entomopathogenic fungus, *Nomuraea rileyi*, was reported to result in suppression of mycosis (Vimala Devi & Prasad 1996). This was attributed to very quick succumbing of the insects to fungal infection in combination treatment with little scope for colonization by the fungus and its subsequent sporulation on the dead insect. In the present study, the effective conidial concentration also decreased when treated together with neem. Thus, a lower dose of the fungus can be used when combined with neem. Neem was found to have a synergistic effect when used together with *B. bassiana* only when the fungal isolate was compatible with neem. In a study of combination treatment of neem with another entomopathogenic fungus *Paecilomyces fumosoroseus* (syn. *Isaria fumosorosea*), an enhanced effect on insect mortality was reported but the effect was less than additive (James 2003). The *P. fumosoroseus* isolate used in this study was moderately inhibited in germination and growth by neem (James 2003).

The enhancing effect of neem when used in combination with a compatible *B. bassiana* isolate could not arise only due to its effect on the growth, metamorphosis and behavior of the insect (Akbar et al. 2005; Filotas et al. 2005), but also due to its oily nature. Oils are believed to enhance the effect of mycopathogens by facilitating conidial adhesion (Prior et al. 1988), spread on the insect cuticle (Ibrahim et al. 1999) and germination (Prior et al. 1988). Oils are reported to enhance conidial germination either through replacement of epicuticular lipids in the insect cuticle with an aqueous fluid (Locke 1984), or by extracting fungistatic compounds from the insect cuticle (Ibrahim et al. 1999).

Acknowledgements

We thank Dr R. A. Humber, USDA, ARS, Ithaca, NY, USA, Dr Kerry O'Donnell, Microbial Properties Research, Peoria, IL, USA and Dr P. N. Chowdhury, Indian Type Culture collection, IARI, New Delhi, India for providing cultures of the fungal isolates. Uma K. Devi is thankful to CSIR Grant No 38(938)/97EMR-II, New Delhi and AP-NL BTU (BTU/P-76) for financial support. Murali C. Mohan is thankful to UGC, New Delhi, India for a Research fellowship. Narasimha P. Reddy is thankful to AP-NL BTU, Hyderabad for a research fellowship.

References

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18:265–267.

Akbar W, Lord JC, Nechols JR, Loughin TM. 2005. Efficacy of *Beauveria bassiana* for red flour beetle when applied with plant essential oils or in mineral oil and organosilicone carriers. Journal of Economic Entomology 98:683–688.

Anderson TE, Hajek AE, Roberts DW, Preisler HK, Robertson JL. 1989. Colorado potato beetle (Coleoptera: Chrysomelidae) effects of combination of *Beauveria bassiana* with insecticides. Journal of Economic Entomology 82:83–89.

Atwal AS, Dhaliwal GS. 1997. Agricultural pests of South Asia and their management. 3rd ed. Hyderabad: Kalyani Publishers. pp 156–164.

Bajan C, Kmitowa K, Popowska-Nowak E. 1998. Reactions of various ecotypes of entomopathogenic fungus *Beauveria bassiana* to the botanical preparations NEEM/trademark/and pyrethroid Fastak. Archives of Phytopathology and Plant Protection 31:369–375.

Bateman RP, Carey M, Moore D, Prior C. 1993. The enhanced infectivity of *Metarhizium flavoviride* in oil formulations to desert locust at low humidities. Annals of Applied Biology 122:145–152.

Butt TM, Jackson C, Magan N. 2001. Fungal biological control agents – Appraisal and recommendations. In: Butt TM, Jackson C, Magan N, editors. Fungi as biocontrol agents. Wallingford: CABI Publishing. pp 377–384.

Depieri RA, Martinez SS, Ayres O, Menezes JR. 2005. Compatibility of the fungus *Beauveria bassiana* (Bals.) Vuill. (Deuteromycetes) with extracts of neem seeds and leaves and the emulsible oil. Neotropical Entomology 34:601–606.

Filotas M, Sanderson J, Wraight SP. Compatibility and potential synergism between the entomopathogenic fungus *Beauveria bassiana* and the insect growth regulator azadirachtin for control of the greenhouse pests *Myzus persicae* and *Aphis gossypii*. Society for Invertebrate Pathology Annual Meeting Proceedings, August 7–11 2005, Alaska. p 81.

Fransen JJ. 1995. Survival of spores of the entomopathogenic fungus *Aschersonia aleyrodis* (Deuteromycotina: Coelomycetes) on leaf surfaces. Journal of Invertebrate Pathology 65:73–75.

Gahukar RT. 2000. Use of neem products/pesticides in cotton pest management. International Journal of Pest Management 46:149–160.

Goettel MS, Inglis CD. 1997. Fungi: Hyphomycetes. In: Lacey LA, editor. Manual of techniques in insect pathology. London: Academic Press. pp 213–249.

Gomez AK, Gomez AA. 1984. Statistical procedures for agricultural research. Singapore: John Wiley and Sons. pp 643–645.

Gupta PP, Paul MS, Sharma SN, Gupta P. 1999. Studies on compatibility of white muscardine fungus *Beauveria bassiana* with some neem products. Indian Phytopathology 52:278–280.

Ibrahim L, Butt TM, Beckett A, Clark SJ. 1999. Germination of oil-formulated conidia of the insect-pathogen, *Metarhizium anisopliae*. Mycological Research 103:901–907.

Inglis GD, Goettel MS, Butt TM, Strasser H. 2001. Use of Hyphomycetous fungi for managing insect pests. In: Butt TM, Jackson C, Magan N, editors. Fungi as biocontrol agents. Wallingford: CABI Publishing. pp 23–69.

James RR. 2003. Combining azadirachtin and *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) to control *Bemisia argentifolii* (Homoptera: Aleyrodidae). Journal of Economic Entomology 96:25–30.

John AI. 1999. The commercial use of azadirachtin and its integration into viable pest control programmes. Pesticide Science 54:285–289.

Lacey LA, Frutos R, Kaya HK, Vail P. 2001. Insect pathogens as biological control agents: do they have a future? Biological Control 21:230–248.

Lee ET. 1992. Statistical Methods for Survival Data Analysis. 2nd ed. New York: John Wiley and Sons.

Locke M. 1984. The structure of insect cuticle. In: Roberts DW, Aist JR, editors. Infection process of fungi. New York: Rockefeller Foundation. pp 38–53.

Mathur YK, Nigam S. Insecticide, antifeeding and juvenilizing effects of neem (*Azadirachta indica* A. Juss.) oil against *Corcyra cephalonica* Staint. and *Epilachna vigintioctopunctata* F. In: Singh RP, Chari MC, Rahej AK, Krans W, editors. Neem and environment, Proceedings of world neem conference, February 24–28 1993, Bangalore, India. pp 505–515.

Newman D. 1939. The distribution of range in samples from a normal population expressed in terms of an independent estimate of standard deviation. Biometrika 31:20–30.

Prior C, Jollands P, Le Patourel. 1988. Infectivity of oil and water formulations of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) to the cocoa weevil, *Pantorhytes plutus* (Coleoptera: Curculionidae). Journal of Invertebrate Pathology 52:66–72.

Rodríguez-Lagunes DA, Tajada AL, Diaz DR, Maciel CR, Mendoza JV, Roamn EB, Colorado SR, Velasco EP. 1997. Compatibility of *Beauveria bassiana* and aqueous extracts of neem (*Azadirachta indica*) to control coffee borer (*Hypothenemus hampei*). Manejo Integrado de Plagas 44:14–19.

Singh S. 2003. Effects of aqueous extract of neem seed kernel and azadirachtin on the fecundity, fertility and post-embryonic development of the melonfly, *Bactrocera cucurbitae* and the oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae). Journal of Applied Entomology 127:540–547.

UNSCEAR (United Nations Scientific Committee on the Effects of Atomic Radiation Report to the General Assembly), 1982. Biological effects of radiation in combination with other physical, chemical or biological agents. Annex L from ionizing radiation: Sources and biological effects. United Nations, New York.

Varela A, Morales E. 1996. Characterization of some *Beauveria bassiana* isolates and their virulence towards the coffee berry borer *Hypothenemus hampei*. Journal of Invertebrate Pathology 67:147–152.

Varma J, Dubey NK. 1999. Prospective of botanical and microbial products as pesticides of tomorrow. Current Science India 76:172–179.

Vimala Devi PS, Prasad YG. 1996. Compatibility of oils and antifeedants of plant origin with the entomopathogenic fungus *Nomuraea rileyi*. Journal of Invertebrate Pathology 68:91–93.