Journal of Biological Control, 24 (3): 238–243, 2010

Research Article

Compatibility of insecticides with *Beauveria bassiana* (Balsamo) Vuillemin for use against *Spodoptera litura* Fabricius

P. RAJANIKANTH[1*], G. V. SUBBARATNAM[2]and S. J. RAHAMAN[3]

[1]*District Agricultural Advisory and Transfer of Technology Centre, (DAATTC), Nalgonda 508 001, Andhra Pradesh. India.*

[2]*Agri-Biotech Foundation, Acharya N. G. Ranga Agricultural University, Rajendranagar Hyderabad 500 030, Andhra Pradesh, India.*

[3]*All India Coordinated Research Project on Biological Control of Crop Pests and Weeds, ARI, Rajendranagar, Acharya N. G. Ranga Agricultural University,Hyderabad 500 030, Andhra Pradesh, India.*

**Corresponding author E-mail*: panuganti_kanth@rediffmail.com

ABSTRACT: Compatibility of six strains of *Beauveria bassiana* with four commonly used insecticides, *viz.*, imidacloprid, spinosad, indoxacarb and chlorpyriphos, was tested to identify and incorporate the compatible strain(s) in IPM against *Spodoptera litura*. All the strains were compatible with imidacloprid and spinosad. Indoxacarb showed no significant inhibition of radial growth of *B. bassiana* strains, but caused significant inhibition of sporulation and spore viability in some strains. Chlorpyriphos was found to be highly incompatible with all the strains and exhibited high inhibition of growth and complete inhibition of sporulation and spore viability.

KEY WORDS: *Beauveria bassiana*, compatibility, side effects, associate control, entomopathogenic fungi, *Spodoptera litura*

(Article chronicle: Received: 25.05.2010; Sent for revision: 09.07.2010; Accepted: 19.07.2010)

INTRODUCTION

The tobacco caterpillar, *Spodoptera litura* (F.) is an important polyphagous crop pest distributed throughout south and eastern World tropics infesting 112 species of plants belonging to 44 families, of which 40 are known from India (Chari and Patel, 1983). Having developed resistance to some of the common insecticides like carbaryl, endosulfan, monocrotophos, etc. (Ramakrishnan *et al*., 1983), management of this pest is a challenge to entomologists.

Biointensive integrated pest management using virulent isolates of entomopathogenic fungi could be a promising effort for managing this pest. Though the use of entomopathogenic fungi in Integrated Pest Management (IPM) is increasing now-a-days, its potential is still left untapped. To achieve full potentiality of entomopathogenic fungi in IPM, incorporation of highly virulent, stable and target-specific isolates which have compatibility with the insecticides targeted against such pests is must. Hence an experiment was planned to identify the compatibility of six different strains or isolates of *Beauveria bassiana* with commonly used insecticides targeted against *S. litura.*

MATERIALS AND METHODS

Three isolates of *Beauveria bassiana*, *viz*., Bb–13, Bb–11 and Bb–5A from the National Bureau of Agriculturally Important Insects, Bangalore, and a commercial isolate coded as Bb-N were used. All the four isolates were passaged through *Spodoptera litura* and the fungus was recovered from the dead cadavers following single spore isolation procedure and identification of the fungi was done based on the characteristics of culture, colony, size of the hyphae and conidia as per the standard identification protocols suggested by St. Germain and Summerbell (1996) on Sabouraud Dextrose Agar medium with yeast extract (SDAY). Field infected larvae of *S. litura* showing the symptoms of *B. bassiana* infection with white muscardine growth over the body surface were collected from *rabi* groundnut and castor fields. Single spore isolation method was followed for subculturing the growing fungus on fresh plates of SDAY medium regularly till the pure cultures were obtained. The culture thus obtained was then host passaged and a pure culture was recovered which was used for the experiment. The two local isolates obtained from two

different localities were named as *B. bassiana* Local–1 (Bb–L–1) and *B. bassiana* Local–2 (Bb–L–2).

The commonly used insecticides, *viz.,* chlorpyriphos (Dursban 20EC 0.05%), imidacloprid (Confidor 200SL 0.0045%), spinosad (Tracer 45SC 0.018%) and indoxacarb (Avaunt 14.5SC 0.0145%) were selected to study the inhibitory effect, if any, on *B. bassiana* strains in terms of radial growth, sporulation and viability following Poison Food Technique (Nene and Thapliyal, 1993).

Incorporation of test insecticides into the media

Sterilized SDAY medium was melted and cooled but before solidification, the test insecticides at field recommended concentration as mentioned were added treatment wise by using micropipette. The medium was shaken vigorously for thorough mixing of the contents and 100 ml was poured into sterile Petri plates of 9.5 x 1.5 cm and allowed to solidify for further tests.

Inoculation of the medium with mycelial mat

Circular discs of 10 mm diameter were cut from vigorously grown cultures of *B. bassiana* using a sterile cork borer and such discs were placed in the middle of each Petri plate on the medium mixed with insecticide. Medium inoculated with the fungus without insecticide served as untreated control. These steps were carried out under aseptic conditions inside an inoculation chamber sterilized with UV radiation. These plates were incubated at 25 $\pm$ 1°C, RH 90 $\pm$ 5% and 12: 12 h photoperiod for 10 days to induce growth and sporulation.

Radial growth of the fungus was measured after 10 days and compared with untreated control using a measuring scale. Circular discs of 10 mm diameter were cut randomly from the 10-day-old uniformly grown culture plates. Each disc was placed in a test tube containing 10 ml of distilled water. The spores present in the disc were allowed to disperse uniformly in water by rotating the test tube on a vortex shaker for one minute, with proper care taken to avoid spillage of the suspension. The suspension was serially diluted and the spores counted with the help of an improved Neubaeur Haemocytometer under a compound microscope at 40x magnification and the number of spores present per ml was calculated using the formula of Aneja (1996).

No. of spores / ml = Total no. of spores in 5 randomly selected squares of Haemocytometer x 5 x 10^4

The readings thus obtained were computed to 10 ml to determine the number of conidia per unit area of 10 mm diameter disc. The viability of conidia was also recorded by harvesting the conidia from the uniformly grown culture plates with the help of a fine brush into sterile distilled water and filtered through a double layered muslin cloth. Approximately 500 µl of uniformly suspended spore solution was placed in the cavities of a cavity slide containing 100 µl of SDY medium. The slides were placed in a Petri plate containing a moistened filter paper at its bottom and incubated at a temperature of 25 $\pm$ 1°C and RH of 95%. The slides were observed after 24 hours under a microscope and the number of conidia germinated and total number of conidia visible in any of the focused region recorded and the per cent conidial viability was calculated. Five replications with a sample size of 10 were fixed for all the treatments studied.

The per cent reduction in radial growth, conidia per unit area and conidial viability was calculated by using the formula,

$$R = \frac{C - T}{C} \times 100$$

Where

R = Per cent reduction of radial growth / conidia per unit area / conidial viability

C = Radial growth / conidia per unit area / Conidial viability of fungi grown on control or untreated medium

T = Radial growth / conidia per unit area / conidial viability of fungi grown on insecticide treated medium

RESULTS AND DISCUSSION

All the test strains of *B. bassiana* were found highly compatible with the insecticides imidacloprid and spinosad by recording no inhibition of growth, sporulation and viability (Table 1). The results concur with the findings of Gardner and Kinard (1998), Neves *et al*. (2001), James and Elzen (2001), Xu *et al*. (2002) and Bhattacharya *et al*. (2004) who observed no deterimental effects of imidacloprid on *B. bassiana.*

Indoxacarb showed no significant inhibition of radial growth of *B. bassiana* strains but caused significant inhibition of sporulation in all the strains except Bb–13 and Bb–N and spore viability in all the test strains. Though the inhibition was statistically significant, it was below 4 per cent, which ensures its compatibility with *B. bassiana* strains. Manjula (2002) reported no significant inhibition of growth of *Nomuraea rileyi* but recorded 100 per cent reduction of sporulation due to indoxacarb, which may be due to the differences of the test pathogen.

The expression of little inhibition in the biological properties of *B.bassiana* strains as observed in the case of indoxacarb may be due to the presence of emulsifiers and other additives in the formulated products of insecticides. Generally wettable powders and flowable formulations cause no inhibition and often increase colony counts, whereas emulsifiable concentrate formulations frequently inhibit B. *bassiana* germination (Anderson *et al*., 1989). Adjuvants in wettable powders and flowable formulations may act as mild abrasives and break up agglomerations of

Table 1. Effect of selected insecticides on the biological properties of different strains / isolates of *Beauveria bassiana*

Strain Bb-13						
Insecticide	Radial growth (cm) after 10 days	Per cent inhibition over control	Conidial concentration / 1 cm diameter x 10^7	Per cent reduction over control	Conidial viability (%)	Per cent reduction over control
Imidacloprid	3.70 ± 0.04[a]	1.05	3.76 ± 0.05[a]	1.62	90.38 ± 0.56[a] (71.93 ± 0.54)	0.94
Indoxacarb	3.68 ± 0.04[a]	2.64	3.73 ± 0.02[a]	2.56	88.23 ± 0.51[b] (69.92 ± 0.45)	3.29
Spinosad	3.72 ± 0.04[a]	1.58	3.80 ± 0.02[a]	0.62	90.40 ± 0.48[a] (71.94 ± 0.46)	0.92
Chlorpyriphos	1.20 ± 0.03[b]	68.25	0.00 ± 0.00[b]	100	0.00 ± 0.00[c]	100.00
Control	3.78 ± 0.06[a]		3.83 ± 0.04[a]		91.24 ± 0.37[a] (72.77 ± 0.38)	
SEM ±	0.043		0.033		0.433	
C D (0.01)	0.127		0.099		1.287	
Strain Bb-11						
Imidacloprid	3.50 ± 0.04[a]	0.56	3.51 ± 0.04[a]	0.45	92.80 ± 0.40[a] (74.43 ± 0.44)	0.26
Indoxacarb	3.44 ± 0.02[a]	2.27	3.01 ± 0.05[b]	14.58	90.12 ± 0.57[b] (71.68 ± 0.54)	3.14
Spinosad	3.48 ± 0.04[a]	1.13	3.42 ± 0.03[a]	2.80	92.56 ± 0.44[a] (74.17 ± 0.48)	0.52
Chlorpyriphos	1.22 ± 0.02[b]	65.34	0.00 ± 0.00[c]	100	0.00 ± 0.00[c]	100.00
Control	3.52 ± 0.04[a]		3.52 ± 0.03[a]		93.05 ± 0.28[a] (74.70 ± 0.32)	
SEM ±	0.034		0.037		0.392	
CD (0.01)	0.101		0.110		1.164	
Strain Bb-5A						
Imidacloprid	4.46 ± 0.02[a]	0	3.84 ± 0.04[a]	2.76	93.34 ± 0.43[a] (75.04 ± 0.49)	0.82
Indoxacarb	4.42 ± 0.04[a]	0.89	3.11 ± 0.05[b]	21.21	90.40 ± 0.49[b] (71.94 ± 0.47)	3.95
Spinosad	4.44 ± 0.02[a]	0.44	3.83 ± 0.03[a]	3.01	93.66 ± 0.35[a] (75.41 ± 0.40)	0.48
Chlorpyriphos	1.48 ± 0.04[b]	66.81	0.13 ± 0.003[c]	96.60	28.54 ± 0.37[c] (32.28 ± 0.24)	69.38
Control	4.46 ± 0.05[a]		3.94 ± 0.05[a]		94.12 ± 0.47[a] (75.98 ± 0.59)	
SEM ±	0.036		0.042		0.427	
C D (0.01)	0.108		0.124		1.269	

Insecticide	Radial growth (cm) after 10 days	Per cent inhibition over control	Conidial concentration / 1 cm diameter x 10^7	Per cent reduction over control	Conidial viability (%)	Per cent reduction over control
Strain Bb-N						
Imidacloprid	3.60 ± 0.03[a]	0.55	3.40 ± 0.03[a]	1.70	92.36 ± 0.49[a] (73.95 ± 0.53)	0.32
Indoxacarb	3.56 ± 0.02[a]	1.65	3.41 ± 0.02[a]	1.64	90.83 ± 0.64[b] (72.38 ± 0.63)	1.97
Spinosad	3.56 ± 0.04[a]	1.65	3.42 ± 0.02[a]	1.09	91.45 ± 0.45[ab] (72.99 ± 0.46)	1.30
Chlorpyriphos	1.18 ± 0.04[b]	67.40	0.00 ± 0.00[b]	100	0.00 ± 0.00[c]	100.00
Control	3.64 ± 0.04[a]		3.46 ± 0.04[a]		92.66 ± 0.40[a]	
					(74.27 ± 0.43)	
SEM ±	0.035		0.025		0.451	
C D (0.01)	0.105		0.075		1.338	
Isolate Bb-L-1						
Imidacloprid	3.08 ± 0.04[a]	1.28	3.04 ± 0.04[a]	2.18	88.54 ± 0.30[a] (70.19 ± 0.27)	0.62
Indoxacarb	3.06 ± 0.04[a]	1.92	2.81 ± 0.05[b]	9.54	86.36 ± 0.37[b] (68.31 ± 0.31)	3.07
Spinosad	3.08 ± 0.05[a]	1.28	3.06 ± 0.05[a]	1.63	88.44 ± 0.27[a] (70.10 ± 0.24)	0.74
Chlorpyriphos	1.12 ± 0.04[b]	64.10	0.00 ± 0.00[c]	100	0.00 ± 0.00[c]	100.00
Control	3.12 ± 0.04[a]		3.11 ± 0.06[a]		89.10 ± 0.30[a]	
					(70.70 ± 0.28)	
SEM ±	0.040		0.047		0.279	
C D (0.01)	0.120		0.142		0.829	
Isolate Bb-L-2						
Imidacloprid	3.42 ± 0.05[a]	0	3.12 ± 0.04[a]	1.91	89.16 ± 0.55[a] (70.77 ± 0.50)	0.86
Indoxacarb	3.38 ± 0.06[a]	1.17	2.92 ± 0.05[b]	8.44	86.60 ± 0.46[b] (68.51 ± 0.38)	3.71
Spinosad	3.36 ± 0.04[a]	1.75	3.09 ± 0.06[a]	2.95	88.73 ± 0.60[a] (70.38 ± 0.56)	1.34
Chlorpyriphos	1.18 ± 0.04[b]	65.49	0.00 ± 0.00[c]	100	0.00 ± 0.00[c]	100.00
Control	3.40 ± 0.04[a]		3.19 ± 0.05[a]		89.94 ± 0.62[a]	
					(71.51 ± 0.59)	
SEM ±	0.046		0.045		0.502	
C D (0.01)	0.138		0.134		1.492	

Figures in parentheses are angular transformed values; figures indicated by the same letters are not significantly differerent as per DMRT

conidia, which would improve the field performance of *B. bassiana*.

The results of the present study suggest imidacloprid, spinosad and indoxacarb can be used with *B. bassiana* in pest management. This combination would give an added advantage where the insecticide-pathogen mixtures introduce multiple mortality factors against the target pest with insecticide making the insect physiology weak to a desired degree which makes it much more susceptible to the attack of the entomopathogens (Fedorinehik, 1974) and also delay the chances of expression of resistance to new insecticides (Georghiou, 1983). This approach in pest management was explored by Steinkraus (1996) and Brown *et al*. (1997) who found that the combination of imidacloprid and *B. bassiana* yielded greater control of adult tarnished plant bugs in cotton over the use of either of them alone.

Chlorpyriphos was found to be highly incompatible with all the strains as it exhibited a high degree of inhibition of growth and complete inhibition of sporulation and spore viability. Similar findings of incompatibility of chlorpyriphos with various entomopathogens were reported by Gupta *et al*. (2002), de-Oliveira *et al*. (2003), Isaiah *et al*. (2005) and Puzari *et al*. (2006). The inhibition of growth and sporulation might be due to the interference of chlorpyriphos in the uptake of carbohydrates and nitrogen from exogenous source (media) which are essential for growth and sporulation of entomopathogenic fungi (Pachamuthu *et al*., 1999).

Of the six strains, the strain Bb-5A could overcome the toxicity of chlorpyriphos to some extent and was able to produce little growth, low sporulation and low spore viability (Table 1). This may be due to the strain variation. Similar variation between the strains was noticed by Anderson and Roberts (1983) while working with six isolates of *B. bassiana* where colony growth and germination of one isolate was found inhibited by carbaryl. Based on the above findings, the strain Bb-5A could be incorporated in IPM as a microbial control component.

REFERENCES

Anderson, T. E. and Roberts, D. W. 1983. Compatibility of *Beauveria bassiana* isolates with insecticide formulations used in Colorado potato beetle control. *Journal of Economic Entomology*, **76**: 1437–1441.

Anderson, T. E., Hajek, A. E., Roberts, D. W., Preisler, H. K. and Robertson, J. L. 1989. Colorado potato beetle: Effect of combination of *Beauveria bassiana* with insecticides. *Journal of Economic Entomology*, **82**: 83–89.

Aneja, K. R. 1996. Experiments in microbiology, plant pathology and tissue culture, pp. 31–34. Vishwa Prakashan, A division of Wiley Eastern Limited, New Delhi, India.

Bhattacharya, S., Dutta, S. and Dhar, T. 2004. *In vitro* compatibility of different entomopathogens to pesticides, plant growth regulators and micronutrients. *Annals of Plant Protection Sciences,* **12**: 19–202.

Brown, J. Z. Steinkraus, D. C. and Tugwell, N. P. 1997. The effects and persistence of the fungus *Beauveria bassiana* (Mycontrol) and imidacloprid (Provado) on tarnished plant bug mortality and feeding, pp. 1302–1305. In: *Proceedings of the Beltwide Cotton Conference*, 6-10 January, 1997 New Orleans, L.A. National Cotton Council, Memphis, TN, USA.

Isaiah, A., Jain, A. and Paul, M. S. 2005. Compatibility of *Beauveria bassiana* with multineem and chemical pesticides. *Annals of Plant Protection Sciences*, **13**: 222–223.

Chari, M. S. and Patel, N. G. 1983. Cotton leaf worm *Spodoptera litura*, its biology and integrated control measures. *Cotton Development*, **13**: 7–8.

de Oliveira, C. N., Neves, P. M. O. J. and Kawazoe, L. S. 2003. Compatibility between the entomopathogenic fungus *Beauveria bassiana* and insecticides used in coffee plantations. *Scientia Agricola*, **60**: 663–667.

Fedorinehik, N. S. 1974. Biological agents for plant protection, pp. 169-307. In: Shumakov, E. M., Gusev, G. V. and Fedorinehik, N. S. (Eds.), *Biological Control Methods for Plant Protection* Moscow, Kolos.

Finney, D. J. 1964. Probit analysis: A Statistical treatment of the sigmoid response curve. 2nd edition. Cambridge University Press, London, 318 pp.

Gardner, W. A. and Kinard, D. J. 1998. *In vitro* germination and growth response of two entomogenous fungi to imidacloprid. *Journal of Entomological Science,* **33**: 322–324.

Georghiou, G. P. 1983. Management of resistance in arthropods, pp. 769–792. In: Georghiou, G. P. and Saito, T. (Eds.), *Pest Resistance to Pesticides*. Plenum Press, New York, USA.

Gupta, R. B. L. Sharma, S. and Yadava, Y. P. S. 2002. Compatibility of two entomopathogenic fungi, *Metarrhizium anisopliae* and *Beauveria bassiana* with certain fungicides, insecticides and organic manures. *Indian Journal of Entomology,* **64**: 48–52.

James, R. R. and Elzen, G. W. 2001. Antagonism between *Beauveria bassiana* and imidacloprid when combined for *Bemisia argentifolii* control. *Journal of Economic Entomology,* **94**: 357–361.

Manjula, K. 2002. Studies on the entomogenous fungus, *Nomuraea rileyi* Samson with special reference to its pathogenicity to *Spodoptera litura* Fabricius and *Helicoverpa armigera* Hubner, Ph.D. Thesis submitted to Acharya N.G. Ranga Agricultural University, Hyderabad, 130 pp.

Nene, Y. L and Thapliyal, P. N. 1993. *Fungicides in plant disease control*. 3rd edition, Oxford and IBH Publishing Company Private Limited, New Delhi, 691pp.

Neves, P. M. O. J. Hirose, E. Tehujo, P. T. and Moino Junior, A. 2001. Compatibility of entomopathogenic fungi with neonicotinoid insecticides. *Neotropical Entomology,* **30**: 263–268.

Pachamuthu, P., Kamble, S. T. and Yuen, G. Y. 1999. Virulence of *Metarrhizium anisopliae* strain ESC-1 to the German cockroach and its compatibility with insecticides. *Journal of Economic Entomology,* **92**: 340–346.

Puzari, K. C. Hazarika, L. K. Pranab Dutta, and Das, P. 2006. *In-vitro* inhibition of *Beauveria bassiana* growth by different commonly used insecticides in rice. *Journal of Biological Control,* **20**: 51–55.

Ramakrishnan, N., Saxena, V. S. and Dhingra, S. 1983. Insecticide resistance in the population of *Spodoptera litura* in Andhra Pradesh. *Pesticides,* **18**: 23–27.

Steinkraus, D. C. 1996. Control of tarnished plant bug with *Beauveria bassiana* and interactions with imidacloprid, pp. 888–889. In: Proceedings of the Beltwide cotton conference, 9–12 January, 1996 Nashville, TN, National Cotton Council Memphis, TN, USA.

Xu, S. T. Ying, S. H. and Feng, M. G. 2002. Biological compatibility of ten commercial pesticides with *Beauveria bassiana* conidia. *Acta Phytophylacica Sinica,* **29**: 158–162.