J. Biol. Control, 18(2): 173-178, 2004

Effect of supplementation of medium with larval extract on the virulence of *Beauveria bassiana* (Balsamo) Vuillemin against *Spilosoma obliqua* Walker

AJAY KUMAR PANDEY* and K. R. KANAUJIA
Department of Entomology
G. B. Pant University of Agriculture and Technology
Pantnagar 263145, Uttranchal, India

ABSTRACT: **Bioassays were conducted with two isolates of *Beauveria bassiana* (Balsamo) Vuillimen, cultured on Sabouraud's dextrose agar (SDA) medium supplemented with host insect larval extract against *Spilosoma obliqua* (Walker) larvae. The LC_{50} values of isolate MTCC 984 and MUCL 32508, grown on SDA medium, were 1.43 and 7.69 x 10^6 conidia ml^{-1}, 2.34 and 11.6 x 10^6 conidia ml^{-1}, and 3.53 and 19.5 x 10^6 conidia ml^{-1} against second, third and fourth instar larvae, respectively. However, the virulence of both isolates increased when grown on SDA medium supplemented with larval extract. The LC_{50} values of isolates MTCC 984 and MUCL 32508 grown on SDA medium supplemented with larval extract were 0.86 and 3.31 x 10^6 conidia ml^{-1}, 1.95 and 6.28 x 10^6 conidia ml^{-1}, and 2.38 and 14.6 x 10^6 conidia ml^{-1} against second, third and fourth instar larvae, respectively, showing thereby higher virulence of the former in terms of LC_{50} and LT_{50} value against *S. obliqua* larvae.**

KEY WORDS: *Beauveria bassiana*, bioassay, larval extract, *Spilosoma obliqua*

INTRODUCTION

Bihar hairy caterpillar, *Spilosoma obliqua* Walker (Lepidoptera: Arctiidae), is a widely distributed polyphagous insect pest causing damage to a large number of cultivated as well as non-cultivated plant species (Bhatacharya and Rathor, 1977). However, continuous use of chemical insecticides in insect pest management resulted in adverse effects like development of resistance, pest resurgence and pollution to environment. Thus, there is a need for alternative methods of pest control like microbial control.

Microorganisms like viruses, bacteria, fungi, nematode and protozoa infecting insect pests and causing epizootic are of great importance for the management of pests. *Beauveria bassiana* (Balsamo) Vuillemin is an important entomopathogenic fungus with a broad spectrum of pathogenicity (Martin *et al.*, 2000), varying degrees of virulence and host specificity (Aguda *et al.*, 1984). Culture medium influences the virulence of fungus. In view of this, an experiment was carried out to assess the pathogenicity of *Beauveria bassiana* cultured on Sabouraud's dextrose agar medium (SDA) with and without larval extract, against *S. obliqua* larvae.

* Present addres: Assistant Profesor, RARS, SKUAST(K), Post Box No. 146, Leh-Ladakh

MATERIALS AND METHODS

The present investigation was carried out in the Department of Entomology, at G. B. P. U. A. & T., Pantnagar on the pathogenicity of *B. bassiana* against *S. obliqua* larvae. Adult males and females of *S. obliqua* were collected from the Crop Research Centre (CRC), Pantnagar and reared in the laboratory.

Two isolates of *B. bassiana viz.*, MUCL 38502 and MTCC 984 were used. The isolates MUCL 38502 and MTCC 984 were obtained from Belgium Coordinated Collection of Microorganisms (BCCM™), Belgium and Institute of Microbial Technology (IMTECH), Chandigarh, respectively. The cultures were grown on Sabouraud's dextrose agar (SDA) medium and conidia were harvested from eighteen days old culture using 100ml of sterilized distilled water having 0.02 per cent Tween-80 as wetting agent (Rombach *et al.*, 1986). Counts of conidia were taken using an improved Neubauer haemocytometer. The two isolates were tested at five different concentrations *viz.*, 5×10^5, 5×10^6, 5×10^7, 5×10^8 and 5×10^9 conidia ml^{-1}.

The larval extracts prepared by using 24 hour starved (25g) larvae of *S. obliqua*, macerated in 25ml distilled water were added to SDA medium in the ratio of 1:10 (extract and medium) in separate flask and autoclaved at 15 lbs/cm^2 pressure for 15 minutes. Medium was poured in conical flask inoculated, separately, with both the fungal isolates of *B. bassiana*. These flasks were incubated at 25± 2°C with 95 per cent relative humidity for 18 days.

The bioassays were performed on second to fourth instar larvae of *S. obliqua*. In each Petri-dish (7.5cm diam) 10 larvae of different instar were placed separately and sprayed under Potter's tower at 40±2 lbs/inch with 2ml of each concentrations. For control, larvae were treated with distilled water containing 0.02 per cent Tween-80. Each treatment was replicated thrice having ten larvae per replication. Mortality was recorded at 24-hour interval and cumulative mortality data were subjected to Probit analysis (Finney, 1972).

RESULTS AND DISCUSSION

The results of bioassay indicate that the virulence of both the fungal isolates change when grown on medium supplemented with larval extract.

Table 1. Dose mortality response of *S. obliqua* larvae to *B. bassiana* isolates

Instar	χ^2 value	Regression equation Y = a + bx	LC_{50} (x 10^6 conidia ml^{-1})	Fiducial limit (x 10^8- x 10^5 conidia ml^{-1})	Relative virulence
MTCC 984					
II	0.39	Y = 3.5185 + 0.2406 x	1.43	1.206 - 2.489	1.00
III	0.18	Y = 3.7640 + 0.1928 x	2.34	3.039 - 8.006	1.63
IV	0.62	Y = 3.8549 + 0.1745 x	3.53	1.097 - 1.188	2.47
MUCL 32508					
II	0.58	Y = 3.1435 + 0.2696 x	7.69	4.543 - 2.914	1.00
III	0.08	Y = 4.1743 + 0.1168 x	11.60	9.019 - 1.500	1.51
IV	0.16	Y = 3.4317 + 0.2151 x	19.50	2.183 - 4.173	2.53

Bio-efficacy of *B. bassiana* isolates cultured on SDA medium

The results of bioassays indicate that older larvae were relatively less susceptible than youner larvae as evident from the LC_{50} and LT_{50} values (Table 1&2). The LC_{50} values of isolate MTCC 984 of *B. bassiana* was 1.43 x 10^6 conidia ml^{-1} against second instar larvae which increased 1.63 and 2.47 times against third (2.34 x 10^6 conidia ml^{-1}) and fourth (3.53 x 10^6 conidia ml^{-1}) instar larvae, respectively. The LT_{50} values of the isolate MTCC 984 were 154.9, 156.4 and 160.2 h for second, third and fourth instar larvae, respectively, thereby indicating lower susceptibility of third and fourth instar larvae over the second instar larvae. The LC_{50} values of isolate MCUL 32508 of *B. bassiana* followed a similar trend and were 7.69, 11.6 and 19.5x 10^6 conidia ml^{-1} against second, third and fourth instar larvae, respectively. The third and fourth instar larvae were 1.51 and 2.53 times less susceptible, respectively, when compared to the second instar larvae. The LT_{50} value was 160.6 h against second instar larvae with slight increase for the third (162.8 h) and fourth (164.4 h) instar larvae. The results are indicative of a decrease in susceptibility to the entomofungal pathogen *B. bassiama* with an advancement in the age of the larvae.

Bio-efficacy of *B. bassiana* isolates cultured on larval extract medium

There was increase in the susceptibility of the host insect to both the fungal isolates cultured on SDA medium supplemented with larval extract. The LC_{50} values of the isolate MTCC 984, cultured on larval extract medium, were 0.86, 1.95 and 2.38 x 10^6 conidia ml^{-1} against second, third and fourth instar larvae, respectively (Table 3). The second and third instar larvae were found to be 2.26 and 2.76 times less susceptible, respectively over the second instar larvae. The LT_{50} values were 154.0, 156.6 and 158.2 h against second, third and fourth instar larvae, respectively (Table 4). The LC_{50} value of isolate MUCL 32508 was 3.31 x 10^6 conidia ml^{-1} against second instar larvae which increased 1.89 and 4.41 times against third (6.28 x 10^6 conidia ml^{-1}) and fourth (14.6 x 10^6 conidia ml^{-1}) instar larvae, respectively (Table 3). Lower LT_{50} value was also observed when cultures were grown on SDA medium supplemented with larval extract. The LT_{50} values of this isolate were 159.1, 159.2 and 160.8 h to second, third and fourth instar larvae, respectively (Table 4). Increase in the LC_{50} and LT_{50} values with the advancement of larval age was observed with both isolates grown on SDA medium supplemented with larval extract.

Table 2. Time mortality response of *S. obliqua* larvae to *B. bassiana* isolates

Instar	χ^2 value	Regression equation Y = a + bx	LC_{50}(h)	Fiducial limit (h)
MTCC 984				
II	0.86	Y=2.5918+3.4363 x	154.9	185.2-134.2
III	0.86	Y=2.6977+3.5081 x	156.4	191.2-137.4
IV	0.49	Y=9.5972+2.7097 x	160.2	214.4-134.4
MUCL 32508				
II	0.17	Y=2.0393+3.9133 x	160.6	205.1-139.2
III	0.40	Y=2.5098+3.3955 x	162.8	204.8-142.0
IV	0.48	Y=2.1432+3.2235 x	164.4	212.3-142.4

Table 3. Dose mortality response of *S. obliqua* larvae to *B. bassiana* cultured on SDA medium with *S. obliqua* larval extract

Instar	χ^2 value	Regression equation Y = a + bx	LC_{50}(x 10^6 conidia ml^{-1})	Fiducial limit (x 10^8- x 10^5 conidia ml^{-1})	Relative virulence
MTCC 984					
II	0.57	Y = 4.8080 + 0.0997 x	0.86	14.290-5.190	1.00
III	0.47	Y = 4.1396 + 0.1367 x	1.95	2.327-16.340	2.26
IV	0.16	Y = 3.5271 + 0.2309 x	2.38	1.988-10.502	2.76
MUCL 32508					
II	0.15	Y = 3.8144 + 0.1870 x	3.31	9.209-1.196	1.00
III	0.70	Y = 3.5931 + 0.2069 x	6.28	6.272-1.199	1.89
IV	0.08	Y = 3.7879 + 0.1691 x	14.60	2.776-7.707	4.41

Table 4. Time mortality response of *S. obliqua* larvae to *B. bassiana* cultured on SDA medium with *S. obliqua* larval extract

Instar	χ^2 value	Regression equation Y = a + bx	LT_{50}(h)	Fiducial limit (h)
MTCC 984				
II	0.24	Y=1.9502 + 3.1772 x	154.0	192.3-133.9
III	0.42	Y=2.1077 + 3.2384 x	156.6	196.1-136.3
IV	0.63	Y=1.2825 + 3.1627 x	158.2	205.6-138.6
MUCL 32508				
II	0.12	Y=2.0374 + 3.964 x	159.1	201.8-138.1
III	0.38	Y=0.0332 + 2.2556 x	159.2	244.9-131.2
IV	0.64	Y=2.0064 + 3.1735 x	160.8	205.9-139.4

Of the two isolates, isolate MTCC 984, grown on SDA medium was found to be 5.37, 4.95 and 5.52 times more virulent to second, third and fourth instar larvae, respectively, when compared to the isolate MUCL 32508. The virulence of both fungal isolates of *B. bassiana*, in terms of LC_{50} and LT_{50} increased when grown on medium supplemented with larval extract (Table 5). Isolate MTCC 984, grown on larval extract SDA medium, was found to be 1.66, 1.20 and 1.48 times more virulent to second, third and

Table 5. Relative virulence of *Beauveria bassiana* isolates against *S. obliqua* larvae

Instar	LC_{50} value ($x10^6$ conidia ml^{-1}) of isolate				Relative virulence			
	MTCC 984		MUCL 32508					
	A	B	a	b	A over B	A over a	a over b	B over b
II	1.43	0.86	7.69	3.31	-1.66	5.37	-2.32	3.84
III	2.34	1.95	11.6	6.28	-1.20	4.95	-1.84	3.22
IV	3.53	2.38	19.5	14.6	-1.48	5.52	-1.33	6.13

Note:
A and a = culture grown on SDA medium
B and b = culture grown on SDA medium supplemented with larval extract

fourth instar larvae, respectively, over the virulence of culture grown on SDA medium. Prasad (1989) has reported that there was variation in pathogenicity of five isolates of three entomogenous fungi against *S. litura* larvae. Similarly, isolate MUCL 32508 was 2.32, 1.84 and 1.33 times more virulent, respectively, when culture was grown on SDA medium supplemented with larval extract. It was observed that isolate MTCC 984 was 3.84, 3.22 and 6.13 times more virulent to second, third and fourth instar larvae, respectively, in comparison to the isolate MUCL 32508 when both the isolates were grown on SDA medium supplemented with larval extract. Increase in virulence of fungal isolates grown in SDA medium supplemented with larval extract could be attributed to the presence of nutrients in larval extract. Among the various carbon sources, chitin and low level of fatty acids were found to be more efficient for conidial germination which indicated that fungus might have used nutrients present in the integument (Smith and Grula, 1981). An increase in virulence of *B. bassiana* was reported when culture was grown on medium containing animal fat (Scharoffenber, 1964).

On the basis of data generated as above it may be concluded that isolate MTCC 984 was more virulent than the isolate MUCL 32508 when the cultures were grown on SDA medium alone or supplemented with larval extract. Susceptibility of *S. obliqua* larvae to the fungal isolates increased when cultured on medium containing larval extract of same host insect.

ACKNOWLEDGEMENTS

The authors are grateful to the Head, Department of Entomology and Dean, G. B. Pant University of Agriculture & Technology, Pantnagar for providing the necessary facilities.

REFERENCES

Aguda, R. M., Litsinger, J. A. and Roberts, D. W. 1984. Pathogenicity of *Beauveria bassiana* on brown planthopper (BPH), whitebacked planthopper (WBPH), and green leafhopper (GLH). *International Rice Research Newsletter*, **9**: 6, 20.

Bhatacharya, A. K. and Rathor, Y. S. 1977. Survey and study of the bionomics of major soybean insect and their control. Research Bulletin No. 107; Department of Entomology. Gobind Ballabh Pant University of Agriculture. & Technology, Pantnagar. 324 pp.

Finney, D. J. 1964. *Probnit Analysis. A statistical treatment of the sigmoid response curve. 2nd ed.* Cambridge University Press, London. 318 pp.

Martin, P. A. W., Schroder, R. F. W., Poprawski, T. J., Lipa, J. J., Hausvater, E. and Rasocha, V. 2000. Temperature effects on the susceptibility of the Colorado potato beetle (Coleoptera: Chrysomelidae) to *Beauveria bassiana* (Balsamo) Vuillemin in Poland, the Czech Republic and the United States. *Journal of Entomological Science,* **35**: 251-258.

Prasad, V. D., Jayaraj, S. and Rabindra, R. J. 1989. Susceptibility of tobacco caterpillar, *Spodoptera litura* Fabr. (Noctuidae, Lepidoptera) to certain entomogenous fungi. *Journal of Biological Control,* **3**: 53-55.

Rombach, M. C., Aguda, R. M., Shepard, B. M. and Roberts, D. W. 1986. Infection of rice plant hopper, *Nilaparvata lugens* (Homoptera: Delphacidae) by field application of entomopathogenic hyphomycetes (Deutero-mycotica). *Environmental Entomology,* **15**: 1070-1073.

Schaerffenberg, B. 1964. Biological and environmental conditions for the development of mycosis caused by *Beauveria* and *Metarhizium*, *Journal of Invertebrate Pathology,* **6**: 8-20.

Smith, R. J. and Grula, E. A. 1981. Nutrition requirements for conidial germination and hyphal growth of *Beauveria bassiana*. *Journal of Invertebrate Pathology,* **37**: 222-230.