J. Biol. Control, 19(2): 129-133, 2005

Effect of different grain media on sporulation, germination and virulence of *Beauveria bassiana* (Balsamo) Vuillemin against *Spodoptera litura* Fabricius larvae

AJAY KUMAR PANDEY* and K. R. KANAUJIA
Department of Entomology
G. B. Pant University of Agriculture & Technology
Pantnagar 263 145, Uttaranchal, India
E-mail: drajay2002@rediffmail.com

ABSTRACT: **A laboratory experiment was carried out to study the influence of different grain based media on sporulation, germination and virulence of *Beauveria bassiana* (Balsamo) Vuillemin against third instar larvae of *Spodoptera litura* Fabricius. Among the six grain based media, *viz.* barley, finger millets, maize, sorghum, soybean and wheat, highest spore production (5.39×10^7 conidia ml^{-1}) and spore viability (86.6 %) was observed in conidia produced from finger millet. The production and viability of conidia increased which ranged from 5.0 to 11.5 per cent and 1.70 to 4.65 per cent, respectively, when the media was supplemented with sucrose. The conidia produced from finger millet showed highest virulence against third instar larvae of *S. litura* with LC_{50} value of 1.56×10^6 conidia ml^{-1} while soybean was lowest with LC_{50} value of 4.98×10^6 conidia ml^{-1}. However, conidia produced from SDA medium showed highest spore production (6.36×10^7 conidia ml^{-1}), viability (93.75 %) and virulence (0.95×10^6 conidia ml^{-1}) against third instar larvae of *S. litura*.**

KEYWORDS: *Beauveria bassiana*, grain media, *Spodoptera litura*, sporulation, virulence

INTRODUCTION

Indiscriminate use of pesticides to control the insect pests of crops has upset the natural ecological balance. This has initiated to use of biological control agent as an important and alternate control practice. Among the microbial control agents, entomogenous fungi like *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschinikoff) Sorokin have proved excellent biocontrol agents against a large number of insect pests. It has a wide range of pathogenicity to insects belonging to different orders. However, selection of suitable media for mass multiplication, and testing of virulence against various insect pests are important step for successful utilization of entomopathogenic fungi. Latch and Fallon (1976) suggested using of grains for mass production of entomogenous fungi. In light of above fact, an experiment was carried out to assess the effect of

* Correspondence Address: Assistant Professor, RARS, SKUAST-K, P. B. No. 146, Leh (Ladakh) 194 101 (J&K)

different grain media on sporulation, germination (viability) and virulence of *B. bassiana* against third instar *S. litura*.

MATERIALS AND METHODS

The present investigation was carried out in the Department of Entomology, G. B. Pant University of Agriculture and Technology, Pantnager to study the effect of different grain media on sporulation and germination of *B. bassiana* (MTCC 984), maize, sorghum, finger millet, soybean, barley and wheat were used. Fifty grams each of maize, sorghum, finger millet, soybean, barley and wheat grains was soaked in distilled water for 1-2 hours in two Erlenmeyer flasks. The soaked grains were washed with fresh water and two grams of sucrose added in first treatment. All the flasks containing medium were autoclaved 1.08 kg/cm^2 pressure for 40 minutes. After cooling, each flask was inoculated with five mm inoculation disc, which were cut from seven day old actively growing cultures of *B. bassiana* (strain MTCC 984), aseptically, in a laminar flow. Flask containing Sabouraud dextrose agar (SDA) medium was also inoculated in similar way as mentioned above, which served as control for comparison. There were four replications for each treatment. The flasks containing different media were incubated at 25±2°C and 95±5 per cent relative humidity for 18 days. For conidial count, homogenous conidial suspension was prepared by adding 0.02 per cent Tween 80 as a wetting agent. Then the flasks were shaken thoroughly to prepare proper suspension. The prepared suspension was passed through a double-layered muslin cloth. The conidial counts were made by serially diluted conidial suspension using improved Neubauer haemocytometer. To observe the conidial viability, one ml of each fungal suspension were poured in Petri-dishes containing very thin layer of SDA medium and kept for 16-24 h at 25±2°C and 95±5 cent relative humidity for germination. Per cent viability of conidia was determined.

To observe the virulence of conidia produced from different grain media, the spore suspension was prepared in 100 ml distilled water containing 0.02 per cent Tween 80. From this stock solution 10^5-10^9 conidia ml^{-1} suspension were prepared and used for bioassay test. Third instar larvae of *S. litura* were used for bioassay test. Ten larvae were placed in Petri-dish (7.5cm diam) for each concentration and 2ml conidia were sprayed through Potter's tower, separately. Each treatment was replicated thrice having ten larvae per replication. The treated larvae were incubated at 25 ±2°C and 95 per cent relative humidity. The mortality due to mycosis was recorded at 24 h interval and continued up to 10 days and cumulative mortality data were subjected to Probit analysis (Finney, 1964). Mortality at the concentration of 10^7 conidia ml^{-1} was used for calculation LT_{50} value.

RESULTS AND DISCUSSION

Effect of gain media on sporulation

There was variation in quantity of spore produced from different grain media. SDA medium produced highest conidia (6.36 x 10^7 conidia ml^{-1}) that were significantly higher than conidia produced from all the grain based media (Table 1). Among all the grain-based media, finger millet produced highest conidia (5.39 x 10^7 conidia ml^{-1}), which was significantly higher than barley and soybean, and was on par with sorghum, wheat and maize. Patel and Kanaujia (1997) reported that sorghum grain medium was best substrate for growth and sporulation of *B. bassiana* and *M. anisopliae*. Among all the test media, SDA medium produced highest conidia (6.36 x 10^7 conidia ml^{-1}), which was 27.8, 15.5, 15.2, 33.0, 32.7 and 25.4 per cent higher than conidia produced from maize, sorghum, finger millet, soybean, barley, and wheat grain medium, respectively.

After addition of two grams sucrose in all the grain media, it was found that production of conidia was slightly increased which ranged from 5.0 to 11.5 per cent over the conidia produced from grain media alone. The finger millet medium produced highest amount of conidia (5.98 x 10^7 conidia ml^{-1}) compared to rest of the grain media. Maglhas *et al.* (1994) reported that media containing four per cent sucrose with one per cent yeast extract was better

Table 1. Effect of grain media on spore production and viability of *B. bassiana* (MTCC 984)

Sl. no.	Grain media	Spore (x 10^7conidia ml^{-1})		Spore viability (%)	
		A	B	A	B
1	Maize	4.59(27.83)	4.82(24.21)	74.13(20.93)	75.63(19.33)
2	Sorghum	5.37(15.56)	5.71(10.22)	86.60(7.63)	89.83(4.18)
3	Finger millets	5.39(15.25)	5.98(5.97)	87.06(7.14)	91.11(2.82)
4	Soybean	4.26(33.01)	4.75(25.31)	68.75 (25.67)	70.70(24.59)
5	Barley	4.28(32.70)	4.76(25.16)	70.74 (24.54)	72.06(23.14)
6	Wheat	4.74(25.47)	5.11(19.65)	71.58(23.65)	72.80(22.35)
7	SDA (control)	6.36	6.36	93.75	93.75
SEM±		0.26	0.28	1.20	1.57
CD (P=0.05)		0.80	0.84	4.25	4.72

* Data in parentheses indicate per cent decrease over control.

A = media without sucrose

B = sucrose added media

for mycilial growth of entomogenous fungi, *M. anisopliae*. In sucrose supplemented grain media, production of conidia on maize, sorghum, finger millets, soybean, barley and wheat medium was increased by 5.01, 6.3, 10.9, 11.5, 11.2 and 7.8 per cent, respectively, over the conidia produced from grain media alone. The SDA medium produced 24.21, 10.22, 5.97, 25.31, 25.16 and 19.65 per cent more than conidia produced from sucrose supplemented maize, sorghum, finger millet, soybean, barley and wheat media, respectively.

Effect of viability of conidia

Different grain media influenced the conidial viability that they produced. The highest conidial viability was 93.75 per cent, which observed in conidia produced from SDA medium. The viability was 20.39, 7.63, 7.14, 25.67, 24.54, and 23.65 per cent higher than viability of conidia produced on maize, sorghum, finger millet, soybean, barley and wheat, respectively (Table 1). Among the grain based media, highest conidial viability was observed in finger millet (87.06%) while lowest was in soybean produced conidia (68.75%) followed by barley (70.74%) and wheat (71.58%). However, Devi *et al.* (2001) reported higher spore production of conidia (2.8 x 10^9/g) on crushed sorghum grain media.

Conidial viability, produced from sucrose supplemented grain media, was slightly increased which ranged from 1.7 to 4.65 per cent. Among all the grain-based media, highest and lowest conidial viability was 91.11 and 70.7 per cent in conidia produced from finger millet and soybean medium, respectively (Table 1). Viability of conidia produced form SDA medium was highest compared to all the test media.

Effect of grain media on pathogenicity

Pathogenicity tests of conidia produced from different grain media was carried out against third instar larvae of *S. litura*. There was variation in virulence of *B. bassiana* conidia produced from different grain media. The conidia produced from finger millets showed higher virulence to *S. litura* larvae with LC_{50} value of 1.56x 10^6 conidia ml^{-1} and LT_{50} value of 145.5 hours followed by sorghum with LC_{50} value of 1.9x10^7 conidia ml^{-1} with LT_{50} value of 155.4 hours (Table 2 & 3).

Sankarnarayanan *et al.* (2001) reported that conidia produced from sorghum medium have more virulence than the conidia produced from broken rice or wheat. Conidia produced from finger millets showed 2.59, 2.94, 1.22, 3.19, and 2.92 times higher virulence than the virulence of conidia produced from maize, sorghum, finger millet, soybean, barley, and wheat, respectively. However, conidia produced from SDA medium showed highest virulence against *S. litura* larvae with LC_{50} value of 0.95 x 10^6 conidia ml^{-1} and LT_{50} value of 139.1 hours. On the basis of LC_{50} value, it showed 4.25, 1.64, 4.83, 2.0, 5.24 and 4.8 times more virulence over the virulence of conidia produced from barley, finger millet, maize, sorghum, soybean and wheat, respectively. Differences in virulence of conidia, produced from SDA and various grain media, may be due to presence of nutrient. Lane *et al.* (1991) reported that conidia of *B. bassiana* produced from carbon and nitrogen limited culture media had equal virulence against *Nephotettix virescens*. Similarly, Hallsworth and Magan (1994) reported that conidia of *B. bassiana*, *Metahizium anisopliae* and *Paecilomyces* produced from carbohydrate added media had more virulence against the larvae of *Galleria mellonella* than control.

Thus, on the basis of data it may conclude that among the grain-based media, highest conidia produced with higher viability and virulence was

Table 2. Dose mortality response of *B. bassiana* against third instar larvae of *S. litura*

Media	χ^2 value	Regression equation (Y = a + bx)	LC_{50} (x10^6conidia ml^{-1})	Fiducial limits (conidia ml^{-1})	Relative virulence
Maize	0.21	Y = 3.9507 + 0.1575 x	4.59(2.94)	1.75 x 10^8 - 1.02 x 10^5	4.83
Sorghum	0.05	Y = 3.8958 + 0.1758 x	1.90(1.22)	7.90 x 10^7 - 4.59 x 10^4	2.00
Finger millets	0.11	Y = 4.2036 + 0.1285 x	1.56(1.00)	2.96 x 10^8 - 8.26 x 10^4	1.64
Soybean	0.09	Y = 4.1513 + 0.1267 x	4.98(3.19)	4.33 x 10^8 - 5.73 x 10^4	5.24
Barley	0.14	Y = 4.0466 + 0.1443 x	4.04(2.59)	2.17 x 10^8 - 7.513 x10^4	4.25
Wheat	0.14	Y = 4.0939 + 0.1360 x	4.56(2.92)	3.09 x 10^8 - 6.76x 10^4	4.80
SDA (control)	0.22	Y = 4.2811 + 0.1205 x	0.95(0.61)	3.90 x 10^8 - 2.12 x 10^3	1.00

* Data in parentheses indicate folds higher virulence of conidia produced from finger millet medium.

Table 3. Time mortality response of *B. bassiana* against third instar larvae of *S. litura*

Media	χ^2 value	Regression equation (Y = a + bx)	LT_{50} (h)	Fiducial limits (h)
Maize	2.044	Y = 4.4234 + 4.2478 x	165.2	93.2 - 293.0
Sorghum	3.722	Y = 3.5757 + 3.9130 x	155.4	136.0 - 177.6
Finger millets	3.919	Y = 2.8085 + 2.6102 x	145.5	127.3 - 166.2
Soybean	1.158	Y = 6.6448 + 5.1281 x	186.5	166.5 - 208.9
Barley	1.748	Y = 2.0643 + 3.1901 x	163.5	137.4 - 195.2
Wheat	4.828	Y = 3.4301 + 3.8130 x	162.5	122.2 - 216.0
SDA (control)	2.046	Y = 2.3508 + 3.4294 x	139.1	121.6 - 159.1

recorded in conidia produced from finger millets grain medium while lowest was in conidia produced from soybean medium. However, conidia produced from SDA medium were superior then all the test media in respect of production, germination and virulence.

ACKNOWLEDGEMENTS

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

REFERENCES

Devi, P. S. V., Chowdary, A. and Prasad, Y. G. 2001. Cost-effective multiplication of the entomopathogenic fungus *Nomuraea rileyi* (F.) Samson. *Mycopathologia,* **151**: 35-39.

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

Hallsworth, J. E. and Magan, N. 1994. Improved biological control by changing polyols/ trehalose in conidia of entomopathogens, pp. 1091-1096. *In: Proceedings Brighton Crop Protection Conference, Pests and Diseases,* Vol. 3. 1994, BCPC Publications, British Crop Protection Council, Bracknell, UK.

Latch, G. C. and Fallon, R. F. 1976. Studies on the use of *Metarhizium anisopliae* to control of *Oryctes rhinoceros. Entomophaga,* **21**: 39-48.

Lane, B. S., Trinci, A. P. J. and Gillespie, A. T. 1991. Influence of cultural conditions on the virulence of conidia and blastospores of *Beauveria bassiana* to the green leafhopper, *Nephotettix virescens. Mycological-Research,* **95**: 829-833.

Maglhas, B. P., Cabrall-de-sousa Dias, J. M. and Ferreir, C. M. 1994. Mycilial production of *Metarhizium anisopliae* in liquid culture using different source of carbohydrate and nitrogen. *Revista-de-Microbiologia,* **25**: 181-187.

Patel, S. P. and Kanaujia, K. R. 1997. Mass multiplication techniques, pathogenicity and feasibility of micropesticides in integrated pest management, pp 222-231. In: *Proceeding of International Conference on Ecological Agriculture Towards Sustainable Development,* Chandigarh, India, November 15-17, 1997.

Sankarnarayanan, C., Hussaini, S. S., Kumar, P. S. and Rangeshwaran, R. 2001. Evaluation of substrate for the multiplication of *Verticillium chlamydosporium* Gooddard and its biocontrol efficacy against *Heterodera cajani* Koshy on pigeon pea. *Annals of Plant Protection Sciences,* **9**: 73-76.