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Evaluation of Pathogenicity to *Spodoptera litura* Fabricius of Different *Beauveria bassiana* Vuillemin Isolates Mass Multiplied on Economically Viable Substrates

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Abstract

Six isolates of *Beauveria bassiana* were mass multiplied using five economically viable substrates, *viz*. sorghum, rice bran, rice husk, press-mud and bagasse to identify the suitable substrate as wells as the vigorous isolate(s) for large scale multiplication. The results of the study showed sorghum as the most suitable substrate for large scale mass multiplication as the conidia harvested from sorghum recorded highest mortality of *Spodoptera litura*. Among six isolates, the isolate Bb-5A was found most virulent as it recorded highest mortality of *Spodoptera litura* mass multiplied on all the substrates.

1. Introduction

An alternate eco-friendly strategy for the management of insect pests has been envisaged to reduce the harmful effects of chemical insecticides on human beings. In this context, crop protection based on biological control of crop pests with microbial pathogens like virus, bacteria, fungi and nematodes has been recognized as a valuable tool in pest management due to their eco-safety, target specificity, no development of resistance, reduced number of applications, higher yields and quality improvement, higher acceptability and value of produce, and suitability for exports. In view of these advantages of bio-pesticides, there has been wider acceptance of these globally. Among all the microbes, entomopathogenic fungi which were found to be promising alternative with advantages such as their entry by contact, replication in target insect, safety to non-target organisms, ease and economical *in vitro* mass culture.

Mass multiplication as suggested is one of the important criteria for commercialization of an identified potent isolate of the fungus. Based on the importance the study was formulated to identify the suitable substrate and isolate for mass multiplication.

2. Materials and Methods

The present experiment was conducted in the Department of Entomology and AICRP on Biological Control of Crop Pests and Weeds, College of Agriculture, Rajendranagar, Hyderabad, India during 2004-2007. Three isolates of *Beauveria bassiana*, viz. Bb-13, Bb-11 and Bb-5A of Project Directorate of Biological Control (PDBC), Bangalore, India and a commercial isolate coded as Bb-N were obtained. All the four isolates were host passaged through *Spodoptera litura* and the fungus was recovered from the dead cadavers following single spore isolation procedure on the Sabourauds Dextrose Agar medium with yeast extract (SDAY). *B. bassiana* infected larvae of *S. litura* showing the symptoms of white muscardine disease with white growth over the body surface were collected from *rabi* groundnut and castor fields. Single spore isolation method was followed for sub-culturing the growing fungus

on to fresh plates of SDAY medium regularly till the pure cultures were obtained. The so obtained culture was then host passaged and pure culture was recovered which was used for the experimental purpose. 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). Different economically viable substrates such as sorghum grain, rice bran, rice straw and sugar industry wastes such as press-mud and bagasse were selected as substrates and pathogenicity of the six isolates mass multiplied on these substrates was evaluated to identify if these substrates could be effectively used for mass multiplication. These substrates were inoculated individually, with all the six isolates of *B. bassiana* following different methods.

2.1. Sorghum

One kg of sorghum grain was washed thoroughly under tap water and soaked in distilled water for about half an hour. The soaked grain was cooked until the grain became soft and could form a paste when pressed between fingers. The cooked grain was air dried in shade for one hour on a filter paper to remove excess moisture.

50 g of the grain was weighed into 250 ml flasks plugged with cotton and autoclaved for 20 min. at 121°C and 15 Ψ. The sterilized grain was inoculated with 10 ml of conidial suspension prepared from 10 mm diameter disc of fungus cut from a vigorously growing 10 days old culture under aseptic conditions. The inoculated grain was incubated for 25 days at 25±1°C.

2.2. Rice bran and press-mud

50 g of rice bran was taken in 250 ml flasks plugged with cotton and autoclaved at 121°C and 15 Ψ for 20 min. The sterilized rice bran flasks were inoculated with 30 ml of conidial suspension prepared by placing 30 mm diameter of fungal disc in 10 ml sterile distilled water. Water was used in excess for preparation of conidial suspension for inoculating rice bran so as to provide adequate moisture required to support fungal growth. The contents of the flask after inoculation were stirred thoroughly using a sterile glass rod to avoid formation of clumps and also for uniform mixing of the inoculum. The flasks were incubated at 25±1°C for 25 days.

2.3. Press-mud

The same procedure followed for rice bran was adopted for press-mud but, only 10 ml of conidial suspension prepared from 10 mm diameter of fungal disc was used for inoculation of press-mud as the substrate contained enough moisture to support fungal growth.

2.4. Rice straw and bagasse

Rice straw cut into small pieces and bagasse when used as substrates did not support growth of *B. bassiana*. These substrates were therefore used separately as physical means of support for the growth of fungus by soaking them overnight in 10% of molasses and 2.5% of yeast extract mixed in 2 l of water separately. The soaked materials was squeezed and air-dried on a filter paper for one hour to absorb the excess moisture from the substrate. 50 g of the substrate was weighed into 250 ml flasks and autoclaved. The sterilized substrate was inoculated with 10 ml of conidial suspension prepared from 10 mm diameter fungal disc and incubated for 25 days at 25±1°C. All the six isolates were tested in the five test substrates and each isolate was replicated thrice.

After 25 days of incubation, the pathogenicity of the fungal isolates produced on various substrates was then evaluated by spraying a standard conidial concentration of 2×10^{10} conidia ml^{-1} drawn (Aneja, 1996) from each treatment on early third instar larvae and recorded the per cent mortality of larvae at 24 h interval till pupation or death. The median lethal time (LT_{50}) of different strains of *B. bassiana* cultured on different substrates at the standard conidial concentration of 2×10^{10} conidia ml^{-1} against third instar larvae of *S. litura* was determined by subjecting the mortality data obtained at regular interval of 24 h to probit analysis (Finney, 1964). A total of five replications with a sample size of 10 larvae $replication^{-1}$ were maintained.

3. Results and Discussion

The *B. bassiana* isolates showed significant difference in their pathogenicity to third instar *S. litura* larvae when cultured on different substrates (Table 1). The isolate Bb-5A followed similar pattern of pathogenicity towards *S. litura* as that exhibited by Bb-11 when cultured on different substrates but the per cent mortalities exhibited by isolate Bb-5A were relatively higher than the isolate Bb-11.

The isolate Bb-N cultured on sorghum grain, rice bran, bagasse and rice straw recorded the mortality of 90, 84, 80 and 74%, respectively which were on par with one another. The isolate cultured on press-mud recorded significantly lowest mortality of 74% as compared to all other substrates.

The isolate Bb-13 cultured on sorghum grain recorded significantly highest mortality of 86%, which was on par with rice bran (86%), rice straw (78%) and Bagasse (78%). Significantly least mortality of 70% was observed due to culturing on press-mud which was however on par with rice straw and bagasse (Figure 1).

The isolate Bb-11 recorded significantly highest mortality of 82% when grown on sorghum grain closely followed by rice bran with non-significant difference from the former. Bagasse, rice straw and press-mud were the next preferred substrates with non-significant variability from one another.

Table 1: Effect of different substrates on the pathogenicity of *Beauveria bassiana* isolates to *S. litura* larvae

Beauveria bassiana isolates	% mortality of *S. litura* larvae on different substrates					SEm±	CD (p=0.05)
	Sorghum grain	Rice bran	Bagasse	Rice straw	Press-mud		
Bb-5A	92.00±3.74^{a} (77.30±5.39)	90.00±3.16^{ab} (73.60±4.39)	86.00±4.00^{abc} (70.35±5.16)	80.00±3.16^{bc} (63.71±2.34)	78.00±3.74^{c} (62.38±2.73)	3.57	10.62
Bb-N	90.00±3.16^{a} (73.60±4.39)	84.00±4.00^{ab} (66.96±2.99)	80.00±3.16^{ab} (63.71±2.34)	74.00±4.00^{b} (59.55±2.55)	74.00±4.00^{b} (59.55±2.55)	3.68	10.95
Bb-13	86.00±2.45^{a} (68.29±1.99)	86.00±2.45^{a} (68.29±1.99)	78.00±3.74^{ab} (62.38±2.73)	78.00±3.74^{ab} (62.38±2.73)	70.00±4.47^{b} (57.02±2.83)	3.46	10.29
Bb-11	82.00±3.74^{a} (65.33±2.81)	80.00±4.47^{ab} (64.00±3.31)	72.00±2.45^{abc} (58.09±1.33)	70.00±3.74^{bc} (56.89±2.00)	68.00±3.74^{c} (55.69±2.35)	3.52	10.46
Bb-L-1	76.00±2.45^{a} (60.75±1.63)	72.00±3.74^{a} (58.22±2.39)	72.00±2.00^{a} (58.09±1.33)	68.00±3.74^{ab} (55.69±2.35)	60.00±3.16^{b} (50.80±1.86)	3.09	9.20
Bb-L-2	80.00±3.16^{a} (63.71±2.34)	76.00±5.10^{a} (61.17±3.50)	70.00±4.47^{ab} (57.02±2.83)	64.00±2.45^{b} (53.15±1.47)	64.00±4.00^{b} (53.21±2.36)	3.95	11.73

Figures in the parentheses are angular transformed values; Figures indicated by same letters are not significantly different from one another as per DMRT

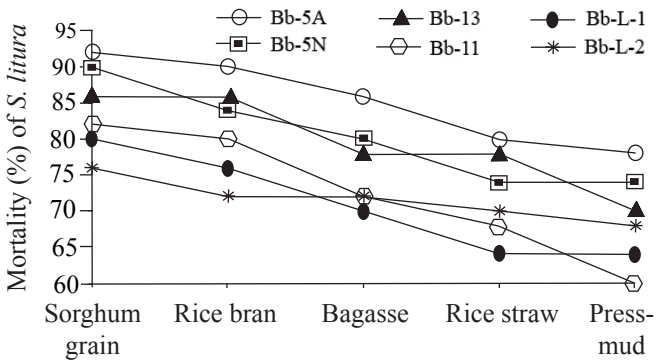

Figure 1: Pathogenicity of *B. bassiana* strains/isolates cultured on different substrates to *S. litura* larvae

The local isolate Bb-L-1 cultured on sorghum grain, rice bran, bagasse and rice straw recorded higher mortality with non-significant difference from one another. The mortality achieved due to culturing on press-mud was significantly low but on par with rice straw. The isolate Bb-L-2 also exhibited the same pattern as that of Bb-L-1.

Among the *Beauveria bassiana* isolates, significantly higher per cent mortality of *S. litura* larvae was exhibited by isolate Bb-5A when cultured on all the five substrates. Of all the five substrates, sorghum grain induced higher pathogenicity towards S. *litura* larvae in all the isolates. The substrate rice bran followed sorghum the isolates cultured on rice straw and bagasse, exhibited moderate pathogenicity while the least pathogenicity was exhibited by isolates cultured on press-mud.

The reason for this can well be explained by considering the nutritional status of the substrates used in mass production of the fungi. Higher the nutritional status, higher would be the spore viability which in turn leads to high virulence.

The research workers working on the issue suggested different opinions. Madelin (1963) and Kuruvilla and Jacob (1981) suggested that virulence of the fungal pathogens was influenced by the media in which they were grown. Sutton et al. (1981) opined that nutrients govern the parasitic nature of the fungus. Lane et al. (1991) reported that blastospores of *B. bassiana* obtained from carbon rich and nitrogen rich media exhibited high virulence. Hallsworth and Magan (1994) reported that conidia of *B. bassiana*, *M. anisopliae* and *Paecilomyces* produced from carbohydrate rich media had more virulence against *G. mellonella* larvae. Kulkarni and Lingappa (2002) attributed that nutrient status of the food medium not only enhances the sporulation rate but also the virulence possibly by altering the physiology of the fungus. Differences in virulence of conidia produced from different media may be due to the variations in nutrient levels (Pandey and Kanaujia, 2005).

The time mortality response of *B. bassiana* strains cultured on different substrates showed relatively low LT_{50} values for the nutritionally rich substrates which induced high pathogenicity and resulted in higher per cent mortality of the third instar *S. litura* larvae (Table 2).

The LT_{50} values for the strains grown on sorghum grain were in the range of 3.30 to 4.04, while the LT_{50} values were in the range of 3.35 to 4.13 when the substrate used for culturing was

Table 2: Time mortality response of *B. bassiana* strains/isolates cultured on different strains/isolates and applied @ 2 x 10^{10} conidia ml^{-1} against third instar *S. litura* larvae

	Sorghum				Rice bran				Rice straw				Press-mud				Bagasse			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Bb-5A	0.55	Y=2.807 +5.386x	3.31	3.05-3.58	0.32	Y=2.682 +5.106x	3.35	3.07-3.62	0.41	Y=3.250 +5.356x	4.04	3.74-4.37	0.32	Y=2.958 +4.875x	4.04	3.72-4.40	0.25	Y=2.854 +5.113x	3.61	3.32-3.91
Bb-N	0.70	Y=2.799 +5.168x	3.48	3.19-3.76	0.97	Y=2.829 +5.046x	3.63	3.34-3.94	1.19	Y=2.851 +4.671x	4.07	3.74-4.45	4.88	Y=3.511 +5.651x	4.18	3.88-4.51	0.80	Y=2.912 +4.976x	3.84	3.54-4.17
Bb-13	0.36	Y=2.995 +5.768x	3.30	3.04-3.58	0.30	Y=2.719 +4.883x	3.60	3.30-3.91	0.45	Y=2.947 +4.878x	4.02	3.69-4.37	3.27	Y=3.443 +5.277x	4.49	4.15-4.89	0.60	Y=2.856 +4.750x	3.99	3.66-4.35
Bb-11	0.67	Y=2.496 +4.488x	3.59	3.28-3.92	0.80	Y=2.805 +4.779x	3.86	3.54-4.20	3.65	Y=3.481 +5.425x	4.38	4.06-4.75	1.26	Y=3.399 +5.146x	4.57	4.22-5.01	0.82	Y=2.536 +4.230x	3.97	3.62-4.38
Bb-L-1	0.59	Y=2.819 +4.644x	4.04	3.71-4.42	0.83	Y=2.677 +4.341x	4.13	3.78-4.55	2.40	Y=3.655 +5.480x	4.64	4.30-5.06	2.48	Y=2.965 +4.326x	4.84	4.41-5.45	0.74	Y=2.951 +4.668x	4.28	3.93-4.70
Bb-L-2	0.60	Y=2.705 +4.692x	3.77	3.45-4.10	0.70	Y=2.510 +4.231x	3.92	3.57-4.31	3.91	Y=3.296 +4.912x	4.68	4.31-5.17	3.69	Y=3.341 +4.962x	4.71	4.34-5.20	1.58	Y=2.627 +4.256x	4.14	3.78-4.57

1: Chi^2 (b); 2: Regression equations, 3: LT_{50} (Days), 4: Fiducial limits (95%)

rice bran. The values of LT_{50} increased (3.61 to 4.28) when the strains were cultured on bagasse. Similar increase in the range of values of LT_{50} was observed when rice straw was used as substrate where the values of LT_{50} were in the range of 4.02 to 4.68. Highest range of LT_{50} values (4.04 to 4.84) was recorded when press-mud was used as substrate.

Among all the six strains the strains Bb-5A, Bb-N and Bb-13 recorded relatively low LT_{50} values when cultured on different substrates compared to Bb-11, BB-L-1 and Bb-L-2. The variations in LT_{50} values for different strains when cultured on a given substrate may be due to the variation in virulence capabilities of the strains.

Among the substrates low LT_{50} values were recorded by sorghum grain followed by rice bran, bagasse, rice straw and press-mud. The order was exactly to that of the increasing order of pathogenicity exhibited by these substrates against *S. litura* larvae. Similar low LT_{50} values were recorded by Pandey and Kanaujia (2005) for the nutritionally rich substrates, i.e. finger millets and sorghum grain which yielded highly viable spores and low LC_{50} values against third instar *S. litura* larvae compared to other substrates.

Results of the present study indicate that sorghum grain as a substrate supports high pathogenicity in entomopathogenic fungus *B. bassiana* and is in accordance with the findings of Vilas-Boas et al. (1996) against *Diatraea saccharalis*, Sankaranarayanan et al. (2001) against *Heterodera cajani*, and Pandey and Kanaujia (2005) against *S. litura*. Similarly, culturing of *Nomuraea rileyii* on sorghum grain favored high virulence against *S. litura* was reported by Kulkarni and Lingappa (2002).

4. Conclusion

It is concluded through this study that of the six isolates the isolate Bb-5A was found highly virulent compared to rest of the isolates and of the five substrates used sorghum was found to be the best and economically viable substrate for mass multiplication of *Beauveria bassiana*. It is implied that the media used for multiplication of entomopathogenic fungi greatly influences the virulence and pathogenicity of the entomopathogenic fungi. The same has already been established through studies carried out by various researchers that higher the nutritional content of the substrate used for multiplication more will be the virulence of the entomopathogenic fungi.

5. References

Aneja, K.R., 1996. Experiments in Microbiology, Plant Pathology and Tissue Culture. Wishwaprakasham, New Delhi, 31.

Finney, D.J., 1964. Probit Analysis: A Statistical treatment of the sigmoid response curve. 2nd edition Cambridge

University press. London, 318.

Hallsworth, J.E., Magan, N., 1994. Improved biological control by changing polyols/trehalose in conidia of entomopathogens. In: Bright, J.R. (Ed.), Proceedings on Crop Protection Conference Pests and Disease, vol. 3, British Crop Protection Council Publications, Bracknell, UK, 1091-1096.

Kulkarni, N.S., Lingappa, S., 2002. Evaluation of food grains for mass production of entomopathogenic fungus *Nomuraea rileyi*. Karnataka Journal of Agricultural Sciences 15, 288-292.

Kuruvilla, S., Jacob, A., 1981. Mass culturing of *Fusarium oxysporum*, an entomogenous fungus of brown plant hopper, *Nilaparvata lugens*. Agricultural Research Journal of Kerala 19, 66-68.

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

Madelin, M.F., 1963. Diseases caused by hyphomycetous fungi. In: Steinhans, E.A. (Ed.), Insect Pathology: An Advanced Treatise. Academic Press, New York, 233-271.

Pandey, M, Kanaujia, K.R., 2005. Effect of different grain media on sporulation, germination and virulence of *Beauveria bassiana* (Balsamo) Vuillemin against *Spodoptera litura* Fabricius larvae. Journal of Biological Control 19, 129-133.

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

Sutton, R.M., Gardner, W.A., Noblet, R., 1981. Effects of casein trehalose and ascorbic acid on in vitro growth of *Nomuraea rileyi*. Journal of the Georgia Entomological Society 16, 420-423.

Vilas-Boas, A.M., Andrade, R.M., Oliveira, J.V., 1996. Diversification of culture media for production of entomopathogenic fungi. Arquivos de Biologia e Tecnologia 39, 123-128.