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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(12): 4072-4075

www.thepharmajournal.com
Received: 02-09-2023
Accepted: 05-10-2023

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Evaluation of efficacy of entomopathogenic fungi, *Beauveria bassiana* (Balsam) Vuillemin isolates against tobacco caterpillar, *Spodoptera litura* (Fab.)

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Abstract
Beauveria bassiana is a known natural enemy of a number of insect pests of crop plants. Molecular markers provide a means for constructing the molecular phylogeny, diversity and link to virulent phenotypes in order to screen different isolates of any given entomopathogens. In this experiment, 13 isolates of *B. bassiana* isolated from different insect hosts and from different geographical origins were collected. Bioassays were conducted by using second instar larvae of *Spodoptera litura* in order to categorize the isolates based on virulence. The percent mortality of 1st instar larvae is high in Vikarabad *Beauveria bassiana* strain followed by native local 1 strain. Similar percent mortality of 2nd instar larvae is observed in Vikarabad strain followed by native local 1, whereas third instar larvae the highest mortality is observed in NB followed by Vikarabad strain.

Keywords: *Beauveria bassiana*, isolates, *Spodoptera litura*, LC_{50} and LC_{90} values

Introduction
Entomopathogenic fungi (EPF) are cosmopolitan natural enemies of arthropod pests and they are effective for the regulation of numerous insect pests in natural ecosystems in an eco-friendly manner. The endo-phytic and epiphytic characteristics of EPF induce plant resistance to insect pests (Bischoff *et al.*, 2009) [1] and microbial disease-causing agents by increasing plant defense responses (Moonjely *et al.*, 2016) [9]. Consequently, they have attracted attention as microbial insecticides to control insect pests because of their high viru-lence, broad host range and can be isolated from wide ranges of soil habitats particularly cultivated and forest soils existence in a wide rangeof habitats (Mishra *et al.*, 2015) [8].
The *Beauveria*, *Metarhizium* and *Paecilomyces* are predominant genera of entomopathogenic fungi widely used as biocontrol agents throughout the world. Among these, the white muscardine fungus *B. bassiana* and the green muscardine fungus M. anisopliae, are the most pronounced fungal entomopathogens for the control of sucking and chewing agricultural insect pests and play a vital role in the integrated pest management strategies. The *B. bassiana* is reported to infect 707 species of insect hosts (Raja Goud, 2009) [10].
To determine its efficacy, host specificity, survival and partial temporal distribution in the field, distinctive markers are needed for the individual strains (Leal *et al.*, 1997) [7]. DNA markers provide more detailed genomic information than do isozymes and they are not influenced by environmental or culture conditions. Molecular markers have been utilized to assess genetic variation among isolates of B. *bassiana* and other entomopathogenic fungi, thereby providing a means to identify strains of interest, determine the origin of isolates, or study the population structure (Castrillo *et al.*, 2003) [2]. Arbitrary primed PCR is relatively easy to perform and the markers are rapid and reliable tools for resolving ambiguity based on morphological characterization. For population level analysis, arbitrary primed PCR is generally used (White *et al*., 1990) [11].
Isolation of Entomopathogenic fungi is frequently based on insect bait method using *Galleria mellonella* larvae from soils and insect cadavers (Idress, 2022) [6], of which insect bait using *G. mellonella* is a very sensitive detection method. Besides in vitro characterization of entomopathogenic fungi through germination, radial growth and sporulation parameters are important in defining the virulence of fungal isolate. Furthermore, identification of EPF based on molecular technique is a prerequisite for distinguishing species more accurately for the successful control of insect pests. Internal transcribed spacer ITS1-5.8S-ITS4 region of ribosomal DNA (rDNA-ITS) is the most widely used for detection and

identification of various *Beauveria* spp.

Materials and Methods

Collected the 13 No. of B*eauveria bassiana* isolates from castor fields of Siddipet, Suryapet, Nalgonda, Karimnagar, Ananthapur, Jagityal, Vikarabad, Rangareddy, Yadadri, Kurnool districts and two native isolates and reared on Sabouraud's Dextrose Agar Yeast (SDAY) medium. The pure cultures thus obtained will further cultured and preserved in SDAY slants as well as in the Paraffin oil to make use of them for DNA extraction.

Details of Beauveria isolates collected in Telangana and Andhra Pradesh states

S.no.	District	Code
1	Siddipet	SD
2	Suryapet	SP
3	Nalgonda	NG
4	Karimnagar	KN
5	Ananthapur	AP
6	Jagityal	JL
7	Vikarabad	VB
8	Rangareddy	RR
9	Yadadri Bhuvanagiri	YD
10	Kurnool	KN
11	Native Isolate	NL 1
12	Native Isolate	NL 2
13	NBAII	NB

Morphological characterization

Slide culture method

Microscopic features of fungal isolates were characterized by slide culture techniques. A bent glass rod was placed into a sterile Petri dish containing a piece of filter paper, and a sterile glass slide was put on the glass rod. A 1x1cm block potato dextrose agar (PDA) cut with a sterile scalpel was then transferred to the glass slide. The 15 days old fungal culture was inoculated with the help of a sterile needle on the four sides of the agar block. Then a coverslip was placed over the block and pressed to ensure adherence. Approximately 2 ml of sterile distilled water was added to the bottom of the Petri dish and incubated at 25^0 C for 2–5 days. After fungal growth, the coverslip was removed with the help of forceps and placed on a drop of lactophenol cotton blue on another clean glass slide and then observed under a microscope with 400 x magnification.

Preparation of entomopathogenic fungi suspension

The spore suspensions of all isolates were prepared according to the procedures described by (Erper *et al.*, 2016) [12]. The isolates were grown on potato dextrose agar (PDA) for 14–20 days at 25 °C. The fungal spores were scraped using a sterile spatula and transferred into 10 ml of sterile distilled water having 0.1% Triton X-100 solution. Suspensions were filtered with layers of cheesecloth to remove the mycelium and vortexed to homogenize the inoculum. Spore concentrations of the filtrates were determined using Hemocytometer at 400 x magnification.

Rearing of *Spodoptera* larvae in the laboratory

The field collected *Spodoptera larvae* were reared on natural food in the laboratory. From this uniform size and growth larvae were obtained by breeding the field-collected insects in the laboratory. Female moths lay up to 300 eggs in masses, which hatch within 3-5 days. The egg masses laid on castor leaves by the moths were carefully transferred onto fresh castor leaves in petriplate or rearing jar. After hatching, the first larvae were transferred to and maintained on fresh castor leaves until they reached the second instar stage. After reaching second instar, homogenous population were selected and subjected to Bioassays.

Mortality data of *Spodoptera litura were* subjected to probit analysis to derive LC_{50} values: The data were subjected to Probit Analysis (Finney, 1971) for calculating the regression lines, LT_{50} values and fudicial limits. Heterogeneity among the populations were performed by following chi square test.

Results and Discussion

Bioassay of *Beauveria bassiana* isolates on 1st, 2nd and 3rd instar larvae of *Spodoptera litura*

Virulence of *B. bassiana* isolates were tested at three instar stages of the *Spodoptera* pest. Ten larvae were taken for each treatment in a plastic container or petriplate of 8 cm in diameter and 10 cm in height, lined with moistened filter paper and small pieces of castor leaves. Two (2) ml of conidial suspension at a concentration of 1 x 10^8 spores/ml were sprayed using a hand atomizer. Three replicates were maintained for treatment with each isolate. The treated larvae were kept in the plastic containers and were fed with castor leaves during incubation at 25 ± 2 °C. During the incubation period the relative humidity were maintained at about 95%. The dead larvae before 24 h were removed from the experiment. The larval mortality were recorded at 6-hourly intervals. The mummified larvae, if any, were kept for re isolation of the fungus in Petri plates lined with moistened filter paper.

The results revealed in Table 1 that, the Vikarabad strain has shown 88.89% mortality with LC_{50} values were 1.0 x 10^9 spores/ml on 1st instar larvae followed by native local 1 strain. Similarly, Vikarabad strain has shown 86.67% mortality with LC_{50} values were 1.0 x 10^8 spores/ml on 2nd stage larvae followed by NBAII strain. On 3rd instar larvae, Vikarabad strain has shown 53.33% with LC_{50} values of 1.3 x 10^{11} mortality on 2nd stage larvae followed by NBAII strain with 60.00 percent mortality.

Table 1: Laboratory evaluation of native isolates of *Beauveria bassiana* at 1×10^{10} conidia ml^{-1} against 1st, 2nd and 3rd instar larvae of *Spodoptera litura*

	percent mortality		
Name of the strain	***1st instar**	***2nd instar**	***3rd instar**
SD	66.67 (54.91)^{bd}	57.78 (49.53)^{bc}	40.00 (39.19)^{cd}
SP	57.78 (49.51)^{f}	40.00 (39.08)^{i}	28.89 (32.30)^{h}
NG	66.67 (54.80)^{bd}	48.89 (44.31)^{bg}	37.78 (37.80)^{ce}
KM	57.78 (49.52)^{f}	46.67 (43.08)^{bh}	44.44 (41.80)^{c}
AP	60.00 (50.81)^{be}	51.11 (45.64)^{bf}	33.33 (35.20)^{cg}
JL	57.78 (49.54)^{f}	40.00 (39.08)^{i}	33.33 (35.20)^{cg}
VB	88.89 (74.01)^{a}	86.67 (69.02)^{a}	53.33 (46.96)^{ab}
RR	42.22 (40.47)^{fg}	40.00 (39.19)^{i}	13.33 (20.98)^{i}
YD	57.78 (49.53)^{f}	40.00 (39.19)^{i}	33.33 (35.20)^{cg}
KN	33.33 (35.09)^{h}	20.00 (26.36)^{j}	13.33 (20.98)^{i}
NL 1	75.56 (60.42)^{b}	55.56 (48.36)^{bd}	33.33 (35.09)^{cg}
NL 2	71.11 (57.52)^{bc}	53.33 (46.92)^{be}	35.56 (36.59)^{cf}
NB	71.11 (57.70)^{bc}	60.00 (50.81)^{b}	60.00 (50.81)^{a}
SE(d)	4.79	4.78	4.20
CD (P=0.05)	9.85	9.83	8.64

Table 2: $LC_{50\ and}$ LC_{90} values of different isolates of *Beauveria bassiana* against 1st instar larvae of *Spodoptera litura*

Name of the isolates	Regression equation (Y=)	R^2	LC_{50} values spores ml^{-1}	Fiducial limits spores ml^{-1}		LC_{90} values spores ml^{-1}	Fiducial limits spores ml^{-1}		Slope (b)
SD	-0.060+0.065X	0.994	2.8×10^{8}	4.2×10^{5}	1.6×10^{10}	8.6×10^{14}	4.6×10^{12}	1.0×10^{21}	0.065
SP	-0.122+0.083X	0.892	7.2×10^{7}	7.7×10^{5}	4.6×10^{8}	1.8×10^{11}	1.6×10^{10}	3.6×10^{14}	0.083
NG	-0.064+0.054X	0.901	4.6×10^{9}	2.3×10^{6}	5.2×10^{12}	3.4×10^{14}	1.0×10^{12}	1.5×10^{66}	0.054
KM	-0.137+0.077X	0.873	2.0×10^{8}	1.6×10^{7}	1.4×10^{9}	1.2×10^{12}	6.6×10^{10}	1.7×10^{15}	0.077
AP	-0.220+0.110X	0.965	5.2×10^{6}	4.2×10^{5}	2.6×10^{7}	1.1×10^{10}	1.7×10^{9}	3.2×10^{11}	0.110
JL	-0.277+0.105X	0.876	3.0×10^{7}	4.4×10^{6}	1.8×10^{8}	9.3×10^{10}	1.0×10^{10}	2.1×10^{12}	0.105
VB	-0.146+0.066X	0.842	1.0×10^{9}	6.6×10^{6}	2.5×10^{10}	1.9×10^{14}	1.2×10^{12}	1.4×10^{25}	0.066
RR	-0.162+0.074X	0.922	6.0×10^{8}	2.5×10^{7}	7.9×10^{10}	4.1×10^{11}	6.0×10^{11}	4.7×10^{19}	0.074
YD	-0.131+0.085X	0.983	2.8×10^{7}	3.4×10^{5}	3.9×10^{8}	2.5×10^{12}	9.8×10^{10}	2.5×10^{15}	0.085
KN	-0.157+0.054X	0.948	2.6×10^{10}	1.5×10^{9}	1.8×10^{12}	1.5×10^{15}	1.2×10^{13}	5.4×10^{19}	0.054
NL 1	-0.173+0.071X	0.949	1.4×10^{9}	1.2×10^{8}	2.6×10^{10}	9.8×10^{13}	1.3×10^{12}	1.1×10^{19}	0.071
NL 2	-0.162+0.070X	0.979	1.1×10^{9}	5.0×10^{7}	2.9×10^{10}	1.1×10^{14}	1.9×10^{12}	3.4×10^{17}	0.070
NB	-0.120+0.042X	0.95	3.3×10^{11}	9.6×10^{9}	3.2×10^{14}	7.6×10^{16}	1.1×10^{14}	2.0×10^{24}	0.042

Fiducial limits are calculated by using equivalent deviate at (P=0.05) level

Table 3: $LC_{50\ and}$ LC_{90} values of different isolates of *Beauveria bassiana* against 2nd instar larvae of *Spodoptera litura*

Name of the isolates	Regression equation (Y=)	R^2	LC_{50} values spores ml^{-1}	Fiducial limits^{-1} spores ml		LC_{90} values spores ml^{-1}	Fiducial limits spores ml^{-1}		Slope (b)
				Lower	Upper		Lower	Upper	
SD	-0.22+0.037X	0.941	5.3×10^{11}	-	-	2.0×10^{21}	9.8×10^{4}	6.1×10^{14}	0.1365
SP	-0.084+0.062X	0.944	1.3×10^{9}	2.1×10^{6}	5.1×10^{10}	3.8×10^{14}	1.6×10^{12}	1.5×10^{30}	0.062
NG	-0.111+0.048X	0.973	6.7×10^{10}	1.3×10^{9}	2.4×10^{13}	6.1×10^{16}	1.0×10^{14}	3.8×10^{23}	0.048
KM	-0.126+0.067X	0.900	9.9×10^{8}	3.9×10^{7}	2.7×10^{10}	3.2×10^{14}	3.4×10^{12}	5.2×10^{18}	0.067
AP	-0.255+0.112X	0.968	7.0×10^{6}	8.4×10^{5}	3.5×10^{7}	1.9×10^{10}	2.8×10^{9}	4.4×10^{11}	0.112
JL	-0.195+0.068X	0.949	2.8×10^{9}	2.4×10^{8}	5.0×10^{10}	6.9×10^{13}	1.6×10^{12}	1.0×10^{19}	0.068
VB	-0.16+0.064X	0.897	4.4×10^{9}	3.6×10^{8}	2.2×10^{11}	3.2×10^{14}	2.0×10^{12}	1.2×10^{23}	0.064
RR	-0.137+0.066X	0.986	1.6×10^{9}	8.1×10^{7}	4.6×10^{10}	4.0×10^{14}	4.0×10^{12}	1.7×10^{19}	0.066
YD	-0.175+0.083X	0.923	1.0×10^{8}	6.9×10^{6}	1.6×10^{9}	3.8×10^{12}	1.2×10^{11}	7.8×10^{14}	0.083
KN	-0.155+0.048X	0.868	8.2×10^{10}	5.8×10^{9}	1.4×10^{13}	2.8×10^{15}	1.5×10^{13}	2.6×10^{21}	0.048
NL 1	-0.171+0.056X	0.841	2.4×10^{10}	1.9×10^{9}	2.7×10^{12}	9.9×10^{14}	6.0×10^{12}	4.5×10^{22}	0.056
NL 2	-0.12+0.051X	0.965	4.5×10^{10}	1.2×10^{9}	1.0×10^{13}	2.7×10^{16}	6.3×10^{13}	1.4×10^{23}	0.051
NB	-0.077+0.025X	0.924	2.3×10^{13}	1.0×10^{11}	6.7×10^{15}	1.3×10^{17}	1.2×10^{15}	3.2×10^{32}	0.025

Fiducial limits are calculated by using equivalent deviate at (P=0.05) level

Table 4: LC_{50} and LC_{90} values of different isolates of *Beauveria bassiana* against 3rdinstar larvae of *Spodoptera litura*

Name of the isolates	Regression equation (Y=)	R^2	LC_{50} values spores ml^{-1}	Fiducial limits spores ml^{-1}		LC_{90} values spores ml^{-1}	Fiducial limits spores ml^{-1}		Slope (b)
				Lower	Upper		Lower	Upper	
SD	-0.146+0.046X	0.890	6.9×10^{10}	3.0×10^{9}	1.2×10^{13}	2.3×10^{15}	1.2×10^{13}	1.3×10^{20}	0.046
SP	-0.142+0.048X	0.901	4.5×10^{10}	1.6×10^{9}	8.4×10^{12}	2.9×10^{15}	1.3×10^{13}	1.6×10^{20}	0.048
NG	-0.093+0.037X	0.979	1.3×10^{12}	1.7×10^{10}	1.9×10^{16}	2.2×10^{17}	7.1×10^{14}	9.7×10^{26}	0.037
KM	-0.082+0.047X	0.877	9.6×10^{10}	1.3×10^{9}	3.6×10^{16}	6.1×10^{17}	1.1×10^{14}	4.5×10^{49}	0.047
AP	-0.164+0.071X	0.904	1.2×10^{9}	1.1×10^{8}	2.0×10^{10}	6.3×10^{13}	8.3×10^{11}	3.2×10^{17}	0.071
JL	-0.128+0.046X	0.934	1.3×10^{11}	5.1×10^{10}	5.3×10^{13}	2.8×10^{16}	6.4×10^{13}	2.9×10^{23}	0.046
VB	-0.135+0.041X	0.866	2.3×10^{11}	8.8×10^{10}	1.0×10^{14}	8.3×10^{15}	3.0×10^{13}	4.6×10^{21}	0.041
RR	-0.16+0.051X	0.892	5.3×10^{10}	4.0×10^{9}	5.3×10^{12}	1.8×10^{15}	1.2×10^{13}	2.9×10^{22}	0.051
YD	-0.204+0.075X	0.938	1.0×10^{9}	1.7×10^{8}	1.1×10^{10}	1.4×10^{13}	3.9×10^{11}	1.3×10^{17}	0.075
KN	-0.057+0.015X	0.720	3.3×10^{13}	1.2×10^{11}	3.5×10^{24}	1.0×10^{18}	8.7×10^{13}	1.7×10^{38}	0.015
NL 1	-0.073+0.043X	0.787	5.6×10^{10}	3.3×10^{9}	8.6×10^{22}	1.9×10^{15}	2.7×10^{12}	6.9×10^{50}	0.043
NL 2	-0.12+0.040X	0.892	5.7×10^{11}	1.7×10^{10}	1.6×10^{15}	9.6×10^{16}	1.1×10^{14}	6.4×10^{25}	0.040
NB	-0.062+0.017X	0.8	3.0×10^{13}	9.8×10^{10}	4.0×10^{22}	2.1×10^{18}	1.5×10^{14}	1.5×10^{35}	0.017

Fiducial limits are calculated by using equivalent deviate at (P=0.05) level

Results and Discussion

The results in table 1 revealed that, the percent mortality of 1st instar larvae is high in Vikarabad *Beauveria bassiana* strain followed by native local 1 strain. Similar percent mortality of 2nd instar larvae is observed in Vikarabad strain followed by native local 1, whereas third instar larvae the highest mortality is observed in NB followed by Vikarabad strain. The above results are supported with the findings of Idress *et al.*, 2022 [6], Holder & Keyhani, 2005 [5].

Acknowledgement

My sincere thanks to RKVY, Govt of India for providing the budget and also SKLTS Horticultural university, Mulugu, Telangana for providing laboratory facilities at College of Horticulture, Rajendrangar, Hyderabad and also scientists from Fruit Research Station, Sangareddy for helping collection of *Beauveria* samples from siddipet, Karimnagar areas.

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