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Beauveria bassiana (Bals.) Vuill. strains pathogenic to *Periplaneta americana* (L.)

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Abstract

A laboratory experiment was conducted in the Physiology Laboratory, Department of Entomology, Assam Agricultural University Jorhat-13 during 2014-2016 to study the comparison of *Beauveria bassiana* (KR855715) strains viz., KR855715, IMI335352 and MTCC-6095 against instars and adults of *Periplaneta americana* (L.) (Blattodea: Blattidae). The biological parameters of *B. bassiana* showed that the native strain, KR855715 radially grew highest (40.15±0.91 mm) and showed highest spore germination (83.13±1.06%) and conidial density (12.02×10⁷ conidia/ml). All the strains were infectious to *P. americana*. However, instars were more susceptible than the adults. 100 percent mortality was recorded in case of 1st and 2nd instars (KR855715) and 40 (IMI335352) and 24 (MTCC-6095) per cent in 1st instars. With respect to 2nd instars, the mortality was found to be 36 (IMI335352) and 18 (MTCC-6095) per cent only. Similarly, mortality of 3rd, 4th, 5th instars and adults varied between 16 (MTCC-6095) to 72 (KR855715), 20 (MTCC-6095) to 68 (KR855715), 16 (MTCC-6095) to 36 (KR855715) and 6 (MTCC-6095) to 26 (KR855715) per cent respectively at 9 days after treatment. The histopathological study revealed the presence of mycelial network of the fungus inside the insect body.

Keywords: adults, *Beauveria bassiana*, instars, *Periplaneta americana*

1. Introduction

The most primitive and successful insect, cockroaches evolved on earth about 300 million years ago [1]. Out of 4500 species of cockroaches, 4 species are well known household pests. *Periplaneta americana* (L.) (Blattodea: Blattidae) is considered as the largest and ubiquitous grubby domestic pest, spread throughout the tropical countries of the world [1, 2]. They are omnivorous, detritophagous and are potential vector of human [2, 3]. Managing *P. americana* with synthetic pesticides is easier but expensive. However, due to their close association with human beings they pose health hazard. Therefore, biological control as an alternative to conventional pesticides is the safest one. Entomopathogenic fungi like *Beauveria bassiana*, has been now regarded as a potential biocontrol tool for controlling pests of various crops and household pests [4]. This mycopesticide could be ideally used for the biocontrol of cockroaches because the habitat of these insects promotes initial fungal infection and its subsequent spread [5]. The fungal spores attach to the roach, germinate and penetrate into the insect body, resulting in the death of the infected roach [6]. It has been reported that *B. bassiana* strains differ in their host range [5]. So, the present work aims to study the pathogenicity of three strains of *B. bassiana* and its ramification in the body cavity of *P. americana* with the use of scan electron microscope.

2. Materials and Methods

2.1 Mass rearing of *Periplaneta americana* (L.)

The experiment was conducted in the Physiology laboratory, Department of Entomology, Assam Agricultural University, Jorhat-13 during 2014-2016. Mass rearing of *P. americana* was done in wooden rearing cages inside which bell jars and plastic containers (8 cm length and 8.5 cm dia.) were placed for rearing adults and nymphs respectively. Crushed dog biscuits and moist sponges were provided as food and water sources respectively. The insect culture was maintained in the Physiology Laboratory of Department of Entomology, Assam Agricultural University, Jorhat-13.

2.2 Sources of *Beauveria bassiana* (Bals.) Vuill. strains

Beauveria bassiana strains namely MTCC-6095 (Institute of Microbial Technology,

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IMTECH, Chandigarh), IMI335352 (Rice Hispa isolate, Assam) and KR855715 (Tea Mosquito Bug isolate, Assam) were collected and maintained in the Physiology laboratory of Department of Entomology, Assam Agricultural University, Jorhat-13.

2.3 Isolation and pure culture

Cadavers were cut into many pieces (0.5 to 1 mm) and surface sterilized with 1 per cent sodium hypo chlorite solution (NaOCl_2) for 30 sec. The sterilized pieces were inoculated on fresh PDA plates and incubated at a temperature of $26\pm 1^\circ\text{C}$ for 15 days. The pure culture was prepared in PDA media in petri plates (Potato Dextrose Agar) and incubated at temperature of $26\pm 1^\circ\text{C}$ for 15 days for complete growth and stored in a refrigerator at 4°C for further course of study.

2.4 Biological parameters of *Beauveria bassiana* strains

2.4.1 Radial Growth

Inoculums of *B. bassiana* strains were produced by growing them on PDA plates for seven days in a BOD Incubator. With the help of a 0.8 cm diameter cork borer, a piece was cut out from the actively growing region of a 7-day-old culture, and the same was placed aseptically in the centre of a fresh Petri plate containing PDA media and incubated in BOD at a temperature of $26\pm 1^\circ\text{C}$. After 3 days of inoculation five orthogonal diameters were measured at an interval of 24 hrs up to 15 days [7].

2.4.2 Conidial Density

The strains of *B. bassiana* were grown on PDA plates for seven days under BOD incubator maintained at $26\pm 1^\circ\text{C}$. Culture tube of each strain was suspended in 10 ml distilled water with Tween-80 (0.023%) in a 20 ml test tube. It was mixed properly by using vortex mixer and sieved through a muslin cloth which served as a stock solution. Different concentrations of each strain were prepared through serial dilution technique. The dilution blanks were labeled as 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} . Conidial density of 1×10^7 conidia/ml was counted for 15 times under light microscope (40X) with Neubauer haemocytometer and means for each strain was calculated separately and data were subjected to ANOVA with Completely Randomized Design [8].

2.4.3 Spore germination (Viability)

The germination test was done by spreading PDA media uniformly on microscopic slides disinfected with 100 per cent ethyl alcohol. Conidial suspension (0.05 ml) of each strain were spread uniformly over the slides and incubated at 26°C for 24 hrs. Germination percentage was recorded by direct examination at 40X with a phase contrast microscope [9].

2.5 Pathogenicity of *B. bassiana* strains against instars and adults of *P. americana*

Three strains of *B. bassiana* viz., KR855715, IMI335352 and MTCC-6095 were tested against instars and adults of *P. americana*. In order to find out the virulence of each strain, concentration of 1×10^7 conidia/ml were taken into consideration and applied through an atomizer @ 10 ml/5 insects. Control bottles were treated with water mixed with Tween- 80 (0.023%). Mortality data was recorded upto 9th days of treatment. The concentration (1×10^7 conidia/ml) for each strain was replicated five times having 5 insects. Mortality data were subjected to ANOVA with Completely Randomized Design (CRD) [8].

After 7 days of incubation the white fluffy growth of the

fungus was observed on the treated larvae and it was further studied under Scanning Electron Microscope (SEM) by following the methods of Jones *et al.*, 1996 [10]. Dead insects were collected and fixed in 3 per cent glutaraldehyde prepared in 0.1M sodium cacodylate buffer for 4 hrs at 40°C . The primary fixation in glutaraldehyde was followed by buffer wash of the samples for 20-30 minutes and secondary fixation in 1 per cent buffered Osmium tetroxide for 30 minutes to 1hr. The fixed samples were dehydrated through increasing concentration of acetone like 10, 30, 50, 70, 90, 100 per cent and were dried. The dried samples were secured horizontally to brass stubbed with double sided adhesive tape. Care was taken to avoid trapping of air bubbles inside the tape. A thin conductive coating of gold was applied to the sample using a fine coat ion-sputter, maintaining a low vacuum (10^{-3} torr). The sample was then observed under Scan electron microscope (SEM) to get the detailed ultra-structure of the surface and ramification of *B. bassiana* in insect cuticle and gut.

2.6 Statistical analysis

The data on mortality were analyzed statistically with Completely Randomized Design (CRD) and subjected to analysis of variance (ANOVA). Before analysis the mortality data were transformed to arc-sine and means were compared with Duncan Multiple Range Test (DMRT) (0.05%).

3. Results and Discussion

3.1 Biological parameters of *Beauveria bassiana*

B. bassiana strains have an innate growth characteristic. The strain, KR855715 radially grew highest (40.15 ± 0.91 mm) followed by IMI335352 (32.46 ± 0.65 mm) and lowest in MTCC-6095 (24.32 ± 1.10 mm) at $26\pm 1^\circ\text{C}$ (Table 1). With respect to conidial density, these strains followed the same trend as in radial growth (Table 2). However, temperature, growth medium and other factors play significant role in growth pattern of the fungus which were reported earlier by Nussenbaum, Kiewnick and Das *et al.*, [11, 12, 13]. Strain wise variation in radial growth of *B. bassiana* was also reported earlier by Senthamizhlselvan *et al.*, [14]. The highest ($83.13\pm 1.06\%$) spore germination was recorded in KR855715 while the lowest ($79.08\pm 1.04\%$) germination was recorded in case of MTCC-6095 after 24

Table 1: Radial growth of three *B. bassiana* strains 15 days after inoculation at $26\pm 1^\circ\text{C}$.

Strains	Mean \pm SD (mm)
KR855715	40.15 ± 0.91^a
IMI335352	32.46 ± 0.65^b
MTCC-6095	24.32 ± 1.10^c
S.Ed (\pm)	0.63
CD(P=0.05)	1.81

*Data presented are the means of 3 replications

*Means followed by the same letter are not significantly different [DMRT, (P<0.05)]

Table 2: Conidial density (1×10^7 /ml) of three *B. bassiana* strains.

Strains	Mean \pm SD
	1×10^7 /ml
KR855715	12.02 ± 1.07^a
IMI335352	6.11 ± 0.69^b
MTCCC-6095	2.47 ± 0.39^c
S.Ed (\pm)	0.63
CD(P=0.05)	1.54

*Data presented are the means of 3 replications

*Means followed by the same letter are not significantly different

[DMRT, (P<0.05)]

Hrs (Table 3). Variability can also vary between strains. Environmental factors like temperature and relative humidity (RH) can determine the viability of a fungus as was evident from the studies of Nussenbaum who reported the optimum temperature for germination of *B. bassiana* spore was 27°C [8]. Similarly Das *et al.*, [13] reported that germination of MTCC-6095 strain of *B. bassiana* varied with temperature. Mishra *et al.*, [15] revealed that spore germination of *B. bassiana* was maximum at 72 hrs and reported that conidial germination varied with the type of surfactant and concentration.

Table 3: Germination of three strains (1×10^7 /ml) of *B. bassiana*.

Strains	Mean±SD (%)
KR855715	83.13±1.06 ^a
IMI335352	80.80±1.44 ^b
MTCC-6095	79.08±1.04 ^c
S.Ed (±)	0.63
CD(P=0.05)	1.54

*Data presented are the means of 3 replications

*Means followed by the same letter are not significantly different [DMRT, (P<0.05)]

3.2 Pathogenicity of *Beauveria bassiana*

All the strains of *Beauveria bassiana* were infectious to *Periplaneta americana*. Instars of *P. americana* were more susceptible to *B. bassiana* as compared to adults which was in conformity with the findings of Mehinto *et al.*, [16]. It is

evident from the present study that the mortality of 1st and 2nd in stars were found to be 100.00 per cent at 2 and 3 DAT respectively in case of KR855715 strain [17] (Table 4). IMI335352 and MTCC-6095 could kill 40 and 24 per cent of the 1st instars. With respect to 2nd instars, the mortality was found to be 36 (IMI335352) and 18 (MTCC-6095) per cent only. Similarly, mortality of 3rd, 4th, 5th instars and adults varied between 16 (MTCC-6095) to 72 (KR855715), 20 (MTCC-6095) to 68 (KR855715), 16 (MTCC-6095) to 36 (KR855715) and 6 (MTCC-6095) to 26 (KR855715) per cent respectively at 9 days after treatment (Table 5). Infectivity can vary with isolates and this capacity is related to the genetic makeup of each strain to produce toxins needed for inducing mortality to the tested insect [18]. The mortality of *P. americana* might be due to the fungal toxin, physical obstruction of blood circulation, nutrient depletion or it might be due to the innate variability of the fungus to invade the host insect which was reported earlier by Das *et al.*, (2012) [19]. Similar results were recorded earlier by Hubner-Campos who found that more than 81.7% mortality of *P. americana* instars were observed in response to *Metarhizium anisopliae* (Metchnikoff) Sorokin, *M. robertsii* and *B. bassiana* on 25 DAT [20]. Faraji *et al.*, reported that due to the treatment of *B. bassiana* the mortality of 3rd instar larvae of Mediterranean flour moth, *Ephestia kuehniella* (Zeller) ranged from 17 to 88 per cent [21]. Elbashir *et al.*, found that due to the isolates ITCC No. 6628, ITCC No. 6645 of *B. bassiana*, the mortality of *Corcyra cephalonica* (Stainton) varied between 31-98 per

Table 4: Effect of *B. bassiana* (1×10^7 conidia/ml) on mortality (%) of 1st and 2nd in stars of *P. americana* (Mean±SE).

Strains	1 st instar					2 nd instar				
	1DAT	2DAT	3DAT	4DAT	5DAT	1DAT	2DAT	3DAT	4DAT	5DAT
KR855715	72±4.89 (58.05) ^a	100±0.00 (91.00) ^a	100±0.00 (91.00) ^a	100±0.00 (91.00) ^a	100±0.00 (91.00) ^a	56±4.00 (48.45) ^a	84±4.00 (66.42) ^a	100±0.00 (91.00) ^a	100±0.00 (91.00) ^a	100±0.20 (91.00) ^a
IMI335352	12±3.74 (20.27) ^b	24±4.00 (29.33) ^b	32±4.89 (34.45) ^b	36±4.00 (36.87) ^b	40±6.32 (39.23) ^b	12±3.74 (20.27) ^b	18±2.00 (25.10) ^b	24±4.00 (29.33) ^b	28±4.89 (31.95) ^b	36±4.00 (36.87) ^b
MTCC-6095	4±2.45 (11.54) ^b	8±4.89 (16.43) ^c	16±4.00 (23.58) ^c	16±4.00 (23.58) ^c	24±4.00 (29.33) ^c	2±2.00 (8.13) ^c	8±4.89 (16.43) ^c	12±3.74 (20.27) ^c	14±4.00 (21.97) ^c	18±2.00 (25.10) ^c
Control (Water+ Twen80)	4±2.45 (11.54) ^b	2±2.00 (8.13) ^c	6±2.45 (14.18) ^c	2±2.00 (8.13) ^d	6±2.45 (4.18) ^d	0±0.00 (0.00) ^c	0±0.00 (0.00) ^c	4±2.45 (11.54) ^c	2±2.00 (8.13) ^d	6±2.45 (14.18) ^d
S.Ed (±)	5	4.69	4.79	4.24	5.58	4.12	4.69	4.24	4.69	3.61
CD (P=0.05)	10.60	9.94	10.17	8.99	11.80	8.74	9.94	8.99	9.94	7.64

*Data presented are the means of 5 replications (5 insects/ replication).

*Means followed by the same letter are not significantly different [DMRT, (P<0.05)]

*Data within the parentheses are angular transformed value.

*DAT= Days After Treatment

Table 5: Effect of *B. bassiana* (1×10^7 conidia/ml) on mortality (%) of 3rd, 4th, 5th instars and adults of *P. americana* (Mean±SE).

Strains	3 rd instar			4 th instar			5 th instar			Adult		
	3 DAT	6DAT	9DAT	3 DAT	6DAT	9DAT	3 DAT	6DAT	9DAT	3 DAT	6DAT	9DAT
KR855715	64±4.00 (53.13) ^a	68±4.89 (55.55) ^a	72±4.89 (58.05) ^a	52±4.89 (46.15) ^a	60±6.32 (50.77) ^a	68±4.89 (55.55) ^a	16±7.48 (23.58) ^a	36±4.00 (36.87) ^a	36±7.48 (36.87) ^a	12±4.89 (20.27) ^a	24±10.73 (29.33) ^a	26±7.48 (30.66) ^a
IMI335352	28±4.89 (31.95) ^b	28±4.89 (31.95) ^b	36±7.48 (36.87) ^b	28±4.89 (31.95) ^b	28±4.89 (31.95) ^b	36±4.00 (36.87) ^b	8±4.89 (16.43) ^b	20±6.32 (26.56) ^b	20±6.32 (26.56) ^b	4±4.00 (11.54) ^b	8±3.58 (16.43) ^a	10±4.47 (18.44) ^a
MTCC-6095	14±4.00 (21.97) ^b	16±4.00 (23.58) ^b	16±4.00 (23.58) ^b	6±2.45 (14.18) ^b	16±4.00 (23.58) ^b	20±0.00 (26.56) ^b	0±0.00 (0.00) ^b	8±4.89 (16.43) ^b	16±4.00 (23.58) ^b	0±0.00 (0.00) ^b	0±0.00 (0.00) ^a	6±4.00 (14.18) ^a
Control (Water+ Twen80)	4±2.45 (11.54) ^b	6±2.45 (14.18) ^c	10±4.47 (18.44) ^c	8±3.74 (16.43) ^b	0±0.00 (0.00) ^c	4±2.45 (11.54) ^c	0±0.00 (0.00) ^c	0±0.00 (0.00) ^b	4±2.50 (11.54) ^c	0±0.00 (0.00) ^b	4±1.78 (11.54) ^a	4±2.45 (11.54) ^a
S.Ed (±)	5.74	5.92	5.96	6.48	6.32	5.00	6.32	6.32	6.32	4.47	5.48	7.00
CD (P=0.05)	12.18	12.54	12.63	13.73	13.41	10.60	13.41	13.41	13.41	9.48	81.61	14.84

*Data presented are the means of 5 replications (5 insects/ replication).

*Means followed by the same letter are not significantly different [DMRT, (P<0.05)]

*Data within the parentheses are angular transformed value.

*DAT= Days after Treatment

Cent within three weeks [22]. Equivalent results were observed by Consolo *et al.*, [23] who revealed that among sixteen fungal isolates of *B. bassiana*, isolate FHD13 caused 70 per cent mortality of 3rd instar *Diabrotica speciosa* (Germar) larvae. Rondelli *et al.*, [24] that the isolate ESALQ-447 of *B. bassiana* caused 68.00 per cent mortality of *Sitophilus zeamais* at 1.7×10^7 conidia/ml concentration. Puzari and Hazarika [25] stated that *B. bassiana*, *Aspergillus flavus* and *Fusarium heterosporum* caused more than 90, 50 and 7 per cent mortality of *Dicladispa armigera* (Olivier), respectively. The highest mortality of the tested insect was observed in the native strain of *B. bassiana* (KR855715) which might be due to highest spore germination (83.13%) and conidial density (12.02×10^7 conidia/ml) [26]. Also the native isolates such as KR855715 were more virulent than the exotic strains [19] and hence this strain had been taken for further study.

3.3 Histopathological study of *Beauveria bassiana* (KR855715)

The histopathological study of *B. bassiana* infecting *P. americana* suggested that the surface of the insect body was totally distorted along with the deformation of the sensilla trichoidea (Fig 1 and 2). Mycellial network of the fungus inside the insect body was also clearly visible under scanning electron microscope (SEM) which was in agreement with the findings of Puzari *et al.*, who reported that white frosty mycellial growth of *B. bassiana* covered the entire body surface including the appendages of *Dicladispa armigera* (Olivier) adult and germ tube entered the host through elytral punctation and ramified over the body surface [8]. Gabarty *et al.*, observed the growth of the fungus, *B. bassiana* on the infected larvae of *Agrotis ipsilon* (Hufn.) with signs of hyphal penetration on insect cuticle as well as proliferation of the cuticle [27]. They also found a dense network of fungus, *M. anisopliae*

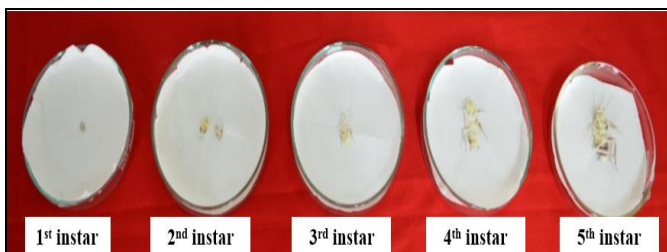


Fig 1: Fluffy growth of *B. bassiana* on the instars of *P. americana*

Which developed green spores on the insect cuticle. Toledo *et al.*, [28] noticed that in *B. bassiana* and *M. anisopliae* infected *Peregrinus maidis* adults, the hydrophobic conidia of

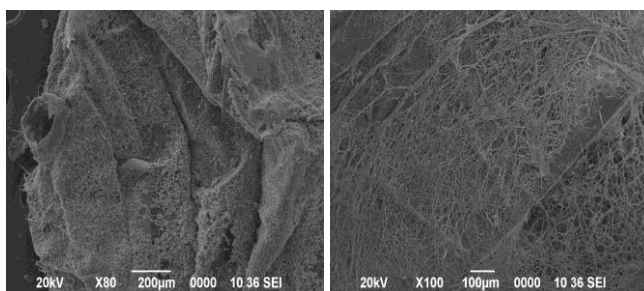


Fig 2: Mycellial mat of *B. bassiana* in *P. americana* (SEM)

Both fungal species were able to attach to all body regions with a preference for surfaces containing hairs.

4. Conclusion

The present study revealed that the native strain of *B. bassiana* (KR855715) was most effective against *P. americana*. Thus, *B. bassiana* proved to be an alternative means of controlling the household pest.

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