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Pathogenicity of *Beauveria bassiana* (Bals.) Vuill. (KR855715) against *Periplaneta americana* (L.)

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Abstract
A laboratory experiment was conducted, to study the pathogenicity of *Beauveria bassiana* (KR855715) against different instars and adults of *Periplaneta americana* (L.) (Blattodea : Blattidae) in the Physiology Laboratory, Department of Entomology, Assam Agricultural University Jorhat-13 during 2014-2016. The study revealed its pathogenicity on both instars and adults of the insect. *B. bassiana* at $1x10^7$ conidia per ml caused cent percent mortality in first and second instars at second and third days after treatment respectively. However, mortality varied from 64 -72 per cent, 52 - 68 per cent and 16 – 36 per cent mortality in 3rd, 4th and 5th instars respectively between third and ninth days after treatment. In adults mortality varied from 12 – 26 per cent between third and ninth days after treatment.

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

1. Introduction
Periplaneta americana (L.) (Blattodea : Blattidae) is the largest as well as ubiquitous and obnoxious grubby domestic pest that spread throughout the tropical countries of the world [1],[2]. They cause unappealing damage to household materials and stored products. They are highly damaging pests worldwide in terms of potential health problems and costs for pest control including their ability to move from sewers into homes and commercial establishment [2]. Managing *P. americana* with chemicals is easier but due to their close association with human beings managing the pest with mere application of pesticide is not an efficient method for its control. *Beauveria bassiana* (Bals.) Vuill., a naturally occurring entomopathogenic fungus has occupied an important place in pest management [3]. It is the most versatile parasite capable of attacking their host and penetrates the host in various developmental stages [4]. This entomopathogenic fungi has been effectively used in controlling several crop pests *viz.* Sweetpotato weevil, *Cylas formicarius* (F.); Whitefly, *Bemisia tabaci* (Genn.); Cotton leaf worm, *Spodoptera littoralis* (Boisd.); Cabbage butterfly, *Pieris rapae* (L.); *Dicladispa armigera* (Olivier) [5, 6, 7, 8]. They play an important role in biological control of insect pests population [9]. They constitute the largest group of insect pests control among the microorganisms [10]. Managing *P. americana* with chemicals is easier. But these are found in close association with human beings and chemicals may have fatal reactions on human beings. So managing the pest with mere application of pesticide is not an efficient method for controlling this pest. However there is scanty of literature on pathogenicity of *B. bassiana* on *P. americana* Keeping this in view, the present investigation was designed to study the pathogenicity of *B. bassiana* against *P. americana*.

2. Materials and Methods
2.1 Laboratory rearing of *Periplaneta americana* (L.)
The present experiment was conducted in the Physiology laboratory, Department of Entomology, Assam Agricultural University, Jorhat-13 during 2014-2016.The mass rearing of *P. americana* was done in wooden rearing cages (90×60 cm) containing belljars (Borosil; 32 cm long, 22 cm dia.) inside which stacks of wooden plates (13.5cm×10.5 cm) were placed for rearing adults. Whereas rearing of nymphs were done inside plastic containers (8 cm length and 8.5 cm dia.). Crushed dog biscuits as food material and moist sponges as water source were provided separately in petriplates (9 cm dia.) inside the belljars for adults and in small plastic corks (1.5 cm length, 2.5 cm dia.) inside the plastic containers for nymphs.

2.2 Sources of *Beauveria bassiana* (Bals.) Vuill. strains
Beauveria bassiana strains, KR855715 (Tea Mosquito Bug isolate, Assam) were maintained in the Physiology laboratory, Department of Entomology, Assam Agricultural University, Jorhat-13.

2.3 Culturing of fungal strains and inoculum preparation
The fungal strains were initially passed through the instars and adults of *P. americana* and re-isolated from the infected cadavers showing typical mycosis and then pure culture was done.

2.4 Cleaning and sterilization of glassware
The glasswares were washed thoroughly with water and wrapped with brown paper and sterilized in hot air oven to prevent contamination during cooling, transportation and storage for future use.

2.5 Preparation of media
2.5.1 Potato Dextrose Agar (PDA) medium
To prepare the PDA medium, 39.9 gm of PDA powder (Himedia) was mixed with 1litre distilled water. The medium was poured into culture tubes and conical flasks, plugged with non-absorbent cotton wool and then sterilized in an autoclave at 121 ºC (15 lb pressure per square inch) for 1 hr.

2.6 Isolation Procedure
Each cadaver were cut into many pieces, each measuring 0.5 to 1 mm in length, and those pieces were then surface sterilized by 1 per cent sodium hypo chlorite solution ($NaOCl_2$) for 30 sec. The sterilized pieces were then transferred to petri plates containing PDA medium (Potato Dextrose Agar) and incubated them at a temperature of 26±1ºC for 15 days for complete sporulation.

2.7 Pure culture
The pure culture was prepared in PDA medium (Potato Dextrose Agar) in culture tubes and incubated them at a temperature of 26±1ºC for 15 days for complete growth and stored in a refrigerator at 4ºC.

2.8 Pathogenicity test of *B. bassiana* strains against instars and adults of *P. americana*
B. bassiana strain (1×10^7 conidia/ml) @ 10 ml/container was mixed with Tween-80 (0.023%) and applied through an atomizer to instars and adults (5 insects/container). Control insects were treated with water mixed with Tween- 80 (0.023%). Mortality data was recorded upto 9th days of treatment. Mortality data were subjected to ANOVA with Completely Randomized Design (CRD).

2.9 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
Infection of insects by entomopathogenic fungi is affected by several parameters including strains of the fungus and stage of the test insect. *P. americana* nymphs were more succeptible to the pathogen compared to adults which was in conformity with the findings of Mehinto [11].

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

Strains	1st instar					2nd 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
Control (Water+ Twen80)	0±0.00 (0.00)^b	0±0.00 (0.00)^c	0±0.00 (0.00)^d	2±2.00 (8.13)^d	2±2.00 (8.13)^d	0±0.00 (0.00)^c	0±0.00 (0.00)^c	0±0.00 (0.00)^d	0±0.00 (0.00)^d	2±2.00 (8.13)^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 2: Effect of *B. bassiana* (1×10^7 conidia/ml) on mortality of 3rd, 4th, 5th instars and adults of *P. americana* (Mean%±SE)

Strains	3rd instar			4th instar			5th 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}
Control (Water+ Twen80)	0±0.00 (0.00)^d	0±0.00 (0.00)^c	2±2.00 (8.13)^c	0±0.00 (0.00)^c	0±0.00 (0.00)^c	0±0.00 (0.00)^d	0±0.00 (0.00)^a	0±0.00 (0.00)^b	0±0.00 (0.00)^c	0±0.00 (0.00)^a	0±0.00 (0.00)^b	0±0.00 (0.00)^b
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	11.61	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

B. bassiana killed 100.00 per cent of 1st and 2nd instars within 2 and 3 days respectively (Table 1). Similarly, mortality of 3rd, 4th, 5th instars and adults was found to be 72, 68, 36 and 26 per cent respectively (Table 2). Infectivity can vary with the genetic makeup of strain to produce toxins needed for inducing mortality to the tested insect [12]. 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 [13]. Similar results were recorded earlier by Hubner-Campos who found that more than 81.7 per cent mortality of *P. amricana* instars were observed in response to *Metarhizium anisopliae* (Metchnikoff) Sorokin, *M. robertsii* and *B. bassiana* on 25 DAT[14]. Faraji 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 [15]. Elbashir 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 cent within three weeks [16]. Equivalent results were observed by Consolo who revealed that among sixteen fungal isolates of *B. bassiana*, isolate FHD13 caused 70 per cent mortality of 3rd instar *Diabrotica speciosa* (Germar) larvae [17]. Rondelli found 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 [18]. Puzari *et al*., (1994) stated that *B. bassiana*, *Aspergillus flavus* and *Fusarium heterosporum* caused more than 90, 50 and 7 per cent mortality of *Dicladispa armigera* (Olivier), respectively [19].

Fig 1: Fluffy growth of *B. bassiana* (KR855715) on adult of *P. americana*

The affected insects became paralysed and sluggish. The dead insects were incubated at a temperature of 26±1°C and white mycelial growth appeared on the body within 5-7 days (Fig 1). The present finding was in conformity with the findings of Hazarika and Puzari [6, 12]. This might be due to the germination of the spore on the cuticle followed by penetration into the haemocoel where secondary growth of the hyphae resulted in death of the insect.

4. Conclusion

American Cockroaches are the pests of household products. Their close association with human habitats makes them difficult to control with application of synthetic chemicals. Entomopathogenic fungi like *Beauveria bassiana* is therefore an attractive alternative to control the pests in comparison to chemical pesticides. The findings of the present study clearly reveals the mortality of *P. americana* on application of *B. bassiana.* It also indicates that the nymphs of *P. americana* are more succeptible than the adults.

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