Larvicidal potential of *Beauveria bassiana* (Balsamo) Vuillemin and *Paecilomyces fumosoroseus* (Wize,) Brown and Smith on *Culex quinquefasciatus* (Say)

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ABSTRACT

The present study aims to determine the pathogenecity of the fungal entomopathogens *Beauveria bassiana* and *Paecilomyces fumosoroseus* to the human lymphatic filariasis (HLF) vector, *Culex quinquefasciatus.* Among the fungal isolates tested, *B. bassiana* caused 100% mortality on 2nd day at 10^8 spores/ml concentration and 97.11% on 5th day at 10^7 spores/ml of 3rd instar larvae of *C. quinquefasciatus.* At 10^7 spores /ml concentration *P. fumosoroseus* inflicted 88.87% and 97.73% mortality on 6th and 8th day respectively. Besides, malformation of wing during the developmental stage was also observed due to the impact of *B. bassiana*. When compared to *P. fumosoroseus, Cx. quinquefasciatus* was highly susceptible to *B. bassiana* and attained complete mortality in two days, whereas, *P. fumosoroseus* need eight days. Efficiency of mortality by both the species though almost similar 100% and 97.73%, short duration of killing by the *B. bassiana* reveals its more virulent nature than *P. fumosoroseus.* DMRT analysis also implies that *B. bassiana* at 10^8 is more efficient when compared to *P. fumosoroseus.*

Key Words: Pathogenicity, *Beauveria bassiana, Paecilomyces fumosoroseus, Culex quinquefasciatus*

INTRODUCTION

Mosquitoes, one of the major arthropods carriers, spread diseases and cause havoc for millions of people in developing countries both among urban and rural populations and the loss in terms of human lives is irrevocable. Malaria, filariasis, encephalitis, dengue and recently chikungunya are the major mosquito borne diseases in India. It is estimated that every year, at least 600 million people suffer (Ravikiran, 2007). *Culex quinquefasciatus* (Say) is a major vector in India as well as in other tropical regions of the world and has been shown to be directly responsible for 80 million annual lymphatic filariasis of which 30 million cases exists in chronic infection. The present proliferation of this disease is not only due to higher number of breeding places in urban area, but also due to increasing resistance of mosquitoes to current commercial insecticides such as organo-chlorides, organophosphates, pyrethroid and carbamates (Das and Amalraj, 1997).

The drug resistance and increasing insecticidal resistance have stimulated the use of alternative larvicides. The EU has withdrawn many pesticides due to the risk they pose to humans and the environment (Carpenter *et al.*, 2008). In recent years interest on mosquito-killing fungi is reviving, mainly due to continuous and increasing levels of insecticide resistance and increasing global risk of mosquito-borne diseases. Historically, both environmental and biological controls of mosquitoes were exclusively aimed at larval stages and as such have been successful in a variety of geographical and ecological settings (Scholte *et al*., 2007). Within the class Dueteromycetes, especially Ascomycetes have entomopathogenic fungi such as *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces fumosoroseus* species. The basic mechanism of pathogenesis behind which was entering through the external integument. Besides, infection through digestive tract was also possible (Goettel and Inglis, 1997). Conidia attach to the cuticle, germinate and penetrate the cuticle. Once in the hemocoel, the mycelium grows and spreads throughout the host, forming hyphae and producing blastospores. Humidity is a key factor for high and rapid killing of insects by entomopathogenic fungi, and further development on cadavers (El Damir, 2006; Lazzarini *et al.,* 2006; Derakhshan *et al.,* 2007).

Of the 31 species of *Paecilomyces* spp., 14 species are known pathogens of arthropods *Beauveria,* is one of the most frequently occurring entomogenous fungal genera as a causative agent of white muscardine disease. Although, this genus has a very broad host range (Roberts, 1974), the natural occurrence of *Beauveria* on

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mosquitoes has been reported only for four times, further Clark *et al.* (1968) and Pinnock *et al.* (1973) have reported infection by *Aedes* (*Ochlerotatus) sierrensis.* Hence, the present study aims at determining the pathogenecity, larvicidal potential and also at finding out any morphological inconvenience encountered by the human lymphatic filariasis (HLF) vector *C. quinquefasciatus* due to the fungal entomopathogens *B. bassiana* and *P. fumosoroseus*.

MATERIALS AND METHODS

The egg rafts of *Culex quinquefasciatus* (Say) are procured from the ICMR (VCRC) Madurai, Tamil Nadu, India and were maintained in the laboratory at 28 ºC ± 2ºC, 75 - 85% RH, 14 L: 10 D photoperiod cycles until hatching. After hatching, the larvae were fed with dog biscuits and yeast at 3:1 ratio. The feeding was continued till the experimental period. Periodically the different larval instars were taken-up for experimental purpose from stock culture.

Isolation and culture of *Paecilomyces fumosoroseus*

P. fumosoroseus was isolated from the soil samples of Azhagar hills, Madurai, Tamil Nadu. Serial dilution was made up to eight dilutions. From each dilution was plated on PDA medium containing streptomycin (1mg/100ml) and incubated for 7 days at 27±2ºC (Haraprasad *et al.*, 2001). After seven days of incubation, pure culture of *P. fumosoroseus* was obtained by streak plate method. *Beauveria bassiana* was produced from the Division of Entomology, UPASI- Tea Research Foundation, Valparai, Coimbatore district, Tamil Nadu. The *B. bassiana* was sub-cultured in Potato Dextrose Agar (PDA) for the further studies. The spores found over the medium were harvested by flooding the plate with sterile distilled water containing 0.02% of Tween 20 and make up to 10 ml. The spore dilution ranging from 10^3 to 10^8 for *P. fumosoroseus* and 10^3 to 10^7 for *B. bassiana* were prepared and counted with the help of Neubaur chamber that was then utilized for the pathogencity studies against *C. quinque fasciatus.*

Bioassay

Bioassay was done following the method of Sholte *et al.* (2003) with minor modifications. Two to four day old mosquito larvae were used for the experiment study. To the bioassay a total of 50 mosquito larvae per replicate were transferred into 250 ml glass beaker containing different concentration of *B. bassiana* and *P. fumosoroseus* conidia and the beaker covered with mosquito net. The set up was maintained at 27 ± 2^0C and 77±4% RH. The total mortality of mosquito larvae was noted at 24 hrs intervals and control was also maintained without conidia. Dead mosquito larvae were collected from the beaker at every 24 hrs, placed on moist filter paper (distilled water) in a paraffin sealed petridish and observed for the fungal growth.

RESULTS

The efficacy of *Paecilomyces fumosoroseus*

The efficacy of *P. fumosoroseus* (Alagar hill isolate) was assessed against *C. quinquefasciatus* at various spore concentrations i.e., 10^3 to 10^8 in different day intervals. Table 1 revealed 97.73% mortality on 8th day of post treatment at 10^8 concentration and maximum of 100% mycosis of *B. bassiana* was noticed at 10^8 spores /ml on 2nd day of post treatment. The mortality of different larval instars of *C. quinquefasciatus* was of low when the concentration decreases in different days intervals. The mean mortality of *C. quinquefasciatus* of during the experimental period ranges from 46.67 - 97.73% at 10^8 spores /ml. Fifty percent mortality were observed at 10^6 spores /ml concentration on 8th day of treatment.

Table 1. Efficacy of *Paecilomyces fumosoroseus* against *Culex quinquefasciatus*

Isolates	Mean mortality of *Culex quinquefasciatus* after treatment (Days)				
	2	4	6	8	Mean Mortality
10^3	0.00 (0.73)^{f}	8.83 (17.32)^{f}	11.13 (19.48)^{f}	17.80 (24.95)^{f}	9.44 (17.89)^{f}
10^4	4.47 (12.19)^{e}	11.13 (19.48)^{df}	13.33 (21.41)^{e}	22.20 (28.12)^{e}	12.76 (20.92)^{e}
10^5	6.67 (14.96)^{d}	22.20 (28.11)^{c}	20.0 (26.56)^{d}	31.30 (34.01)^{d}	17.27 (24.56)^{d}
10^6	11.13 (19.49)^{cf}	46.67 (43.09)^{b}	31.13 (33.91)^{c}	51.13 (45.64)^{c}	28.89 (32.51)^{c}
10^7	17.80 (24.95)^{b}	64.47 (53.41)^{a}	55.53 (48.17)^{b}	68.87 (56.08)^{b}	47.21 (43.40)^{b}
10^8	46.67 (43.09)^{a}	1.67 (7.42)^{c}	88.87 (70.51)^{a}	97.73 (81.33)^{a}	74.43 (59.62)^{a}
Control	0.00 (2.86)^{f}	11.13 (19.48)^{f}	2.33 (8.78)^{b}	4.67 (12.48)^{a}	2.16 (8.45)^{d}

Each value is the mean of three replications; Figures in the parenthesis are arc sine transformed values; a-g represents the level of treatment i.e., a= best treatment; g=poorest treatment

The efficacy of *Beauveria bassiana*

The impact of *B. bassiana* (UPASI isolate) against *C.quinquefasciatus* in different spore dilution at various time intervals of post treatment was assessed (Table 2). The efficacy of entomopathogenic fungus on *C.quiquefasciatus* shows 100% mortality on second day of treatment at 10^8 spores /ml was noticed and the control was maintained separately. The mortality of *C. quinquefasciatus* was found to be very low at low concentrations. The maximum mean mortality was observed at 10^8 spores /ml. Table 2 indicates that 50% mortality was noticed at 2nd day of treatment with 10^7 spores /ml. Besides, spores have epizootic in the developmental stages and prevent *C. quinquefasciatus* from adult emergence (Plate I).

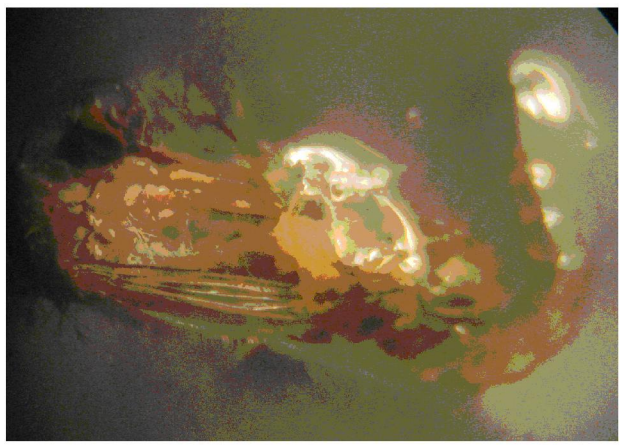

Plate 1 . Malformation due to *B. bassiana* infection

The mean mortality for all days ranges from 33% - 52.07% in the different concentration of spores of *B. bassiana*. The efficacy of both fungi *P. fumosoroseus* and *B. bassiana* indicates the maximum virulence and it was clearly evidenced that, *B. bassiana* highly pathogenic towards *C.quiquefasciatus* at all spore concentrations other than *P. fumosoroseus*.

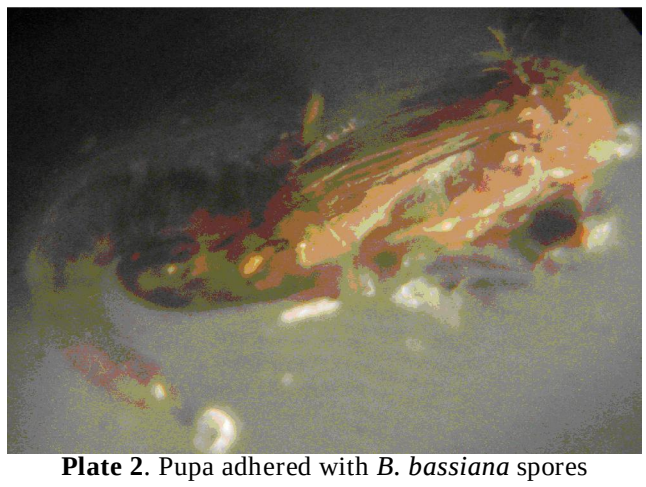

Plate 2. Pupa adhered with *B. bassiana* spores

DISCUSSION

Insect-pathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin shows much promise as low environmental impact alternatives to chemical pesticides (Butt *et al.*, 2001). Much effort has been invested in the development of strains for the control of arthropod pests of agricultural, medical and veterinary importance (Blanford *et al*., 2005; Georgis *et al.*, 2006; Scholte *et al.*, 2003, 2005).

The Hyphomycetes *B. bassiana* and *M. anisopliae* kill adult mosquitoes in the laboratory (Scholte *et al*., 2003). However, practical application methods need to be improved. Conidia of *B. bassiana* are effective in killing mosquito larvae when applied as a conidial dust to the water surface of breeding sites (Clark *et al*., 1968). Conidia are hydrophobic, thus floating on the water surface and contact mosquito larvae that feed below the surface mainly at the tip of the siphon, although Miranpuri and Khachatourians, (1991) reported that the head to be an equally important infection site.

Table 2. Efficacy of *Beauveria bassiana* against *Culex quinquefasciatus.*

Isolates	Mean mortality of *Culex quinquefasciatus* (days after treatment)					
	1	2	3	4	5	Mean Mortality
10^3	6.67 (14.96)^{e}	22.22 (28.12)^{e}	35.56 (36.61)^{e}	40.00 (39.23)^{e}	42.22 (40.52)^{e}	29.33 (32.79)^{e}
10^4	8.87 (17.32)^{d}	26.67 (31.09)^{d}	37.78 (37.96)^{d}	48.89 (44.36)^{d}	51.11 (45.63)^{d}	34.66 (36.06)^{d}
10^5	15.56 (23.24)^{c}	31.11 (33.90)^{c}	42.22 (40.52)^{ce}	55.56 (48.19)^{c}	57.78 (49.47)^{c}	40.44 (37.48)^{c}
10^6	22.22 (28.12)^{b}	37.78 (37.93^{b}	42.22 (40.52)^{be}	46.67 (43.09)^{b}	62.22 (52.07)^{b}	42.22 (40.52)^{b}
10^7	31.11 (33.90)^{a}	48.89 (44.36)^{ad}	64.44 (53.39)^{a}	75.58 (60.38)^{a}	91.11 (72.65)^{a}	62.22(52.07)^{ab}
10^8	64.44 (53.39)^{a}	100 (99.98)^{a}	-	-	-	-
Control	0.00 (2.86)^{c}	1.67 (7.42)^{c}	3.00 (9.97) ^{c}	6.67 (14.96)^{e}	6.67 (14.96)^{e}	3.60 (10.92)^{c}

Each value is the mean of three replications; Figures in the parenthesis are arc sin transformed values; a-g represents, the level of treatment i.e., a= best treatment; g=poorest treatment, Duncan's Multiple Range Test DMRT analysis was performed (AGRESS software) to find out the best treatment among treated fungi against *B. bassiana* and *P. fumosoroseus*

In the present study, two fungal isolates of *P. fumosoro seus* and *B. bassiana* were evaluated against *C. quinquefasciatus* Say at different spore concentration such as 10^3 to 10^8 and 10^3 to 10^7 respectively. 100% percent mortality was found on 2nd day of treatment at 10^7 spore concentration by *B.bassiana* against *C. quinquefas ciatus*, whereas, only 97.73% as a highest mortality was found in *P. fumosoroseus* at concentration of 10^8 on 8th day. Recent experiment by Suman *et al*. (2008) revealed that, all the *Fusarium pallidoroseum* treated female *C. quinquefasciatus* were killed within 4 days of exposure at a concentration of 1.11×10^{10} conidia per m^2. Furthermore *B. bassiana* found to be effective against *C. quinquefasciatus* at all the concentration (10^4 to10^7spores /ml) than *P. fumosoroseus*, and fifty percent mortality obtained even at lowest (10^4) spore concentration. Hussein *et al*. (2002) revealed that, the effective dose (ED_{50}) to repel *C. quinquefasciatus* for the commercial product, *N*, *N*-Diethyl-*meta*-toluamide (DEET) has been documented as 0.0007 mg /cm. Similarly LC_{50} value of Permethrin 10 EC has been documented as $0.36 \cdot 10^5$ (% v/v) against larvae of *C. quinquefasciatus* (Shanmugasundaram *et al*., 2001).

Alves *et al*. (2002) reported that *M. anisopliae* had lost its effect on the *C. quinquefasciatus* after 3 days. Recent studies have also indicated that adult *C. quinquefas ciatus* and *Anopheles gambiae* were infected under laboratory condition. Both species proved susceptible and succumbed to infection with unformulated dry and oil formulated conidia, with LT_{50} values ranging from 4 - 6 days (Scholte *et al*., 2003). Suman and Soam (2008) studies revealed that the third instar larvae of *C. quinquefa sciatus* were 25-fold more susceptible to *Chrysosporium lobatum* (0.47×10^3 conidia /ml) than the first and second instars. The present study also noted that the fungus has also involved in the developmental impairment in *C. quiquefasciatus* (Plate 1) and the emergence of the *C. quiquefasciatus* was suppressed by fungus (*B. bassiana*) evidenced by the observations of conidia of the *B. bassiana* from partially emerged dead mosquitoes. The results of the study reveals high pathogenic effect of *B. bassiana* against *C. quiquefasciatus* than *P. fumosoroseus*. The study may be further extended for its effective usage in controlling mosquito vectors.

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