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Full Length Research Paper

Pathogenicity of *Beauveria bassiana* RBL1034 Against *Anopheles gambiae* s.l.: First Evidence from Cameroon

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Malaria vector control has been successfully achieved by the use of chemical insecticides. However, the evolution of insecticide resistance is rendering the standard approaches unsustainable. Biological control agents such as entomopathogenic fungi are being considered for adult African malaria vectors. In the present study, laboratory trials were carried out to determine the pathogenicity of *Beauveria bassiana* RBL1034 to adult *Anopheles gambiae* s.l. Both male and female *A. gambiae* were exposed to targets inoculated with dry conidia of *B. bassiana* RBL1034 and the infection/mortality rates and relationship with concentration of conidia per inoculated target were determined. *B. bassiana* RBL1034 was observed to be pathogenic to both male and female A. gambiae with an average mortality of 80% in the treated compared to 10% in the untreated group. The median lethal time (MLT_{50}) in treated and untreated groups was 8 days and >10 days, respectively. Mosquito mortality increased as conidial concentration increased. Fungal sporulation was observed in 87% of the mosquito cadavers in the treated group while no sporulation was observed in the control. This study indicates that dry conidia of *B. bassiana* RBL1034 are pathogenic to adult *A. gambiae* and could be a potential biological control agent for these mosquitoes.

Key words: Entomopathogenic fungi, Pathogenicity, *Beauveria bassiana* RBL1034, Biological control, *Anopheles gambiae*.

INTRODUCTION

Each year, malaria kills up to 3 million people and can be debilitating for people infected with the disease (WHO, 2006). More than 90 percent of malaria illnesses and death occur in sub-Saharan Africa and it has been estimated that malaria retards economic growth in Africa by one-third when compared with non malaria's areas (USAID, 2006).

Vector control is an important component of World Health Organisation Global Strategy for Malaria Control, whose main objective is to break the transmission of malaria parasite using indoor residual spraying or pyrethroid impregnated materials (bednets and/ or curtains). However, these efforts are threatened by the evolution of resistance in the main malaria vector, *Anopheles gambiae* s.l. towards the commonly used insecticides for malaria control (Chandre et al., 1999; Hemingway and Ranson 2000, Hougard et al., 2003, Etang et al., 2003). Interest in alternative non-chemical control methods including biological control strategies has therefore increased over the years to argument the limited arsenal of malaria vector control tools (Shiff, 2002).

Many biological control agents including several entomopathogenic fungi have been evaluated against larval stages of mosquitoes. Recently, laboratory and fields trials carried out in Tanzanian villages show that an entomopathogenic fungus is virulent to adult *A. gambiae* (Scholte et al., 2003, 2005). An entomological inoculation rate model in the same study suggested that this vector control method could significantly reduce malaria transmission intensity.

Beauveria is one of the frequently isolated entomopa-

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thogenous fungi with a cosmopolitan distribution and mosquitoes have been listed as one of the natural host of *B. bassiana*. Conidia of *B. bassiana* are effective in killing mosquito larvae when applied as conidia dust in breeding sites (Clark et al., 1968). Besides infecting larvae, the fungus has also proved to be virulent to adult mosquitoes (Sholte et al., 2003).

Malaria remains a major public health concern in Cameroon despite years of vector control and public health activities. Moreover, in both high and low trans-mission areas, people's livelihoods and food security are affected by the impact of malaria on agricultural yields. This has serious implications even at the national level, since agriculture remains the key economic development activity. Insecticide resistance that has been reported in the main malaria vectors in Cameroon and there is need to evaluate other vector control strategies such as the use of biological control agents. The objective of this study was therefore, to determine the pathogenicity of indigenous isolate of the fungal entomopathogen, *B. bassiana* RBL1034 to the malaria vector, *A. gambiae*.

MATERIALS AND METHODS

Study area

The study was carried out in the Nkolbisson neighbourhood (03°52.19'N; 011°27.22') in Yaounde. It has an annual rainfall of 133.9 mm and maximum and minimum temperatures of 28 and 19.4°C, respectively. This areas area is characterized by two rainy seasons; August to November and March to June and two dry seasons; November to March and June to August (Centré Météoro-logique de Nkolbisson, 1975 - 1989).

Field collection of mosquitoes

Mosquitoes were collected as larvae or pupae from breeding sites in the study area between March and November 2006. Breeding sites varied from tyre tracks, irrigation pools, and shallow water to open concrete water tanks. Larvae or pupae were collected using the dipping method with the aid of copper ladle, transferred into plastic jars with perforated lids for ventilation and transported to the laboratory for rearing.

Laboratory rearing of mosquitoes

Larvae and pupae were cultured in plastic containers, 5 cm height by 27 cm width by 36 cm long with a large surface area and a water depth not more than 4 cm at 25 ± 2^{o}C and 70 - 75% relative humi-dity. A 12 h light and dark cycle was be maintained. Pupae were separated daily into wood framed mosquito cages (40 x 40 cm) till adults emerge. The adults were fed on 10% sucrose solution for 1 - 2 days before being used for bioassays.

Morphological identification of *A. gambiae* mosquitoes

A. gambiae s. l. was morphologically identified using the keys of (Gilles and de Meillon, 1968; Hervy et al., 1998). This is based on the morphological differences between anopheline and culicine mosquitoes as well as among the different species of the genus *Anopheles*.

Fungus

B. bassiana RBL1034 isolate was isolated from a stemborer, *Busseola fusc*a (courtesy, Ngue Benel) from a maize farm in Yaounde. The phylogenetic position of isolate RBL1034 within the *B. bassiana* morphospecies was inferred according to the approach and methods of (Rehner et al., 2006). This analysis placed the isolate in "Asia_4", an informal designation for an Asian lineage that also includes closely related counterparts in equatorial Africa patho-genic on the coffee berry borer, *Hypothenemus hampei* (Rehner et al., 2006). Conidia were inoculated on potato dextrose agar (PDA) and placed in an incubator to grow. A mass production of conidia was obtained by inoculating sterile boiled rice with pure spores of *B. bassiana* RBL1034 and allowed to grow at 25°C. Eight days after the onset of sporulation, conidia were harvested using a Myco-Harvester Version V and stored in the dark at 4°C until ready to use.

Bioassays

Bioassay 1

Bioassays were done following the method of (Sholte et al., 2003) with minor modifications. Non blood fed mosquitoes aged 1 - 2 day old were used. Prior to the bioassays a total of 60 male and female mosquitoes per replicate were transferred into paper cups covered with mosquito netting material and keep for at least 1 h in order to eliminate any injured mosquito before the bioassays were carried out. Climatic conditions were 27 ± 3°C and 77 ± 4% RH. A wooden cuboid (2 cm^3) attached to a hook screw (No.8) was wrapped with a yellow tissue paper (LABELL, Belgium) and used as a target. A hundred mg of dry pure conidia of *B. bassiana* RBL1034 was weighed unto an aluminium foil and the target was gently rolled over it to entirely dust the surface. The impregnated target was carefully attached to a wood framed mosquito cage (30 x 30 cm) using a string. The mosquitoes were transferred from the paper cups into the cage using an aspirator. A feeding source (10% sucrose soaked in cotton pad) was placed at 2 cm away from the suspensor. This was replaced after every three days. Mosquitoes landing on the target as a resting surface after feeding will even-tually be exposed to dry conidia through tarsal contact, the mouthparts or legs. The target remained in the cage until the end (10 days) of the bioassay resulting in continuous exposure to the conidia. The experiment was replicated four times on different days. A control was set up with a similar target without conidia. An esti-mation of conidia density in 100 mg of the inoculum was done using the dilution technique giving a total density of 3.6x 10^8 colony forming units (cfu). Dead mosquitoes were collected from the cages daily, placed on moist filter paper (distilled water) in a paraffin sealed petri dish and observed for fungal growth within 4 days.

Bioassay 2

This assay was done to determine the minimum *B. bassiana* RBL1034 conidia density/mm^2 on surfaces of varying sizes that is required to give a maximum mortality in the *A. gambiae*. The experimental procedure was the same as in Bioassay 1 except that the volume and surface of the suspensor was varied (2, 4, 6 and 8 cm^3).

Data analysis

Abbott's formula (Abbott, 1925) was used to correct for natural mortalities in all the bioassays. Results of bioassay tests were analysed for mortality/survival relationships using descriptive statistics.

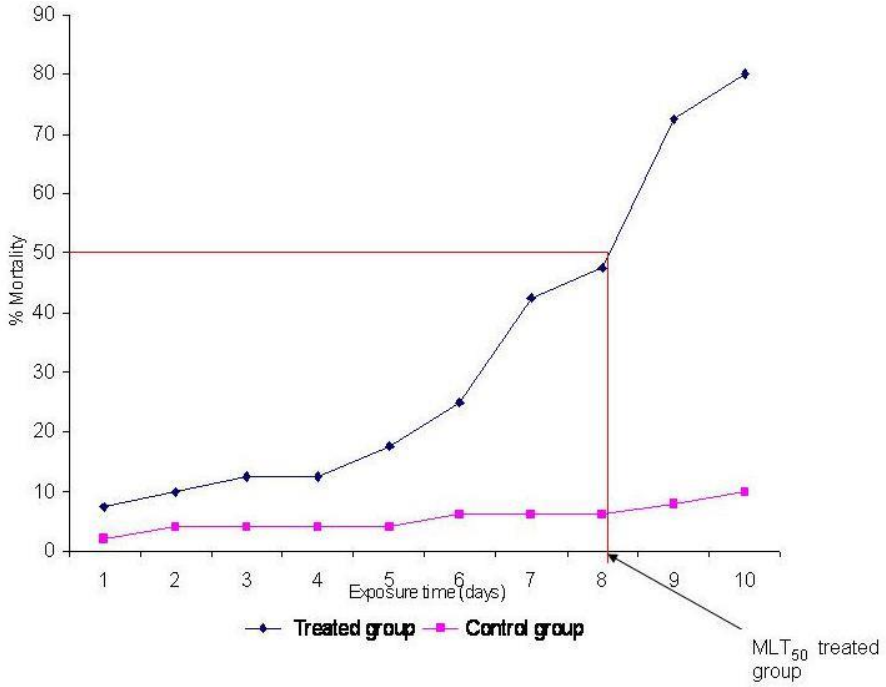

Figure 1. Percentage mortality of adult *A. gambiae* s.l. exposed to dry conidia of *B. bassiana* RBL1034 for 10 days.

RESULTS AND DISCUSSION

In Bioassay 1 *B. bassiana* RB1034 was observed to be pathogenic to both male and female *A. gambiae* mosquitoes. Mortality was observed to increase with time (Figure 1) and the males died faster than the females. Mosquitoes were observed to rest mostly on the edges of the inoculated target. Total mortality in the treated group ranged from 77 – 97% with an average mortality of 80% compared to 10% in the control group (Figure 1). The median lethal time (MLT_{50}) was 8.5 days and greater than 10 days for treated and control group respectively (Figure 1). Relative to the control group, fungal sporulation was observed in 87% of the mosquito cadavers in the treated group (Figure 2). Fungal sporulation was first observed primarily on the mouths parts, thorax and forelegs before eventually spreading to other parts of the insect cada-vers. In Bioassay 2, the mortality in the treated group was observed to increase with increased density of dry conidia, reaching a maximum at $15x10^3$ CFU/mm^2 (Figure 3).

The main objective of malaria vector control is to break the transmission of malaria parasite. Therefore reducing the life span of the female *A. gambiae* will have a considerable impact on the transmission risk of the malaria parasite. Sholte et al. (2003) reported that an entomopathogenic fungus *Metarrihzium anisopliae* was pathogenic to both sexes of *A. gambiae* and *Culex quinquefasciatus* and mosquitoes in the treated group died significantly earlier that those in the control group. Similarly, in the

Figure 2. *B. bassiana* RBL1034 growing on mosquito cadavers 3 days after death following exposure to fungus impregnated target.

present study the daily survival rate was observed to decrease in the treated compared to the control group i.e., when 80% of the infected specimen had died at the end of the experiment, 90% of the uninfected group were still alive indicating that these mosquitoes are susceptible to conidia of *B. bassiana* RBL1034. This was also noted

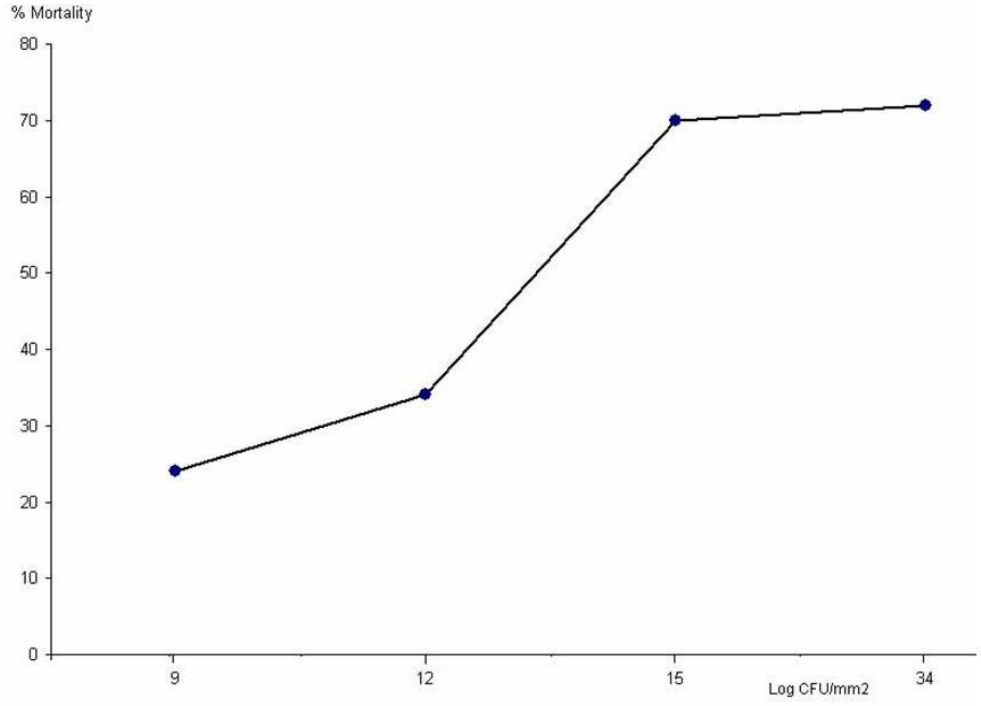

Figure 3. Relationship between the concentrations of conidia of *B. bassiana* RBL1032 (x 10^3 cfu/mm^2) on treated surfaces and percentage morality in adult *A. gambiae* s.l. The % mortality was the same for all the spore concentration values above 15 x 10^3 cfu/mm^2.

in the difference between the LT_{50} values for the treated and control groups. Auto dissemination of fungal spores has been reported in *A. gambiae* (Scholte et al., 2004). Fungal sporulation was observed on both male and female *A. gambiae* which suggests that horizontal infec-tions may be acquired from both infected females and/or males. Blanford et al. (2005) showed that exposure to surfaces treated with fungal entomopathogens following an infectious blood meal reduced the number of mosquitoes able to transmit malaria by a factor of about 80. The procedure used in the present study envisaged *B. bassiana* impregnated targets hung on walls or ceilings and mosquitoes using these as resting surfaces could get infected with fungal spores. The mosquito therefore succumbs to the fungus before or after a blood meal thereby reducing the transmission of malaria. Moreover, unlike entomopathogenic bacteria where the mosquito has to ingest the pathogen, infection with conidia of *B. bassiana* is by contact in which case the fungus germinates, penetrating the mosquito and growing within it leading to eventual death.

Chemical insecticides are the mainstay of mosquito control programmes in most parts of the world. When compared with the results obtained in the present study using the *B. bassiana* RBL1034 the primary consideration is efficacy and cost. However, the major advantages of the use of entomopathogens are their safety to non-target organisms, reduced pesticide residues in food, preserved natural enemies and resultant increased bio-diversity (Lacey et al., 2001). Moreover, the use of fungal biopesticides could supplement chemical-based control strategies in integrated vector management programmes especially in areas where resistance to insecticides have been reported. *B. bassiana* may be particularly promising as it has already been used as an agricultural biopesticide (Li et al., 2001; Donald and McNeil, 2005).

Since earlier studies (http://ipmofalaska.homestead.com/files/beauveria.html) showed that it is safe, it might be a good candidate for malaria vector control programmes. Another possible advantage is that the use of indigenous isolate(s) for insect control would avoid possible unanticipated risks associated with introductions of exotic entomopathogens.

Results from the present study indicate that dry conidia of *B. bassiana* RBL1034 is pathogenic to adult *A. gambiae* under laboratory conditions and could be a po-tential biocontrol agent for these mosquitoes. The formulation of the fungus for its use in human habitation and its stability and viability needs to be investigated.

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