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Larvicidal Action of *Beauveria bassiana*against Yellow Fever Mosquito *Aedes aegypti*

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ABSTRACT
The main objective of this study is to evaluate the larvicidal action of entomopathogenic fungi *Beauveriabassiana*on third and fourth instar of larvae of *Ae.aegypti* for its effectiveness. The entomopathogenic fungi *Beauveria bassiana* (Bals-Criv) Vuill (1912) available as a preparation marketed as Green Beauveria bassiana is used in this study. The preparation contains 2 x 10^8 spores/ml and is marketed by Greenlife Biotech Laboratory, Coimbatore. The fungul solution was diluted for testing it against *Ae.aegypti* larvae. In the present study various concentrations of *B.bassiana*inoculum in concentration ranging from 4-52 (µl/dl) and 10-130 (µl/dl) were used to control third and fourth instar larva of mosquitoes.Mortality of *Ae.aegypti*larvas were recorded every 12h till 72h for both third and fourth instar larvae. At the concentration of 52 µl/dl 100% mortality of *Ae.aegypti* during the third instar was observed whereas 130 µl caused 100% mortality in fourth instar larvae.In the present study followed the general pattern, that the probit analysis of *Ae.aegypti* for third instar larvae showed the decrease in LC50 from 24h (day1) to 72 (day3) is 37.15, 25.70, 19.05 µl/dl. Likewise, fourth instar larvae showed the decrease in LC50 from 24h day1 to 72h (day3) is 89.12, 60.26, 46.67 µl/dl.To recapitulate, increasing concentration and duration of exposure to *B. bassiana*are responsible for the mortality of *Ae.aegypti*. According to the instar of*Ae.aegypti*development(3rd and 4th instar), increased concentration is required to achieve absolute mortality. These fungal isolate*B. bassiana* are potential benefits environmental safety, target-specificity, efficacy, biodegradability and suitability in various fields

Keywords: *Beauveria bassiana*, Yellow fever, *Aedes aegypti*, mortality.

1. INTRODUCTION

Mosquitoes are large and abundant group except Antarctica distributed throughout the tropical, temperate and well beyond Arctic Circle (Harbach, 2007,Hall and Tamir, 2022). They are most diverse group but least known in tropical forests. There are 3,490 species of mosquito species formally recognized; only a few dozen of them is dangerous. They kill over millions of people by spreading various diseases and regarded as one of the humanities's deadliest animal (Hall and Tamir, 2022).

Structurally they are slender, long-legged insects possess long proboscis and has of scales on most parts of the body. Their free-living aquatic larvae are distinguished by the presence of a distinct head bearing mouth brushes and antennae, a bulbous thorax, posterior anal papillae and either a pair of respiratory openings (subfamily Anophelinae) or an elongate siphon (subfamily Culicinae) borne near the end of the abdomen (Harbach,2007).

Since vast surface of earth was occupied by human many native species habitats were encroached. As a consequence, some blood-requiring insects switchin taking blood meals from non-human animals, zoophagy to anthropophagy (*ie.* taking blood from humans). Thus, humans are challenged with infectious diseases once confined to animals (Powell and Tabachnick, 2013). *Aedes aegypti* live in close association with humans, developing preferentially in urban and suburban areas where human hosts are easily available.*Ae.aegypti* is the principal mosquito vector of dengue virus, yellow fever virusandChikungunya, the prior one cause more human morbidity and mortality than any other arthropod-borne viral disease (Harrington, 2001,Powell and Tabachnick, 2013).

Most of the studies conducted for targeting adult mosquitoes however results of larval control of mosquitoes

were highly effective (Bukhari et al 2011). Chemical insecticides (for example organophosphates and pyrethroids), microbial control (bacteria) and physical elimination of breeding grounds are being some of the techniques currently used to control larval mosquitoes (Pereira et al 2009). Insecticides are being the main method of controlling mosquitoes often involves using broad spectrum chemical insecticides (ffrench-Constant 2005). These insecticides often affect non-target organisms and create environmental health problems (Federici et al. 2007, Mishra et al. 2011). The rise in the mosquito resistance and increasing spread of mosquito-borne diseases refocused interest on entomopathogenic fungi as useful alternative to conventional methods (ffrench-Constant 2005). As an alternate biological control of mosquitoes being eco-friendly cost effective and have great potential for effective and extended use of in the control.*B.bassiana* is an entomopathogenic fungi attractive candidate for biological control and can be easily and economically produced and the conidia have a long storage life (Geden et al 1995). Recent studies have shown the potential of this fungus as next generation agents for the control of mosquitoes (Bukhari et al 2011).

2. MATERIALS AND METHODS

The larvicidal action of the fungus *Beauveria bassiana*was tested against the third and fourth instar larvae of *Ae.aegypti.Beauveriabassiana* (Bals-Criv) Vuill (1912) available as a preparation marketed as Green Beauveria bassiana is used in this study. The preparation contains 2 x 10^8 spores/ml and is marketed by Greenlife Biotech Laboratory, Coimbatore. The fungul solution was diluted for testing it against *Ae.aegypti* larvae.

Toxicity Studies

The toxicities of *B. bassiana*against *Ae.aegypti* larvae were tested using the static bioassay protocol (Sparague, 1973). Exactly 10 larvae, each were exposed to 10-13 concentrations of the microbial preparations. The larvae in each cup were observed at 12, 24, 36, 48, 60 and 72 hours. The number of larvae dead at these intervals was recorded as percent mortality values. Simultaneously controls were maintained and mortality values were compared and corrected.

Profit Analysis

Profit analysis was carried out using methods suggested by Finney (1952). The concentration of microbial toxicants was converted into log concentration values and present mortality into profits. Using linear regression analysis, the percentage of mortality was calculated. From this, nh LC_{50} values and their confidence intervals were calculated. The LC_{50} values were used for assessing the toxicity of two microbial pesticides used in this study against the common tiger mosquito *Ae.aegypti*.

3.RESULTS AND DISCUSSION

The present study records the larvicidal action of the fungus *Beauveria bassiana*was tested against the third and fourth instar larvae of *Ae.aegypti*.The third instar of *Ae.aegypti* were allowed into water taken in paper cups, carrying *B.bassiana*in concentration ranging from 4-52 (µl/dl). Similarly, fourth instar of *Ae.aegypti*was allowed at increasing concentration of 10 (µl/dl). Once the experiment was started, mortality was recorded once in every 12h till 72h. Each instar had a life span of 72h.

3rd Instar

When third instar larvae were exposed to *B. bassiana*, no mortality was recorded at 4 (µl/dl), 8 (µl/dl) 10 % mortality was recorded after 72 h (Table 1).

24 hours (Day 1)

At the concentrations of 4,8,12 and 16 (µl/dl) no mortality was recorded within 24h. Whereas, 20 (µl/dl) caused death of 20% mortality, followed by 24, 28, 36 (µl/dl) caused 30 mortality. Similarly, 40 and 44 (µl/dl) caused mortality of 40%, 48 (µl/dl) caused death of 50 %, 52 (µl/dl) caused death of 100 % mortality.Based on the results of probit analysis of 24h response of third instar *Ae.aegypti* (b value is 4.54, x value is 1.57 and hence the LC_{50} is 37.15 (µl/dl) with the confidence interval 37.02 and 46.35.

48 hours (Day 2)

Inoculums of 12(µl/dl) caused mortality after a day at 48h caused 10 % mortality. Followed by 16 (µl/dl) 20 % mortality, 20 (µl/dl) 40% mortality, 24 (µl/dl) 50 % mortality, 28 (µl/dl) 60% mortality, 32 (µl/dl) 50%, 36

(µl/dl) 60%, 40 (µl/dl) 70%, 44 (µl/dl) 60%, 48(µl/dl) 100%.Based on the results of probit analysis of 48h response of third instar *Ae.aegypti*LC_{50} is 25.70 (µl/dl) (b value is 4.03, x value is 1.41 and confidence interval of 26.27 – 34.34).

72 hours (Day 3)

Response of third of instar to*B. bassiana* 8 (µl/dl) after two days of exposure leads to 10% mortality at 72h, followed by increasing concentration of 8, 12, 16, 24, 28, 32, 36,40, 44 (µl/dl) caused increasing mortality rate of 10%, except 32 and 36 (µl/dl) mortality rate was equal (80%). Based on the results of probit analysis of 72 h response of third instar *Ae.aegypti* the calculated value of the LC_{50} is 19.05 µl/dl (b value is 4.21, x value is 1.28 and confidence Interval 17.78 and 26.22).

4th Instar

When fourth instar larvae were exposed to *B. bassiana*inoculums with the concentration ranging from 10 – 130 (µl/dl), no mortality was recorded at 10 (µl/dl) till 72 h of exposure (Table 2).

24 Hours (Day 1)

The mortality response of fourth instar *Ae.aegypti* has been documented at the concentrations of 10 to 40 (µl/dl) no mortality was recorded for 24h. With the concentration of 50 (µl/dl) 20 % mortality was achieved. Whereas, concentration 60 and 70 (µl/dl) equally 30 % mortality was recorded. At the concentration of 80 (µl/dl) 40 % mortality were observed. On the contrary, 90 (µl/dl) caused only 30% mortality. Again, 100 and 110 (µl/dl) equally 40 % mortality was recorded. Concentration of 120 and 130 (µl/dl) had 50 and 100 % mortality respectively.Based on the results of probit analysis of 24h response of fourth instar *Ae.aegypti* LC_{50} is 89.12 µl/dl (b value 4.42, x value 1.95 and confidence interval is 89.06 and 98.02.

48 Hours (Day 2)

*B. bassiana*concentration of 30 (µl/dl) had effect on 10% death at 48h, followed by 40 (µl/dl) had 30% mortality, 50 (µl/dl) 50% mortality, even after increasing concentration 60, 70, 80, 90, 100 the mortality rate was only 60. Concentration of 100 and 120 proved 70 and 100 % mortality. Based on the results of probit analysis of 48h response of fourth instar *Ae.aegypti* (b value is 3.76, x value is 1.78 and hence the LC_{50} is 60.26 µl/dl confidence interval ranged from 58.63 to 66.16).

72 hours (Day 3)

Lowest concentration of 20 (µl/dl) *B. bassiana*had effect on fourth instar larvae of *Ae.aegypti* only on third day with 10 % mortality. Then the mortality showed increasing tread 30 (µl/dl) had 30% mortality, 40 (µl/dl) 40%, 50 (µl/dl) 60%. However, even increasing concentrations of 60, 70, 80 (µl/dl) equally 70 % mortality. Likewise, 90 and 100 (µl/dl) had 80 % mortality. Concentration of 110 (µl/dl) resulted in 90% mortality after 72 h. Based on the results of probit analysis of 72h response of fourth instar *Ae.aegypti* LC_{50} is 46.67 µl/dl (b value is 3.09, x value is 1.67 and confidence interval is 44.06 and 50.24).

DISCUSSION

Mosquitoes are responsible for the transmission of many medically important pathogens and parasites such as viruses, bacteria, protozoans, and nematodes.Which cause serious diseases such as malaria, dengue, yellow fever, Chikungunya fever, encephalitis or filariasis and threatens the human life (Becker et al. 2010). Accordingly developing therapeutics and vaccines for diseases over the last few decades took place. However efficient vector control strategies are still the primary method used for control and prevention of mosquito-borne diseases (Huang etal. 2017). Complete elimination of disease causing vectors is impractical reduction in numbers of vectors is an important part of disease control.

Mosquito control can be achieved in three ways by means of chemical and biological control and environmental management. Mosquito control has long history, involves screening houses, oiling water, draining standing water, distributing larva- eating minnows these trials during 1910 proved successful (Stapleton, 2004, Brühl et al. 2020). Prior to the appearance of resistance in mosquitoes various organophosphorus and chlorinated hydrocarbons were widely used in the mosquito control (Mulla and Darwazeh, 1975). However, the use of these chemical pesticides has ledto several problems, human health effects, environmental pollution, affecting beneficial non-target species insects, landscapes and communities (Devine and Furlong, 2007, Bravo et al. 2011).

Biological control of mosquito larvae were using mosquito fish, *Gambusia affinis* (Bence, 1988), elephant mosquito species *Toxorhynchites* spp. (Collins and Blackwell, 2000), copepods (Marten etal. 1994) and mermithid nematodes (Platzer,2007) success depends on various factors and each one has limitations (Huang etal. 2017). At this juncture, WHO (2005) specific and standardized procedures and guidelines for testing larvicidesin order to control the mosquito were provided.

More than 100 years ago there are two entomopathogenic fungal species *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin and *Beauveria brongniartii* (Saccardo) Patch were described and they have been considered as fungi that can and should be used for control of pest insects. There are naturally occurring fungi fungi*Coelomomycesindiana*and *C. anopheles*first known infections of anopheline larvae had been found in India and described by Iyengar (1935). In the early days of biological control and especially microbial control, there was no concern forpossible side-effects or safety considerations of these two species(Zimmermann, 2007).

Pereira et al (2009) stated that the biology of the vector *Ae. aegypti*, appear to favor the use of entomopathogenic fungi. In the present study *B. bassiana*least concentration of 8µl during 3rd instar development stage of *Ae.aegypti* caused 10% mortality at 72h (day 3). At the concentration of 52 µl/dl within for 12h and 24h (day 1) of observation caused 50% and 100% mortality respectively. Whereas, 10 (µl/dl) concentration cause no mortality for the 4th instar larvae of *Ae.aegypti*. However, 20 µl caused 10% mortality at 72h (day 3). At the concentration of 130 µl within for 12h and 24h (day 1) of observation caused 60% and 100% mortality.

Overall, increasing concentration and duration of exposure to *B. bassiana*are the predicted factors responsible for the mortality of *Ae.aegypti*. In addition, according to the instar (3rd and 4th) of *Ae.aegypti*development, increased concentration is required to achieve the increased mortality. The result of the present study was in agreement with research carried out in houseflies. Mishra et al.(2011) reported bioassay ofhousefly and reported absolute mortality of larval housefly at all concentration within 3 to 5 days of two entomopathogenic fungi. Likewise for housefly larvae were obtained by Watson etal.(1995) and also adult houseflies were susceptible to *B. bassiana*(Geden et al 1995).

Lethal Concentration denoted as LC_{50}, is the concentration of a substance that is lethal to 50% of the organisms in a toxicity test. LC_{50} can be determined for any exposure time, common durations are 24, 48, and 72 hours. In general, smaller LC_{50} value, more toxic is the chemical. The opposite is also true larger the LC_{50} value, the lower the toxicity (Boyd, 2005).In the present study also followed the general pattern, that the probit analysis of *Ae.aegypti* for third instar larvae showed the decrease in LC_{50} from 24h (day1) to 72 (day3) is 37.15, 25.70, 19.05 µl/dl. Likewise, fourth instar larvae showed the decrease in LC_{50} from 24h (day1) to 72h (day3)is 89.12, 60.26, 46.67 µl/dl.

4. CONCLUSION

It is evident from the present attempt to use *Beauveria bassiana*as biological control agents against *Ae. aegypti* mosquitoes. In our study various concentrations of *Beauveria bassiana*inoculum in concentration ranging from 4-52 (µl/dl) and 10-130 (µl/dl) were used to control third and fourth instar larva of mosquitoes. Mortality of *Ae.aegypti*larvas were recorded every 12h till 72h for both third and fourth instar larvae. For each 12h mortality of *Ae.aegypti* was noted. At the concentration of 52 µl/dl 100% mortality of *Ae.aegypti*during the third instar was observed within 24h. Whereas 130 µl caused 100% mortality in fourth instar larvae. Overall, increasing concentration and duration of exposure to *B. bassiana*are the predicted factors responsible for the mortality of *Ae.aegypti*. In addition, according to the instar (3rd and 4th) of *Ae.aegypti*development, increased concentration is required to achieve absolute mortality. Use of organophosphorus, chlorinated hydrocarbons as well as chemical pesticides has been to control mosquitoes. Which intern created problems to human and environmental pollution, affecting non-target species, landscapes and communities. However, these entomopathogenic fungal species *B. bassiana*are highly effective in controlling population at its larval stage. It has potential benefits environmental safety, target-specificity, efficacy, biodegradability and suitability in various fields.

Table 1. Response of third instars*Ae.aegypti*to *Beauveria bassiana*

Sl.No	Concentration µl/dl	Hours of Observation					
		Hours 12	Hours 24	Hours 36	Hours 48	Hours 60	Hours 72
1	4	-	-	-	-	-	-
2	8	-	-	-	-	-	10
3	12	-	-	-	10	10	20
4	16	-	-	10	20	30	30
5	20	-	20	30	40	40	50
6	24	10	30	40	50	50	60
7	28	20	30	50	60	60	70
8	32	20	20	40	50	70	80
9	36	20	30	50	60	70	80
10	40	30	40	60	70	80	90
11	44	30	40	50	60	80	100
12	48	40	50	70	100	-	-
13	52	50	100	-	-	-	-

Table 2 Response of fourth instar*Ae.aegypti*to *Beauveria bassiana*

Sl.No	Concentration µl/dl	Hours of Observation					
		12	24	36	48	60	72
1	10	-	-	-	-	-	-
2	20	-	-	-	-	-	10
3	30	-	-	-	10	20	30
4	40	-	-	10	30	30	40
5	50	-	20	40	50	50	60
6	60	10	30	40	60	70	70
7	70	20	30	50	60	60	70
8	80	20	40	50	60	60	70
9	90	20	30	50	60	70	80
10	100	30	40	50	60	70	80
11	110	30	40	50	70	80	90
12	120	30	50	70	100	-	-
13	130	60	100	-	-	-	-

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