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Evaluation of botanicals against: Mosquito Larvae to the Extracts of Fungus Beauveria Species

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ABSTRACT

The larvicidal activity of Beauveria species on mosquito Culex quiquefaciatus, Armegeries subalbatus and Anopheles species was done, penicilliumis a large genus of filamentous fungi widely distributed in soil and in associated with plants. It was observed that more toxicity of *Beauveria e*xtract towards the larvae of *Anopheles* species than *Culex quinquifaciatus*, The toxicity was lesser in *Aremgeries subalbatus*, The toxicity range was observed between 100 ppm to 250 ppm. The toxicity on mosquito may be due to the toxins, and secondary metabolites of Beauveria species. Cyanide also creates toxicity on larvae, Beauveria based mosquito control can be done after field trials under controlled environment.

***Keywords*:** Beauveria species, *Culex quiquefaciatus, Armegeries subalbatus* and Anopheles

1. INTRODUCTION

Mosquitoes are one of the medically important arthropod vectors of disease. Mosquitoes transmit many dreadful diseases like malaria, filariasis, Japanese encephalitis and dengue fever affecting in the socio- economi status many nations (Service, 1983). Mosquitoes are more tropical and subtropical regions create nuisance to human by biting and sucking blood.

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Mosquitoes are classified as Dipteran insects with few genera and many species. Mosquitoes belong to the *Genera Anopheles, Culex Amergeres, Aedes, Monsonia* are some examples of human concern. Only the female mosquito bite and disease spread in man. Through the larval stage of the mosquito is not infectious or parasitic it contaminates all natural fresh water bodies and storage tanks water. Several studies have been done for their diversity, survivability and disease spread (Mariappan and Reddy 1982).

*Culex quinquefasciatu*s is an important mosquito species and it causes several diseases, including West Nile Virus, yellow fever, filariasis, Japanese encephalitis and avian malaria. More than 50 percent of the people receive filariasis is through bites of C. *quinquefaciatus* (Butt, *et. all.* 2013).

Control or eradication of mosquitoes is an essential step to give healthy environment to man. National and International Organizations have taken several steps to eradicate mosquitoes by chemical mode or insecticide mode of control of adult mosquito and larval populations through surfactants, oil or toxic chemicals (Georg et al., 2013). Recently transgenic approach has been made to prevent the transmission of malarial disease through *Anopheles* species (Chinnasamy *et al*., 2017). A new type of toxin isolated from Xenorhabdus innexii bacteria called XLT lipoprotein has a potent Mosquitoes activity (Farenhorstand Knols, 2010).

Mosquito control any at any stage by physical, chemical or biological mean is necessary to prevent is existence disturbance to man and disease cause. Approaches for control of mosquito are started only when an outbreak of mosquito based nuisance or any trace of mosquito based nuisance or any trace of mosquito based disease is observed. Biological control methods are better way as it is less harmful to the environment and other organism associated with particular a ecosystem. In the present study fugal mediated mosquito larval control was analyzed against the mosquitoes *Culex quinquifaciatus Anopheles species* and *Armigeres subalbatus*. (Chinnasamy *et al*., 2017).

These mosquitoes are prevalent in rainy seasons and winter seasons. Culex is seen throughout the year. Bacterial mandated control of mosquitoes is a promising level to an extent when used bacteria like Bacillus thurgiensis species, and Bacillus sphaericus. Fungi and fungal derived products are highly toxic to mosquitoes, yet have low toxicity to non-target organisms. According the use of entomphathogenic fungi and their dried products may be a promising approach to biological control of mosquitoes (Khodyre *et al.*, 2014).

Fungi belong to several genera are most frequently associated with insect diseases including mosquitoes. The genus Coelomomyces is composed of a large group of obligatory parasitic aquatic fungi, the overwhelming majority of with have been reported from mosquito larvae. All of them are overwhelming majority of which has been reported from mosquito larvae. All of them are obligate parasites and require a mosquito host and a copepod host to complete their life cycle. There are several isolates of the fungus *Lagaeniduium giganteum*, with are pathogenic for mosquito larvae. They are facultative parasites and not tolerant to organically polluted water or brackish water. Significant number of adults of Culexpipiens are infected with Entomophthora species. Entomophthor aquatic infecting Aedes larva was also reported. (Butt, *et.* 2013).

Investigation of the potential of some isolates of Beauveria bassisana for larval mosquito control was not promising. Another species, Beauveria brongniartii was found to be virulent against a wide variety of mosquito larvae. Culicinomy cesclavoporous were originally found as contaminants in laboratory populations of mosquitoes.

They are facultative parasites and unlike other Fungi Imperfect, conidia are produced on submerged mosquito larvae. The fungus is a facultative parasite and all mosquito species tested are susceptible to its conidia. (Achonduh *et al*., 2008)

The secondary metabolites of Aspergillus, Penicillium, Fusarium, Paecilomyces, Rhizopus, Amanta, Syncephalastrum, Monilia and Tolypocladium species have been reported to be toxic to mosquitoes (Farenhorst *et al*., 2010). The biological properties of the secondary metabolites of fungi range from antibiotics to myotoxins (Farenhorst *et al*., 2010). These have been reported to cause retardation of growth, low level of fecundity, loss of fertility, mortality, repellency, etc.

In the present study the larvicidal activity of Beauveria species on selection mosquito was done. Beauveria is one of the well – know nentomogenous fungal genera and has a cosmopolitan distribution. Although the genus has a very broad host range (Chinnasamy *et al*., 2017), the natural occurrence of Beauveria on mosquitoes has also been reported. (Chinnasamy *et al.,* 2017) Conidia of Beauveria bassiana are effective in killing mosquito larve that feed below the surface, mainly at the tip of the siphon, although Miranpuri and

Khachatourians (1991) found the head to be an equally important infection site.

In laboratory tests the fungus proved virulent againstlarvae of *Culexpipiens, Culextarsalis, Culextritaeniorhynchus* and Anopheatus albimanus. Susceptible species were prone to infection only shortly after molting. Laboratory studies showed that besides *Ochlerotus sierrensisles* the fungus, was pathogenic for larvae of Aedes aegypi, (Pinnock *et al.*, 1937).

Besides infecting larvae, the fungus proved by species of this genus: Beauvericin, Bassianin, Bassianolide, Beauverolides, and Tenellin from B. *bassiana*, and Oosporein from Beauveria brongniartii (Achonduh *et al*., 2008).

A dichloromethane extract from mycelium showed activity when assayed against Aedes aegypi larvae at 100 ppm (Achonduh *et al*., 2008 Chinnasamy *et al*., 2017). This extract contained Beauvericin and two analogues (Beauvericin A and B). The larvicidai properties of Beauvericin had already been reported by Grove and Polpe (1980). The effective stages of the fungus against larvae are conidia and blasto conidia, the latter stage being far more pathogenic (Miranpuri and Khachatourians1991). A poilot factory in Krasnodar (former Soviet Union) was able to produce 22 tons of Boverin annually (Achonduh *et al*., 2008).

This limitation, along with the high dosage needed is serious drawbacks for mosquito larval control. Formulation with oil may overcome some of the problem. Beauveria brongniartii was considered a potential biological control agent since this species is pathogenic for larvae of several tree- hole breeding mosquito species, including Aedesasegypi, the large doses required for significant mortality rates of moquite larve was not considered practical (Chinnasamy *et al.,* 2017).

2. MATERIALS AND METHODS

2. 1. Mosquito Species

Mosquito Species used for toxicity bioassay are Culex quinquifasciatus, Anopheles species and Armegere subalbatus.

2. 2. Armigeres subalbatusis

Armigeres subalbatusis a large mosquitoes and produce an immediate allergy reaction in man when it bites. These are large mosquito and size reaches up to 1 cm. The larvae are found in cold climate conditions. They are more active at the time of morning 5 am to 7 am and similarly in the evening 5 pm to 7 pm. They have others hosts like cattle, sheep and other small mammal.

2. 3. Culex quinqufasciatus

*Culex quinquifasciatusi*s genus of mosquito, and is important in that several species serve as vector of important diseases, such as West Nile virus, Filariasis, Japanese encephalitis, St, Louis encephalitis and avian malaria. The adult mosquito can measure from 4-10 millimeter

2. 4. Anopheles species

Anopheles gambiae commonly transmit parasites of the genus Plasmodium, Which cause malaria in human in endemic aress. Some species of Anopheles also can serve as the vectors for canine heart worm *Dirofilaria immitis*, the filarisasis – causing species *Wuchereria bancrofti* and *Braugia malayi.* This mosquito is seen during rainy seasons.

2. 5. Mosquito larvae

Mosquito larvae have a well – developed head with mouth parts used for feeding, a large thorax and nine segmented abdomen. They have no legs, Except Anopheles all other possess respiratory siphon. It respires through the spiracles. The larvae spend most of their feeding on algae, bacteria and other microorganisms in the surface micro layer. They dive below the surface only when they are disturbed. The larvae occur in wide range of fresh water habitats.

2. 6. Beauveria

Beauveria is a gene of asexually – reproducing fungi allied with the ascomycete family Clavicipitaceae. Its several species are typically insect pathogens. The sexual states (teleomorphs) of Beauveria species, where known, are species of Cordyceps. Beauveria species are white entomopathogenic fungi. They from unicellular conidia thatare typically hydrophobic and very small.

The conidia are formed holoblastically from basally inflated conidiogenous cells. After conidium production, the conidiogenous cell elongates before producing another conidium atop a small denticle. The result is the formation of a distinctive, slender, zig- zag rachis. Colonies of Beauveria species are typically white or off- white on artificial culture media. Species of Tritirachium resemble Beauveria species in having a zig – zig conidiogenous cells, but differ in lacking conspicuous denticles and in producing yellow – brown to purple colonies.

Beauveria species are commonly found associated with insects, including soil and private dwellings. *B. bassiana*, (Fig. 1) The most widely known member of this genus, has been developed as a biological pesticide for various insect pests.

2. 7. Taxonomy of Beauveria

Kingdom: Fungi

Phylum: Ascomycota

Class: Sordarionycetes

Order: Hypocreales

Family: Clavicipitaceae

Genus: Beauveria

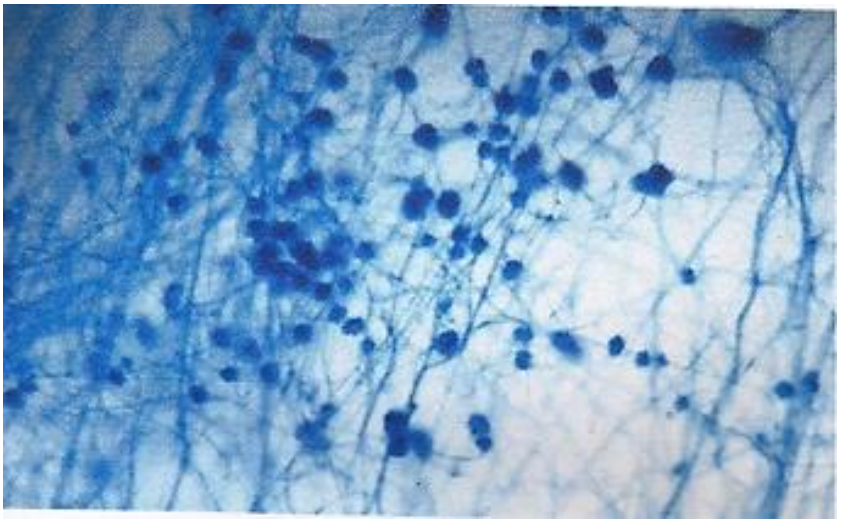

Fig. 1. *B. bassiana*

2. 8. Collection of Mosquito Larvae

The mosquito larvae of *Culex quinquifasciatus*, *Anopheles species* and Armegeres subalbatus larvae were collected from several places in Tiruchirappalli area. Samples of different instars were collected from small stream, stagnant water, ponds and river during winter season December to January. Collected larvae were identified and acclimatized for experiments, Larvae were conducted from these metabolites as per the standard methods and protocols of World Health organization. (WHO 2008, WHO 2013)

2. 9. Collection and Culture Methods For Beauveria species

Beauveria species collected from soil sample were allowed to grow Potato Dextrose Broth (200 g potato, 20 g dextrose, and 1000 ml distilled water) was used to grow the fungi. All opful of fungal growth from an potato dextrose agar plate was transferred to 100 ml of growth medium in a 500 ml was filtered through Whatman No- 1 filter paper the mycelia mass was discarded and the culture filtrate was used as test material for larvicidal activity. The filter paper, the mycelia mass was discarded and culture filtrate was used as test material for larvicidal activity. The filtrate was stored under refrigerator conditions, The bioassays were conducted from these metabolites as per the standard methods and protocols of the World Health Organization. (WHO 2008, WHO 2013).

2. 10. Staining of Fungi for Identication

Erlichs Hematoxlin stain in the formulation (Hematoxlin 2 gms, Alochol 100ml, aceticacid 20 ml, glycerin 100 ml and aluminum ammonium sulfate in excess)

2. 11. Preparations of Inoculum sample for toxicity, Bio assay

Beaueria samples were press said earlier. There are types of samples were used for this toxicity study. One is the cell free extract from the mature stage culture or the culture of declining phase. Second the whole mature culture as such was used. Their dried weight was used to express their concentrations. The concentration was expressed in parts per Million (PPM) or milligram per liter.

2. 12. Toxicity Bioassay for Mosquito Larvae

Mosquito larvae *Culex quinqfasciatus* of first instars and fourth instars stage were used experiment. Ten larvae per 100 ml per water are used and *Penicillium* sample solution and the cell free extract sample were added for toxicity test. The setup was kept for 2 days. Observations were made for larval mortality after 48 hours, Same experiment was repeated for other mosquito larval species (Anopheles and Armigeres Species). The metabolites which caused larval mortality in as follows. Plastic cups with 250 ml tap water added with 50 larvae each. The larval mortality was scored after 24 hours and 48 hours. The LC_{50} value was done by Probit analysis Finney DJ (1971).

3. RESULTS

3. 1. Toxicity of Beauveria Species

The toxicity of Beauveria species was observed in early stages of culture and also in mature period. The toxicity of the culture increases as the culture reaches the later stages of growth periods. Lethal concentration was less when used whole culture than the filtrate or extract of Beauveria culture.

3. 2. Toxicity of Beauveria to Culex quiquefasciatus

The toxicity bioassay done for to Culex quiquefasciatus with Beauveria species showed that the mosquito larvae were susceptible to the fungal products. The lethal concentration at 50% (LC_{50}) was observed for beauveria sample at150 ppm for First instar. The toxicity was at 200 ppm for fouth instar larvae showed inactivity during this period and morality was observed later.

Table 1. Toxicity bioassay for larval Culex quiquefasciatus agiast Beauveria species.

Concentration Beauveria	Percentage of Mortality		Concentration Beauveria sample filtrate	Percentage of Mortality	
	Instar -I	Instar -IV		Instar -I	Instar -IV
50ppm	Nill	Nill	50ppm	Nill	Nill
100ppm	10%	10%	100ppm	10%	10%
150ppm	40%	30%	150ppm	30%	20%
200ppm	60%	50%	200ppm	60%	40%
250ppm	100%	80%	250ppm	70%	70%
300ppm	100%	100%	300ppm	80%	80%

3. 3. Toxicity of Beauveria to Anopheles species

Table 2. Toxicity bioassay for larval Anopheles species against Beauveria species

Concentration Beauveria	Percentage of Mortality		Concentration Beauveria sample filtrate	Percentage of Mortality	
	Instar -I	Instar -IV		Instar -I	Instar -IV
50ppm	10%	Nill	50ppm	Nill	Nill

100ppm	20%	30%	100ppm	10%	20%
150ppm	60%	40%	150ppm	30%	20%
200ppm	80%	70%	200ppm	50%	40%
250ppm	100%	100%	250ppm	90%	70%
300ppm	100%	100%	300ppm	100%	80%

The toxicity bioassay done for Anopheles species with Beauveria species showed that the mosquito larvae were susceptible to the fungal products. The lethal concentration of 50% (LC_{50}) was observed as 100 ppm for First instar larvae, The toxicity was at 150 ppm for forth instar larvae (Table 2). And it was as 200 ppm in filtrate treated larvae.

3.4 Toxicity of of Beauveria to Armegeres subalbatu species

The toxicity bioassay done for *Armegeres subalbatu* species with Beauveria species showed that the mosquito larvae were susceptrible to the fungal products. The lethal concentration at 50% (LC_{50}) was observed as 100 ppm for First instar larvae, The toxicity was at 150 ppm for forth instar larvae (Table 3). And it was as 250 ppm in filtrate treated larvae.

Table 3. Toxicity bioassay for larval Armegeres subalbatu species Beauveria species.

Concentration Beauveria	Percentage of Mortality		Concentration Beauveria sample filtrate	Percentage of Mortality	
	Instar -I	Instar -IV		Instar -I	Instar -IV
50ppm	Nill	Nill	50ppm	Nill	Nill
100ppm	30%	10%	100ppm	20%	10%
150ppm	50%	30%	150ppm	40%	20%
200ppm	60%	50%	200ppm	50%	30%
250ppm	90%	70%	250ppm	60%	60%
300ppm	100%	80%	300ppm	90%	80%

4. DISCUSSION

Control of mosquitoes by physical, chemical or biological mean is necessary to prevent mosquito bites and their associated diseases in man. Biological control methods are better way as it is less harmful to the environment and other organism associated with a particular ecosystem. In the present study fugal mediated mosquito larval control, particularly Beauveria based analysis was done. Its toxic effect was assessed on mosquitoes Culex quiquefaciatus, Anopheles, and Armegeres species. All the three mosquitoes have showed their susceptibility to Beauvria species.

In the previous studies, culture filtrates of Beauveria showed toxicity against larvae of *CulexAedes and Anopheles* (George Jetal. 2013; Gayathri *et al*., 201). Their results also showed some isolates of Beauveria were determined to control of the larvae and adults of *Culex quinquefasciatus.* 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 the digestive tract was also possible (Scholte *et al*., 2007). Within the class Dueteromycetes, especially Ascomycetes have entomopathogenic fungi such as Metarchizium anisopliae, Beauveria bassiana and which was entering through the external integument. Besides, infection throughout digestive tract was also possible (Goettel and Inglis, 1997). Conodia 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 blasto spores in controlling the larvae. Vijayan and Balaraman (1991) studied the ovicidal, larvicidal and adulticidal activities of the metabolites of fungi and actinomycetes against *Culex quinquefasciatus, Anopheles stephensi and Aedes aegypti*.

The mortality of larvae may be due to the presence of toxin compounds, degradative enzyme like proteases, chitinases other regulatory metabolites of the fungus. In different studies the culture filterates of different soli fungi viz., *Aspergillus*, *Penicillum*, *Trichoderma* and some *Actinomycetes* were successfully done for their larvicidal activity. Further investigations are currenty being done to isolate the specific metabolites and toxins those are responsible for mosquitocidal activity.

5. CONCLUSION

The toxicity on mosquito may be due to the toxins, and secondary metabolites of Beauveria species. Cyanide also creates toxicity on larvae, Beauveria based mosquito control can be done after field trials under controlled environment. Further investigations are currenty being done to isolate the specific metabolites and toxins those are responsible for mosquitocidal activity.

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