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Bio-control of mosquito larvae using filamentous fungi-environmental study

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ABSTRACT

Fungal species are useful for medical and agriculture purpose as a bio- control agent. In aquatic environments, temperature, salinity and organic pollution are the important factors. The objective of the current project was to investigate the possibilities of controlling the mosquito population using fungal cultures. to investigate the possibilities of controlling the mosquito population using fungal cultures. In the view of rampant increase in the incidence of dengue fever transmitted through mosquitoes it is absolutely essential to control the mosquito population. There are different species of mosquitoes of which dengue virus is transmitted by the Aedes aegypti, found in habitats like stagnant clear waters, backyards which are common in urban areas. In the present work water samples were collected from in and around Mumbai, the water samples were examined for its nutritional status by analyzing them physically, chemically and biologically. The fungal cultures obtained from water samples were predominantly Aspergillus niger, Aspergillus terreus, and Penicillium, Trichoderma, Curvularia, Sprolegmia and Achlya. As per the results it was observed that the Curvularia was the most effective on the larvae as the mortality rate was 100%. The death of the larvae could be possibly due to the organic acids produced by the fungi. Microscopic observation of the larvae shows the growth of the fungi on larvae ultimately killing it. This is the most effective bio-control.

Keywords*— *Bio-Control, Asperigillus Niger, Aspergillus terreus, Penicillium, Curvalaria, Trichoderma

1. INTRODUCTION

Fungi belong to the second-largest group of organisms next to insects encompassing the most diverse organisms with a global estimate of 1.5 million species This conservative estimate is based on plant-fungus ratio (1:6 in temperate region) considering existence 2 of 250,000 plant species. This ratio has been predicted be up to 1:33 in tropics. Currently the number of known fungi is about 100,000 (6.6%) indicating gaps in our knowledge on fungal resources especially in tropics. The recommendations made for assessment of biodiversity of plants and animals are also applicable to fungi. Although significant proportion of fungi is yet to be discovered, they are important in ecological processes especially the nutrient turnover and energy flow.

The acquisition of knowledge concerning proper control of mosquitoes and mosquito borne diseases requires that studies be made of the biology, physiology, anatomy, genetics, taxonomy and ecology of the insect. *Aedes aegypti*, a vector of dengue, is widely distributed in the tropical and subtropical zones. About two-thirds of the world's population lives in areas infested with dengue vectors, mainly *A. aegypti*. Dengue viruses infect over 100 million people every year. Possibilities to reduce dengue incidence are studied world-wide. Vector control is one of the options, as interruption of transmission of dengue parasites is clearly the most effective disease control strategy. In laboratories, mosquito colonies are needed in order to conduct studies on vector biology, vector-parasite interactions, insecticide susceptibility, vaccine studies etc.

The life cycle of *Aedes Aegypti* includes four stages egg, larva, pupa and adult mosquito; it takes 8 to 10 days for complete metamorphosis. Adult mosquito has black and white markings on body. Larvae rest 45 degree from the surface of water. They are commonly found in containers with clean water. They prefer dark place. (Plate 8) All living organisms needs water, world's most precious resource, every day the energy you depend, the cotton you wear, the food you eat from fresh water habitats. More than 10% of all known animals and about 50% animals of fish species the massive role water plays for people and nature. Less than 1% of the world's water id fresh and accessible. Fresh water species decline 76% between 1970 and 2010. Representing a sharper decline than that measured in either terrestrial or marine biomes. Since 1900 more than 50% types of wet lands are disappeared. There are such fungal species which is useful for medical and agriculture purpose as a bio- control agent. In aquatic environments, temperature, salinity and organic pollution are the important factors.

2. OBJECTIVE

2.1 Primary Objective

To observe effect of filamentous Fungi on mosquito larvae.

2.2 Secondary Objective

- To screen species of fungi available for bio-control of mosquito larvae.
- To screen fungi as bio-control agents could be better option compared alternative expensive methods which are traditionally used by government.
- Examine the nutritional status of water samples, observing micro flora and micro fauna in water sample.
- Culturing and isolation of fungi.

3. MATERIALS AND METHODS

3.1 Sample Collection

Samples were collected from 3 different areas out of which one is polluted, second is from lake and the third one is fresh water. These water samples are collected from 3 sites, sample 1 from Rural area, sample 2 from Suburban area, sample 3 from Urban area. These samples were collected in plastic container and stored at room temperature. (Plate 1) The mosquito larvae were collected from stagnant water near the forest, in plastic container which was covered with cloth to maintain aeration. They were appropriately stored for conducting bio-control experiment.

Collected water samples analyzed for following parameters. Physical parameters: Colour, Turbidity and Odour. Chemical parameters: pH, Total Hardness, Nitrates, Chlorides, Dissolved Oxygen (D.O), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Biological parameters: qualitative analyses of planktons (zooplankton and phytoplankton). Qualitative analysis of elements in the water samples were carried out by ICP AES method at IIT Bombay.

3.2 Culture methods

Water samples were cultured for growth of fungus in PDA (Potato Dextrose Agar), 500ml of media - 100 grams of Potato, 10 grams of Dextrose, 10 grams of Agar and 50mg of Ampicillin (to prevent growth of bacteria) were used. Petri dishes were rinsed with absolute alcohol and then autoclaved. After inoculation of water samples the plates were incubated for 7 days. The fungal cultures were isolated for pure culture, after getting pure culture all the cultures were maintained in slants and kept in fridge for preservation. PD Broth cultures were prepared from pure culture and incubated for 20 days. The culture filtrates were then examined for physical and chemical properties. (Plate 4 & 5)

3.3 Bait technique

Wheat and sesame seeds were taken in small quantity and autoclaved then aseptically transferred into petri dish containing different water samples. The seeds were observed for fungal growth under stereo zoom microscope, then transfer to a sterile PDA plates. The fungi were isolated and stored as master slants. (Plate 6)

3.4 Extraction of aflatoxins from culture filtrates

Using Whatman No.1 filter paper, filters the medium through Buchner funnel. Extract culture filtrate with equal volume of chloroform. Pool the chloroform extract and concentrate to dryness in water bath. Add chloroform for Chromatographic separation. Spot 25µL of the extract onto TLC plates coated with silica gel- HR 250 and develop the plate in acetone: chloroform (12:88, v/v). The plates were visualized under UV light short and long wave length.

3.5 Bio Assay

(a) Spore Suspension: 20 days spore suspension centrifuged at 2500rpm at 10 mins. In a set of test tubes 5 ml of sterile water, mosquito larvae and 2ml of spore suspension added. The test tubes were kept for 7 days observation. (Plate 9)

(b) Culture Filtrate: Six Selected isolated fungal cultures were grown in PD broth. The cultures were filtered using Whatman No. 1 filter paper. Culture filtrates were used for the bio assay

A set of 6 test tubes containing 5ml of water and mosquito larvae and 2ml of culture filtrate of each fungal isolates were kept for 7 days of observation. (Plate 9). Time activity for larval movement was observed for 1 minute per sample. In this experiment the objective was to find out how many flicks appears per minute and how many times they come towards the surface.

4. OBSERVATION AND RESULT

A study was conducted to investigate that fungi can be use a bio-control against mosquito larvae. 3 different water samples were taken. Physical and Chemical characteristics were analyzed. **Physical characteristic** colour, odour and turbidity were determined. Sample 1 showed greyish brown colour with no specific odour and 2.08NTU turbidity. Sample 2 showed greenish brown colour with no specific odour and 2.04NTU turbidity Sample 3 showed brownish colour with no specific odour and 2.12NTU turbidity (Table 1).

Table 1: Physical Characteristics

Sample	Color	Odor	Turbidity
1	Greyish brown	Not specific	2.08 NTU
2	Greenish Brown	Not specific	2.04 NTU
3	Brownish	Not specific	2.12 NTU

4.1 Chemical characteristic

pH, dissolved oxygen, chemical oxygen demand, total hardness, nitrates and nitrites using standard analytical methods (Table 2).

Table 2: Chemical Tests

Tests	Sample 1	Sample 2	Sample 3
pH	7.5	6.5	7
Dissolved O_2	3.02 mg/l	3.52 mg/l	8.15 mg/l
COD	126 mg/l	297 mg/l	113 mg/l
HARDNESS	110 mg/l	120 mg/l	20.04 mg/l
NO_3-N	130.6 µg atoms/lit	12.24 µg atoms/lit	4.08 µg atoms/lit
NO_2-N	240.81 µg atoms/lit	24.48 µg atoms/lit	12.24 µg atoms/lit

In sample 1 it was observed to have pH 7.5, Dissolved oxygen 3.02mg/l, chemical oxygen demand 126mg/l, total hardness 110mg/l, nitrate 130.6µg atoms/lit, nitrite 240.81µg atoms/lit.
In sample 2 it was observed to have pH 6.5, Dissolved oxygen 3.52mg/l, chemical oxygen demand 297mg/l, total hardness 120mg/l, nitrate 12.24µg atoms/lit, nitrite 24.48µg atoms/lit.
In sample 3 it was observed to have pH 7, Dissolved oxygen 8.15mg/l, chemical oxygen demand 113mg/l, total hardness 20.04mg/l, nitrate 4.08µg atoms/lit, nitrite 12.24µg atoms/lit.
The qualitative elemental analysis by ICP AES. Water samples were observed to contain B, Ba, Ca, Cl, Fe, Hg, K, Li, Mg, Mn, Na, S, Si, Sr and Zn. Of the total elements present in samples lithium was found in sample 1 and mercury were found in all the 3 samples as a heavy metal. (Table 3).

Table 3: Total Elements Present in the Samples

FUNGI	SAMPLE 1	SAMPLE 2	SAMPLE 3
Aspergillius nigar	✓	✓	✓
Aspergillius terreus	✓	-	-
Penicillium	-	✓	-
Trichoderma	✓	-	-
Curvularia	✓	-	-
Achlya	-	✓	-

In water samples micro-fauna and micro-flora found are Protozoans having species *Paramecium*, *Trachelomonas*, *Chlamydomonas*, *Phagus*, *Euglena* and *Peranema*, Bacteria of species *Closeterium* and *Cyanobacteria*, Algae of species *Spirulina*, *Staurastum*, *Hymenamonas* and *Chlorella*, Diatoms of *Suriella*, *Mesimmopedia*, *Scenedesmus*, *Pediastrum*, *Micractinum*, *Stylonychia*, *Microcystis* and *Ankistrodesmu.* (Plate 2 & 3)

In the three water samples the fungi were observed. Observations of the fungi were done on the basis of their colony characteristics and microscopic characteristics under light microscope. The fungi were identified using standard flora and manuals. The pure cultures were stored as master slants and digital images

Aspergillus niger, Aspergillus terreus, and Penicillium, Trichoderma, Curvularia, Sprolegmia and Achlya were observed in the cultures of water sample and bait technique. In water sample 1 *Aspergillus niger, Aspergillus terreus, Trichoderma* and *Curvularia* were observed. In water sample 2 *Aspergillus niger, Penicillium, and Achlya* were observed. In the water sample 3 *Aspergillus niger* was observed. (Table 4).

Table 4: Myco Diversity

ELEMENTS	SAMPLE 1	SAMPLE 2	SAMPLE 3
Boron	✓	✓	✓
Barium	✓	✓	✓
Calcium	✓	✓	✓
Chlorine	✓	✓	✓
Iron	✓	✓	✓
Mercury	✓	✓	✓
Potassium	✓	✓	✓
Lithium	✓	-	-
Magnesium	✓	✓	✓
Manganese	✓	✓	✓
Sodium	✓	✓	✓
Sulphur	✓	✓	✓
Silicon	✓	✓	✓
Strontium	✓	✓	✓
Zinc	✓	✓	✓

It was observed that the only aflatoxin found is ochratoxin in *Trichoderma*.
To check the effect of fungal cultures on the mosquito larvae, the fungi were tested on the third instar larvae of *Aedes aegypti* mosquito.

The life cycle of *Aedes Aegypti* includes four stages egg, larva, pupa and adult mosquito, it takes 8 to 10 days for complete metamorphosis. Adult mosquito has black and white markings on body. Larvae moves under the water and frequently surfaces for respiration. They rest at a 45 degree angle from the surface of water. They are commonly found in containers with clean water. They prefer dark place.

There are three larval stages in mosquito namely 1st instar, 2nd instar and 3rd instar depending on the growth of the larvae to the maximum size.

Aspergillus niger (1), A. niger (2), A. niger (3), Aspergillus terreus, Penicillium, Trichoderma and Curvularia were selected for Bio-control experiment. (Plate 4)

The observation of larvae on each day on the basis of movement of the larvae to the surface of water and the speed of movement under water. (Table 5)

There was gradual reduction of the movement of larvae leading to death of the larvae.

The microscopic observation of dead mosquito larvae showed the presence of fungi inside and around the body of larvae.

Table 5: Flicks and Movement Towards Surface Per Minute.

Test Tubes	Flicks per minute	Surface per minute
Control	81/min	1/min
Spore suspensions	43/min	2/min
Culture filtrate	52/min	3/min

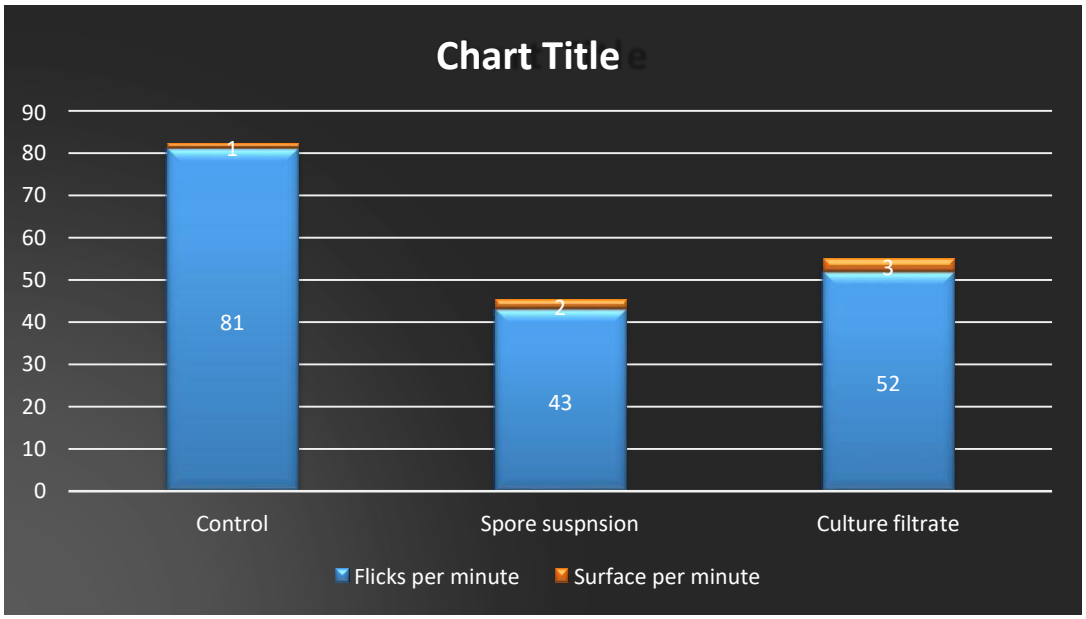

Fig. 1: Flicks and Movement towards Surface per Minute

Table 6: Percent Mortality of Mosquito Larvae Day Wise

% MORTALITY SPECIES	Spore suspension				Culture filtrate			
	Day 2	Day 4	Day 6	Day 8	Day 2	Day 4	Day 6	Day 8
A.terreus	-	-	-	-	-	-	-	-
A.niger (1)	-	**25%**	-	-	-	-	-	-
A.niger (2)	-	-	-	-	-	-	-	-
A.niger (3)	-	-	-	**50%**	-	-	-	**50%**
Curvularia	**100%**	-	-	-	**100%**	-	-	-
Penicillium	-	-	-	**50%**	-	-	-	**50%**
Trichoderma	**75%**	-	-	-	-	-	-	-

Mortality rate of mosquito larvae was analyzed to check the reaction of each species of fungus in two different ways, one in spore suspension and the other one in their culture filtrate. Highest percentage of mortality was observed in both spore suspension and culture filtrate of *Curvularia* on day 2 which is 100% while *Aspergillus terreus* showed 0% percentage of mortality in all spore suspension and culture filtrate, while *Aspergillus niger (1)* showed 25% mortality rate in spore suspension on day 4, *Aspergillus niger (2)* showed 0% mortality rate in both spore suspension and culture filtrate, *Aspergillus niger (3)* showed 50% mortality rate in both spore suspension and culture filtrate on day 8, *Penicillium* showed 50% mortality rate in both spore suspension and culture filtrate on day 8 and *Trichoderma* showed 75% mortality rate in spore suspension on day 2. (Table 6)

5. DISCUSSION AND CONCLUSION

The objective of the current project was to investigate the possibilities of controlling the mosquito population using fungal cultures. In the view of rampant increase in the incidence of dengue fever transmitted through mosquitoes it is absolutely essential to control the mosquito population. There are different species of mosquitoes of which dengue virus is transmitted by the *Aedes aegypti*, found in habitats like stagnant clear waters, backyards which are common in urban areas. In the present work water

samples were collected from in and around Mumbai, the water samples were examined for its nutritional status by analyzing them physically, chemically and biologically. In the three samples that were tested it was observed the pH was 6.5 to 7, all the elements necessary for a living organism were present. In sample collected from rural area, that was near the residential area Lithium and Mercury were detected. Whereas sub-urban lake and water from urban area did not show any trace of heavy metals. These conditions are suitable for various planktonic organisms. This was evident by the phytoplanktons and zooplanktons found in different the water samples. The fungal cultures obtained from water samples were predominantly *Aspergillus niger, Aspergillus terreus, and Penicillium, Trichoderma, Curvularia, Sprolegmia and Achlya.* The cultures were isolated and stored as pure master slants. Of the isolated cultures *Aspergillus niger (1), A. niger (2), A. niger (3), Aspergillus terreus, Penicillium, Trichoderma and Curvularia* were selected for investigating the biocontrol abilities against the larvae of *Aedes aegypti.* The larvae were treated with the spore suspension and culture filtrate of these fungal cultures. As per the results it was observed that the *Curvularia* was the most effective on the larvae as the mortality rate was 100%. This was followed by *Trichoderma t*hat showed mortality rate as 75%, *Aspergillus niger (3)* 50%, *Penicillium* 50%, *Aspergillus niger (1)* 25% and no effect in culture filtrate and spore suspension of *Aspergillus niger (2)* and *Aspergillus terreus.* The death of the larvae could be possibly due to the organic acids produced by the fungi. Microscopic observation of the larvae shows the growth of the fungi on larvae ultimately killing it. This is the most effective bio-control. (Plate 10)

6. REFERENCES

[1] Banu, A. Najitha, and C. Balasubramanian. "Myco-synthesis of silver nanoparticles using *Beauveria bassiana* against dengue vector, *Aedes aegypti* (Diptera: Culicidae)." *Parasitology research* 113.8 (2014): 2869-2877.

[2] Banu, A. Najitha, C. Balasubramanian, and P. Vinayaga Moorthi. "Biosynthesis of silver nanoparticles using *Bacillus thuringiensis* against dengue vector, *Aedes aegypti* (Diptera: Culicidae)." *Parasitology research* 113.1 (2014): 311-316.

[3] Bidochka, Michael J. "Monitoring the fate of biocontrol fungi." *Fungal biocontrol agents: progress, problems and potential. CAB International, Wallingford, United Kingdom* (2001): 193-218.

[4] Bills, Gerald F., and Jon D. Polishook. "Microfungi from *Carpinus caroliniana*." *Canadian Journal of Botany* 69.7 (1991): 1477-1482.

[5] Carruthers, Raymond I., Alan J. Sawyer, and Kirsten Hural. "Use of fungal pathogens for biological control of insect pests." *RESEARCH AND EDUCATION IN THE FIELD* (1991).

[6] Castrillo, L. A., M. H. Griggs, and J. D. Vandenberg. "Vegetative compatibility groups in indigenous and mass-released strains of the entomopathogenic fungus *Beauveria bassiana*: likelihood of recombination in the field." *Journal of invertebrate pathology* 86.1-2 (2004): 26- 37.

[7] Clarkson, John M., and A. Keith Charnley. "New insights into the mechanisms of fungal pathogenesis in insects." *Trends in microbiology* 4.5 (1996): 197-203.

[8] Duan, Zhibing, et al. "A phosphoketolase Mpk1 of bacterial origin is adaptively required for full virulence in the insect-pathogenic fungus *Metarhizium anisopliae." Environmental microbiology* 11.9 (2009): 2351-2360.

[9] Eley, Kirstin L., et al. "Biosynthesis of the 2-Pyridone Tenellin in the Insect Pathogenic Fungus

[10] *Beauveria bassiana*." *ChemBioChem* 8.3 (2007): 289-297.

[11] Ericsson, Jerry D., et al. "Spinosad interacts synergistically with the insect pathogen *Metarhizium anisopliae* against the exotic wireworms *Agriotes lineatus* and *Agriotes obscurus* (Coleoptera: Elateridae)." *Journal of economic entomology*100.1 (2007): 31-38.

[12] Ernst-Jan Scholte, Bart G.J. Knols, Robert A. Samson, Willem Takken; Entomopathogenic fungi for mosquito control: A review, *Journal of Insect Science*, Volume 4, Issue 1, 1 January 2004, 19

[13] Fang, Weiguo, et al. "Agrobacterium tumefaciens-mediated transformation of *Beauveria bassiana* using an herbicide resistance gene as a selection marker." *Journal of invertebrate pathology* 85.1 (2004): 18-24.

[14] Fang, Weiguo, et al. "Implication of a regulator of G protein signalling (BbRGS1) in conidiation and conidial thermotolerance of the insect pathogenic fungus *Beauveria bassiana*." *FEMS microbiology letters* 279.2 (2008): 146-156.

[15] Feng, M. G., T. J. Poprawski, and George G. Khachatourians. "Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: current status." *Biocontrol science and technology* 4.1 (1994): 3-34.

[16] Hall, R. A., and B. Papierok. "Fungi as biological control agents of arthropods of agricultural and medical importance." *Parasitology* 84.4 (1982): 205-240.

[17] Hegedus, Dwayne D., and George G. Khachatourians. "The impact of biotechnology on hyphomycetous fungal insect biocontrol agents." *Biotechnology advances* 13.3 (1995): 455- 490.

[18] Imam, Hashmat et al. "The Basic Rules and Methods of Mosquito Rearing (*Aedes Aegypti*)." *Tropical Parasitology* 4.1 (2014): 53–55. *PMC*. Web. 11 Feb. 2018.

[19] Isaka, Masahiko, et al. "Bioactive substances from insect pathogenic fungi." *Accounts of Chemical Research* 38.10 (2005): 813-823.

[20] Jackson, Jennifer C., Laura A. Higgins, and Xiaorong Lin. "Conidiation color mutants of *Aspergillus fumigatus* are highly pathogenic to the heterologous insect host *Galleria mellonella*." *PloS one* 4.1 (2009): e4224.

[21] Jackson, Mark A., Christopher A. Dunlap, and Stefan T. Jaronski. "Ecological considerations in producing and formulating fungal entomopathogens for use in insect biocontrol." BioControl 55.1 (2010): 129-145.

[22] Khan, Sehroon, et al. "Entomopathogenic fungi as microbial biocontrol agent." *Molecular Plant Breeding* 3.1 (2012).

[23] Kirkland, Brett H., Greg S. Westwood, and Nemat O. Keyhani. "Pathogenicity of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* to *Ixodidae* tick species *Dermacentor variabilis, Rhipicephalus sanguineus,* and *Ixodes scapularis*." *Journal of medical entomology* 41.4 (2004): 705-711.

[24] Klingen, Ingeborg, and Solveig Haukeland. "The soil as a reservoir for natural enemies of pest insects and mites with emphasis on fungi and nematodes." *An ecological and societal approach to biological control*. Springer, Dordrecht, 2006. 145-211.

[25] Klingen, Ingeborg, Jørgen Eilenberg, and Richard Meadow. "Effects of farming system, field margins and bait insect on the

occurrence of insect pathogenic fungi in soils." *Agriculture, ecosystems & environment* 91.1-3 (2002): 191-198.
[26] Leathers, Timothy D., Subhash C. Gupta, and Nancy J. Alexander. "Mycopesticides: status, challenges and potential." *Journal of Industrial Microbiology* 12.2 (1993): 69-75.
[27] Magan, Naresh. "Physiological approaches to improving the ecological fitness of fungal biocontrol agents." *Fungi as biocontrol agents* (2001): 239-252.
[28] Maurer, P., et al. "Genetic diversity of *Beauveria bassiana* and relatedness to host insect range." *Mycological Research* 101.2 (1997): 159-164.
[29] Merritt, R. W., R. H. Dadd, and E. D. Walker. "Feeding behavior, natural food, and nutritional relationships of larval mosquitoes." *Annual review of entomology* 37.1 (1992): 349-374.
[30] Meyling, Nicolai V., and Jørgen Eilenberg. "Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: potential for conservation biological control." *Biological control* 43.2 (2007): 145-155.
[31] Muspratt, J. "Destruction of the larvae of Anopheles gambiae Giles by a *Coelomomyces*
[32] fungus." *Bulletin of the World Health Organization* 29.1 (1963): 81.
[33] Muspratt, J. "On *Coelomomyces* fungi causing high mortality of *Anopheles gambiae* larvae in Rhodesia." *Annals of Tropical Medicine & Parasitology* 40.1 (1946): 10-17.
[34] Nnakumusana, E. S. "Laboratory infection of mosquito larvae by entomopathogenic fungi with particular reference to *Aspergillus parasiticus* and its effects on fecundity and longevity of mosquitoes exposed to conidial infections in larval stages." *Current Science* (1985): 1221- 1228.
[35] Ortiz-Urquiza, Almudena, Zhibing Luo, and Nemat O. Keyhani. "Improving mycoinsecticides for insect biological control." *Applied microbiology and biotechnology* 99.3 (2015): 1057- 1068.
[36] Pedrini, Nicolás, Rosana Crespo, and M. Patricia Juárez. "Biochemistry of insect epicuticle degradation by entomopathogenic fungi." *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 146.1-2 (2007): 124-137.
[37] Roberts, Donald W., and Ann E. Hajek. "Entomopathogenic fungi as bioinsecticides." *Frontiers in industrial Mycology*. Springer, Boston, MA, 1992. 144-159.
[38] Roberts, Donald W., and Raymond J. St Leger. "*Metarhizium spp*., cosmopolitan insect- pathogenic fungi: mycological aspects." *Advances in applied microbiology* 54.1 (2004): 1-70.
[39] Rohlfs, Marko, and Alice CL Churchill. "Fungal secondary metabolites as modulators of interactions with insects and other arthropods." *Fungal Genetics and Biology* 48.1 (2011): 23- 34.
[40] Rohlfs, Marko. "Density-dependent insect-mold interactions: effects on fungal growth and spore production." *Mycologia*97.5 (2005): 996-1001.
[41] Salunkhe, Rahul B., et al. "Larvicidal potential of silver nanoparticles synthesized using fungus *Cochliobolus lunatus* against *Aedes aegypti* (Linnaeus, 1762) and *Anopheles stephensi* Liston (Diptera; Culicidae)." *Parasitology research*109.3 (2011): 823-831.
[42] Samson, Robert A., Harry C. Evans, and Jean-Paul Latgé. *Atlas of entomopathogenic fungi*. Springer Science & Business Media, 2013.
[43] Sandhu, Sardul Singh, et al. "Myco-biocontrol of insect pests: factors involved, mechanism, and regulation." *Journal of pathogens* 2012 (2012).
[44] Scholte, Ernst-Jan, et al. "Entomopathogenic fungi for mosquito control: a review." *Journal of Insect Science* 4.1 (2004).
[45] Senthil-Nathan, Sengottayan. "A review of biopesticides and their mode of action against insect pests." *Environmental sustainability*. Springer, New Delhi, 2015. 49-63.
[46] Shahid, Ali Ahmad, et al. "Entomopathogenic fungi as biological controllers: new insights into their virulence and pathogenicity." *Archives of Biological Sciences* 64.1 (2012): 21-42.
[47] Soni, Namita, and Soam Prakash. "Efficacy of fungus mediated silver and gold nanoparticles against *Aedes aegypti* larvae." *Parasitology research* 110.1 (2012): 175-184.
[48] Soni, Namita, and Soam Prakash. "Synthesis of gold nanoparticles by the fungus *Aspergillus niger* and its efficacy against mosquito larvae." *Rep Parasitol* 2 (2012): 1-7.
[49] Sridhar, K. (2010). Aquatic fungi in the Western Ghats – Current status and future concerns. . 10.13140/2.1.3175.4241.
[50] Sundaravadivelan, Chandran, and Madanagopal Nalini Padmanabhan. "Effect of mycosynthesized silver nanoparticles from filtrate of *Trichoderma harzianum* against larvae and pupa of dengue vector *Aedes aegypti* L." *Environmental Science and Pollution Research* 21.6 (2014): 4624-4633.
[51] Veerakumar, Kaliyan, et al. "Mosquito larvicidal properties of silver nanoparticles synthesized using *Heliotropium indicum (Boraginaceae)* against *Aedes aegypti, Anopheles stephensi,* and *Culex quinquefasciatus* (Dipthera: Culicidae)." *Parasitology research* 113.6 (2014): 2363- 2373.
[52] Vega, Fernando E., et al. "Entomopathogenic fungal endophytes." *Biological Control* 46.1 (2008): 72-82.
[53] Xu, Yuquan, et al. "Biosynthesis of the cyclooligomer depsipeptide bassianolide, an insecticidal virulence factor of *Beauveria bassiana*." *Fungal Genetics and Biology* 46.5 (2009): 353-364.
[54] Ying, Sheng-Hua, Ming-Guang Feng, and Nemat O. Keyhani. "A carbon responsive G-protein coupled receptor modulates broad developmental and genetic networks in the entomopathogenic fungus, *Beauveria bassiana*." *Environmental microbiology* 15.11 (2013): 2902-2921.
[55] Zattau, William C., and Thomas McInnis Jr. "Life cycle and mode of infection of *Leptolegnia chapmanii* (Oomycetes) parasitizing Aedes aegypti." *Journal of invertebrate pathology*50.2 (1987): 134-145.

