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Research Article

Study on Liquid Chromatography and Mass Spectrometry of *Memnoniella echinata* Metabolites against Vector of Dengue and Chikungunya

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Abstract

Aedes aegypti and *Ae.albopictus* are vectors for transmitting Dengue, Chikungunya, and Yellow fever. These fevers are growing public health problem globally. *Memnoniella echinata* is a cosmopolitan fungus. This fungi is exceptionally good model for bio control and more significant as bio agent. Recently, especially fungi, has emerged as a current and future candidate for mosquito control. In the study *M. echinata* was cultured in the Potato Dextrose Broth (PDB) medium. The metabolites were filtered with the Whatman-1 filter paper. These purified metabolites were tested in the statistically significant concentrations of 50, 100, 150, 200, 250 ppm against first, second, third, and fourth instar larvae of *Ae. aegypti* and *Ae. albopictus*. The bioactive compounds have been isolated with the Thin Layer Chromatography (TLC) plates with chloroform and methanol used as solvent system for analysis. These compounds were detected with the Liquid Chromatography Mass Spectrometry (LC-MS/MS) analysis. The LC_{50}, LC_{90} and LC_{99} values were 84, 188, 205 ppm against first and second instar. However, in case of third instar 115, 191, 209 ppm and 125, 196, 214 ppm was observed against fourth instarlarvae of *Ae. aegypti*. Thus, the LC_{50}, LC_{90} and LC_{99} values were 84, 188, 205 ppm against first and second instar of *A. albopictus*. The third instar LC_{50}, LC_{90} and LC_{99} values were 84, 192, 210 ppm, and fourth instar 89.2, 196, and 215 ppm respectively after exposure for 24 hours. These new results significantly support broadening the current dengue and Chikungunya vectors control paradigm beyond chemical larvicides.

Keywords: *Memnoniella echinata*; Metabolites; LC-MS/MS analysis; *Aedes aegypti, Ae. Albopictus*

Introduction

Aedes aegypti and *albopictus* are vectors for dengue Chikungunya, and Yellow fever. In recent years, dengue and Chikungunya transmission has increased predominantly in urban and semi-urban areas as major public health concern. Globally, the incidence of dengue has grown in recent decades. Approximately 2.5 billion people over40% of the world's population is now at risk from dengue. Currently there may be 50–100 million dengue infections worldwide every year [1]. The entomopathogenic fungus to control mosquito adults and larvae is not a new issue [2]. In contrast to bacteria, fungi are adulticidal agents that could be developed for domestic use to reduce vector densities and impair their vectorial capacity. Globally, chemical spraying methods have used for control *Ae. aegypti* and *Ae. albopictus* during dengue and Chikungunya epidemics. Therefore new alternative is urgently needed. The entomopathogenic fungi have used for control of *Ae. aegypti* and *Ae. albopictus*. The virulence of entomopathogenic fungi has confirmed against adult *Ae. aegypti* [3,4]. *Metarhizium anisopliae* is well known entomopathogenic fungi. *M. anisopliae* strain IP 46 was isolated from soil which shows pathogenicity against all stages of *Ae. aegypti* including the egg [5-8]. Field studies have encouraged because significant and rapid larval mortality caused by *Leptolegnia chamanii* against *Ae. Aegypti* [9]. *Lecanicillium muscarium* has isolated from a dead culicid mosquito, this found pathogenic to adults of *Ae. aegypti*, *An. arabiensis* and *Cx. quinquefasciatus* under laboratory conditions demonstrating how naturally occurring fungal pathogens of culicids might have potential for mosquito control [10]. *Aspergillus clavatus* isolated from an African locust causes >95% mortality after 24 hours against *An. gambiae*, *Ae. aegypti*, and *Cx. quinquefasciatus* larvae [11]. The oil based formulations of conidia of *M. anisopliae* have enhanced ovicidal of activity at high humidity in the control of *Ae. aegypti* [12].

The effectiveness and deploy ability of such fungi under field conditions have yet to be explored. The fungal toxins are the true chemical in nature. The entities of fungal metabolism are not known until recent times. The fungi have isolated, a Glomeromycete (possibly Entrophospora sp.) and a Dothideomycete (possibly Phaeosphaeria sp.), for bioactive secondary metabolites. The six new compounds consisting of clearanols and disulochrin have purified [13]. A new fungi *Chrysosporium lobatum* strain BK-3 has isolated from Assam India. This fungal strain has produced two bioactive compounds [14]. Several metabolites from entomopathogenic Deuteromycetes have been investigated. Moreover, *Beauveria* spp. was known to produce beauvericin, a depsipeptide metabolite which has shown toxicity to a number of invertebrates [15]. Similarly, proteases have produced

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by entomopathogenic fungi to degrade cuticle and assist entry into the host were similar to proteases used by insects to degrade their own cuticle during molting [16]. A number of enzymes have known from entomopathogenic fungi, such as the proteases, lipases, and chitinases that assist in cuticular breakdown. These enzymes can be thought of as bioactive and there has been increasing interest in use of these enzymes in mosquito control. Entomopathogenic fungi also produce insecticidal toxins. The early literature on toxins from entomopathogenic fungi was reviewed by Roberts and Stressed [15,17].

Memnoniella echinata is a cosmopolitan fungus. This commonly found in tropical and sun tropical areas especially on cellulose based materials. The maximal spore germination temperature is 37 °C. *M. echinata* releases different mycotoxins. Accordingly in present study we investigated the efficacy of *M. echinata* metabolites against *Ae. aegypti* and *Ae. albopictus* larvae in laboratory and Liquid Chromatography Mass Spectrometry (LC-MS/MS) analysis for compounds.

Materials and Methods

Collection and culture of *Memnoniella echinata* (MTCC 604)

The discovery of bioactive compounds usually begins with isolation and identification of the fungi and growing them at various temperature regimes in a variety of selective and non-selective culture media. The *M. echinata* (MTCC 604) was obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbiology, Chandigarh, India. The Potato Dextrose Broth (purchased from Himedia) M403, 24.0 gm was suspended in sterile water. The medium was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. The broth was supplemented with 50 µg/ml chloramphenicol as a bacteriostatic agent. *M. echinata* colonies grown on the Sabouraud Dextrose Agar plates were transferred to each flask using the inoculation needle. The conical flasks inoculated with *M. echinata* were incubated at 24±2°C for 15 days (Figure 1).

Maintenance of mosquito larvae in laboratory

Aedesaegypti and *Ae.albopictus* larvae were reared in the laboratory at a temperature of 25±2°C, 70±5% relative humidity and LD 14:10 h. The different larval instars were maintained in separate enamel containers (25-cm length×15-cm width×5-cm depth), at a density of 200 larvae per container. All larvae were fed 0.4 mL/ container of a 5% (w/v) autoclaved suspension of freeze-dried yeast in distilled water on day 1 and day 2. Larvae were reared in deionised water at pH 7.0. To counteract evaporation, water was added daily.

Bioassays

The larvae were collected from a mosquito colony maintained at insectaria at the Department of Zoology, Dayalbagh Educational Institute. The bioassays were performed as per the standard procedures recommended by World Health Organization with some modifications [18]. The first, second, third and fourth instars larvae of *Ae. aegypti* and *Ae. albopictus* have been tested (50, 100, 150, 200, 250 ppm) at selected concentrations. These experiments were carried out in triplicate using 20 larvae for each replicate assay. The replicates were run simultaneously yielding a total of 60 larvae for each dosage. The larvae were placed in 50 ml disposable plastic cups containing 15 ml of test solution and fed on larval food during all testing. Mortality and survival was established after 24h of exposure. The number of the dead larvae in the three replicates was expressed as the percentage mortality for each concentration. The negative control was deionised water while the positive control was the Chitinase of *S. griseus* C6137 purchased from Sigma-Aldrich. After determining the mortality of larvae in this same range of concentrations were used to determine LC_{50}, LC_{90} and LC_{99} values. Batches of 20 first, second, third and fourth in star larvae were transferred by means of strainers, droppers to Schott Duran beakers, each containing 200ml of triple deionised water. Unhealthy or damaged larvae or pupae were removed and replaced. The depth of the water in the cups or vessels should remain between 5cm and 10cm, deeper levels may cause undue mortality.

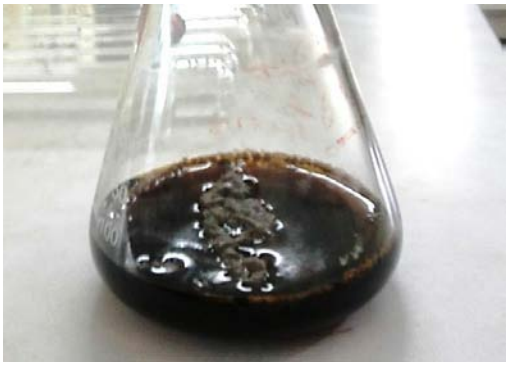

Figure 1: The culture of *Memnoniella echinata* in the Potato Dextrose Broth medium maintained in the laboratory.

Statistical Analysis

Data from all replicates have been pooled for probit analysis [19]. The LC_{50}, LC_{90} and LC_{99} values were calculated from a log dosage probit mortality regression line using computer software programs IBM SPSS 19.0. The bioassays have been repeated at least three times, using new solutions and different batches of larvae each time. Standard deviation or confidence intervals of the means of LC_{50} values are calculated and recorded.

Thin layer chromatography (TLC)

The TLC was used to monitor the identity of each extracts and fractions, additionally to screen the qualitative purity of the isolated compound. It was also developed to optimize the solvent system that would be applied for column chromatography. The Analytical TLC was performed on precoated TLC plates with Si gel 60 F254 (0.2 mm, Merck) and RP-2 (0.2 mm, Merck) using diverse solvent systems for mostly semi-polar compounds. However, solvent system containing EtOAc: Acetone: n-Hexane: Formic acid (25:4:18:0.3, v/v) was mostly used, unless otherwise stated. The compounds were then detected by their UV absorbance at wavelength 254 and 366 nm and/or by spraying the TLC plates with spraying reagents followed by heating at 110 °C. The solvent systems: SS1: EtOAc: Acetone: n-Hexane: Formic acid (25:4:18:0.3, v/v), SS2: DCM: EtOAc (65:35, v/v).

Column chromatography (CC)

What man no.1 filtered metabolites were subjected to repeated separation through column chromatography using appropriate stationary phase and the solvent system previously determined by TLC? The column chromatography was carried out on silica gel 60

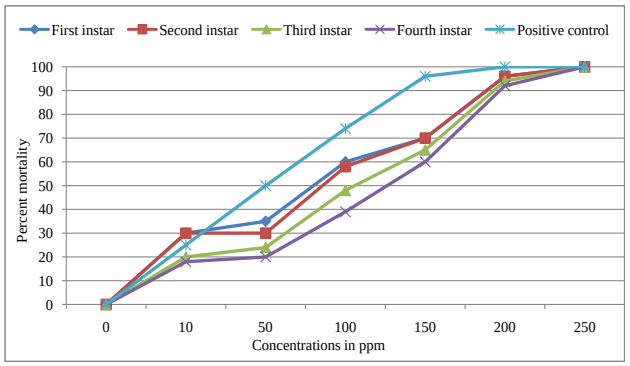

Figure 2: Effect of culture filtrates of *Memnoniella echinata*against *Aedes aegypti* at different concentrations after exposure of 24hours.

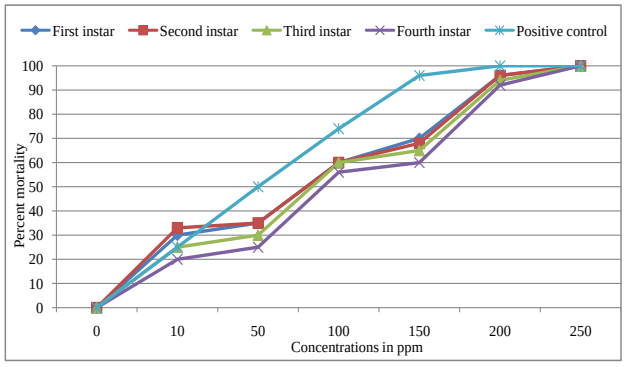

Figure 3: Effect of culture filtrates of *Memnoniella echinata* against *Aedes albopictus* at different concentrations after exposure of 24h.

(0.015-0.040 and 0.040-0.063 mm mesh size, Merck) and Sephadex LH-20 (25-100 µm mesh size, Amersham Biosciences). Further purification of the fractions was later performed on another CC, preparative TLC or semi-preparative HPLC using C-18 column. The separation systems were a) Stationary Phase: Silica gel Solvent systems: SS1 : EtOAc: Acetone: *n*-Hexane (25:4:18, v/v), SS2: DCM: EtOAc (65:35, v/v), SS3 *n*-Hexane: EtOAc (gradient), SS4: DCM: EtOAc (gradient), SS5: DCM:MeOH (10:1, v/v), SS6: Chloroform: MeOH (95:5, v/v). b) Stationary Phase: Sephadex LH-20 and the Solvent systems: SS1 MeOH:H^2O (gradient), SS2:MeOH, SS3: DCM:MeOH (10:1 to 4:1, v/v).

Liquid chromatography mass spectrometry (LC-MS/MS)

Ultra-High performance Liquid Chromatography Mass spectrometry (Waters ACQUITY®) was used to identify the interesting peaks from extracts and fractions as well as to evaluate the purity of isolated compounds. The gradient used started with 10:90 (MeOH: H2O to 100% MeOH) in 35 min. Column C-18 was used for the isolation of pure compounds from fractions previously separated using column chromatography. Each injection consists of about 2-5 µl of the fraction dissolved in 1 µl of the solvent system. The solvent system consisting of MeOH or MeCN and aqua bidestillata was pumped through the column at a flow rate of 1 ml/min. The eluted peaks which were detected by the online UV detector were collected separately in round bottom flasks.

Results and Discussion

Fungi have significant attention in recent years to produce novel compound. The fungi are toxins resources and pathogenic against adult mosquitoes, pupae and larvae. In the present investigation the metabolites of *M. echinata* has shown significant efficacies at all tested concentrations against larvae of *Ae. aegypti* and *Ae. albopictus*. The LC_{50}, LC_{90} and LC_{99} values were 84, 188, 205 ppm against first in star, 86, 188, 205 ppm against second instar. Moreover, third instar 115, 191, 209, ppm and 125, 195, 215 ppm were observed against fourth instar of *Ae. aegypti*. Moreover, the LC_{50}, LC_{90} and LC_{99} values were 84, 188, 206 ppm against first and second instar of *Ae. albopictus*. The third instar LC_{50}, LC_{90} and LC_{99} values were 84, 192, 210, and fourth instar 89.2, 196, and 215ppp. Moreover, significant percent mortality has observed at selected concentration against larvae of *Ae. aegypti* and *Ae. albopictus* (Figure 2,3 Table 1). The compounds were detected in metabolites of *M. echinata* when pass through TLC plates in the chloroform and methanol as solvent system. Figure 4 has demonstrated the excellent match between the observed MS/MS spectrum from 150-400 *m/z* at 1: scan ES+5.61e6. Moreover, Figure 5 has shown the MS/MS spectrum from 150-500 *m/z* at 1: scan ES+1.11e7. The chromatographic pecks predicted proteins and enzymes from the metabolites of *M. echinata* one of the sample.

The chemical insecticides have been replaced for malaria control with residual sprays of fungal bio pesticides has been suggested in areas of high insecticide resistance [20]. *M. anisopliae* has been practically introduced in rural Tanzanian village houses. The black cotton sheets were when impregnated with *M. anisopliae* spores in sunflower oil and hung from the ceiling of traditional houses to provide a resting substrate for mosquitoes after taking their blood meals. This led to the infection of about a quarter of the mosquitoes collected from these sheets and to considerably faster death compared to controls. They claimed that the implementation of such control method would lead to significant reduction in malaria transmission intensity by about 75% [6].In the present study the metabolite of *M. echinata* Figure 6 has shown significant efficacies against larvae of *Ae. aegypti* and *Ae. albopictus*at all the concentrations (10, 50,100,150, 250 ppm). The percent mortalities were minimum 20% and maximum 100% after exposure in 24 hours. In our laboratory the culture filtrates of *Culicinomyces clavisporus*, *Trichophyton ajelloi*, and *Lagenidium giganteum* have been found highly pathogenic against *Ae. aegypti*, *Ae. stephensi*, and *Cx. quinquefasciatus* larvae and adults also [21,22]. The entomopathogenic fungi were acted through external contact without the need to be ingested [23]. The spraying of a biopesticide contained natural isolate of *B. bassiana* on clay tiles with subsequent transient exposure of *An. stephensi* could lessen malaria transmission potential to zero within a feeding cycle [24]. Moreover, fungal efficacy against mosquitoes under field conditions are needed before their full potential as alternatives to insecticides can be determined [25]. The

Table 1: Bioassays of *Memnoniella echinata* culture filtrates against *Aedes aegypti Ae. albopictus* after exposure of 24 hours.

Instars	*Aedes aegyptiAedes albopictus*					
	LC_{50}	LC_{90}	LC_{99}	LC_{50}	LC_{90}	LC_{99}
First instars	84	188	205	84	188	206
Second instars	86	188	205	84	188	206
Third instars	115	191	209	84	192	210
Fourth instars	125	195	215	89.2	196	215

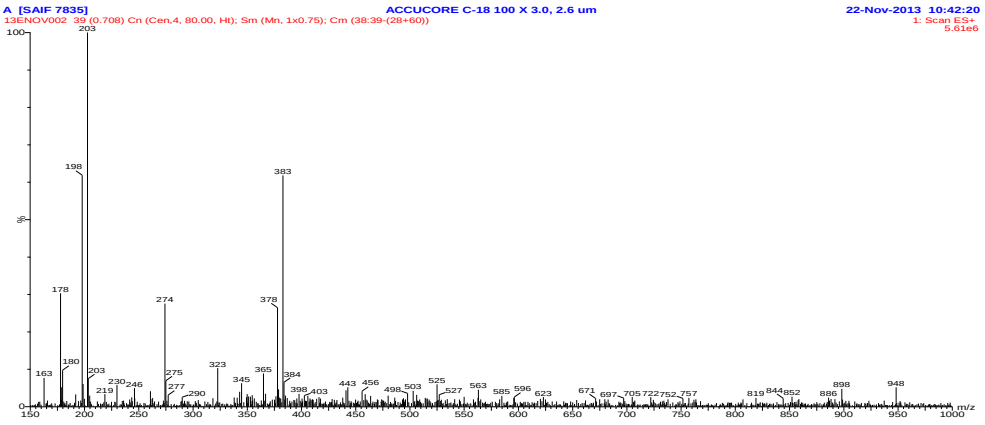

Figure 4: Liquid Chromatography Mass spectrometry (LC-MS/MS) spectra of culture filtrates of *Memnoniella echinata* scan at ES+5.61e6 by C-18 column.

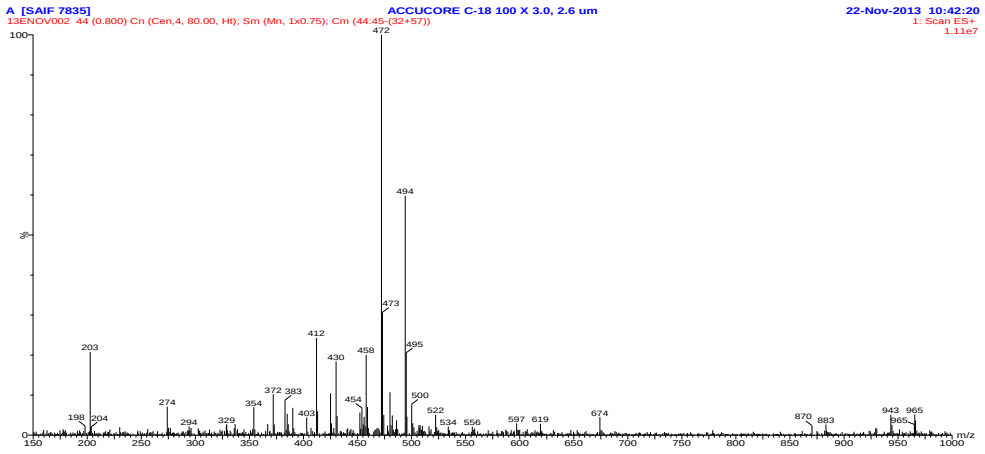

Figure 5: Liquid Chromatography Mass spectrometry (LC-MS/MS) spectra of culture filtrates of *Memnoniella echinata* scan at ES+1.11e7 by C-18 column.

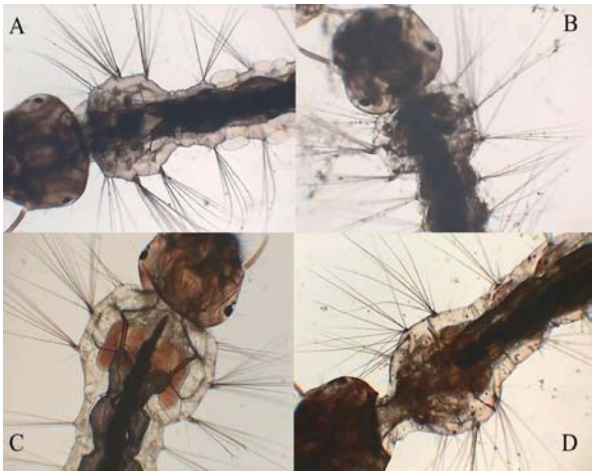

Figure 6: (A) *Aedes aegypti* larvae before exposure. (B) *Ae. Aegypti* larvae after exposure. (C) *Ae. albopictus* larvae before exposure. (D) *Ae. albopictus* larvae after exposure of culture filtrate of *Memnoniella echinata*.

insecticide resistant Anopheles mosquitoes were susceptible to *B. bassiana* infection. The latter finding was important in considering the involvement of fungi in IVM activities in situations where resistance to synthetic insecticides [26]. In present study *M. echinata* has tremendous potential for isolation of compounds in the metabolites. Present study revealed that the LC_{90} and LC_{99} values of 195 and 215pp after exposure of 24 hours. Our study significantly depicted *M. echinata* culture filtrates have significant mosquito larvicidal activity against *Ae. aegypti* and *Ae. albopictus*. Thus the LC-MS/MS spectrum of a complex combination has depicted that overlaid distributions of several different compound. These combinations of compounds have affected the all instar larvae of *Ae.aegypti* and *Ae. albopictus* on all tested significant lethal concentrations.

A sub-lethal concentration of Imidacloprid (IMI) was not significantly alter the daily survival rates of mosquito was identified to be 0.1 ppm. This sub-lethal concentration was combined with *M. anisopliae* conidia (1×109 conidia mL). Both the combined treatment and the conidia alone were able to reduce the survival of *Ae. aegypti* compared with untreated treated mosquitoes. Importantly, mosquito survival following exposure to the combined treatment for 6 and 12 hours was significantly reduced when compared with mosquitoes exposed to conidia alone [27]. In the present efficacy study the metabolites of *M. echinata*was highly susceptible against all instar of *Ae. aegypti* and *Ae. albopictus* after exposure for 24 hrs. The significant LC_{90} values 188-196 have demonstrated the biomolecule of *M. echinata* can apply for controlling larvae of *Ae. aegypti* and *Ae.albopictus*.Moreover, the pathogenicity

of nineteen hypocrealean entomopathogenic fungi from seven different genera was tested in adult *Ae.aegypti*. All fungi proved to be pathogenic, and *Isariafumosorosea*, *Lecanicilliummuscarium*, *Lecanicilliumpsalliotae*, *M. anisopliae*, *M.lepidiotae*, *M.majus*, *M.frigidum*, *Paecilomycescarneus*, and *Paecilomyceslilacinus* caused total mortality within 15 days of exposure of mosquitoes to the fungal culture. All fungi developed on dead individuals. The high susceptibility of adults to most tested strains underlines the interest of entomopathogenic fungi especially those of the genera *Metarhizium, Isaria*, *Paecilomyces* and *Lecanicillium* for biological control of *Ae. aegypti* [28]. *M. anisopliae* IP 46 conidia mixed with soil was tested for effectiveness against *Ae.aegypti* eggs. The mycelium and new conidia developed first on eggs between 4.8 and 15 days respectively after incubation of fungus-treated soils at 3.3×103 up to 3.3×105 conidia/g soil at 25°C and relative humidity close to saturation. After 15 day incubation, 53.3% of the eggs exposed to soil with 3.3× 105 conidia/g showed external development of mycelium and conidia [29]. The *B. bassiana* and *M. anisopliae* have decreased disease transmission by reducing mosquito vector longevity and also occur worldwide. Ninety three isolates of entomopathogenic fungi representing six species (*B. bassiana*, *M. anisopliae*, *Isariafumosorosea*, *I. farinosa*, *I. flavovirescens*, and *Lecanicillium* spp.) were screened as potential biological control agents of *Ae.aegypti* [30]. The efficiency of *M. anisopliae* impregnated cloths, with and without imidacloprid [IMI]) was evaluated against adult *Ae. aegypti* in simulated human dwelling [31]. The *B. bassiana* caused a reduction in the life span of *Ae. aegypti* and hindered dengue virus replication in the mosquito midgut. This infection was induced the expression a variety anti-microbial and dengue virus restriction factor genes [32]. Sub lethal effects of *Leptolegniachapmanii* are entomopathogenic microorganismon fecundity, number of gonotrophic cycles, fertility, and relationship between wing length and fecundity in *Ae. Aegypti* females. That survived infection with *L. chapmanii* laid fewer eggs, had a smaller number of gonotrophic cycles, had shorter wings, and were less fertile than controls. This is the first study on the sublethal effects experienced by specimens of *Ae. Aegypti* that survived infection with zoospores of *L.chapmanii* [33]. Beauvericin was first isolated from *B. bassiana*, which is a common and commercial entomopathogenic mycoinsecticide [34]. Beauvericin was confirmed as the active compound from *B. bassiana* against *Artimiasalina*, which was considered a model organism to study insecticidal activity. Subsequently, the insecticidal effect of beauvericin on a microgram level was investigated on *Calliphoraerythrocephala*, *Ae.aegypti*, *Lygus* spp., *Spodopterafrugiperda* and *Schizaphisgraminum* [35-37]. A significant progress has been made in understanding proteins and enzymes involved with penetration of larvae cuticle. The *M. echinata* can be more effective with combined action of present numerous toxins and enzymes of the culture filtrate.

Conclusion

The bioassays demonstrated that the metabolites of *M. echinata* were highly pathogenic against all instar of *Ae. aegypti* and *Ae. albopictus.* This stage arrangement of pathogenicity can be recommended that this fungus is developmental stage specific parameter. The findings in study emphasize a first step towards characterizing the complex field conditions that can allow interactions between mosquito larvae and *M. echinata*.They underscore the potential of mycolarvicides for the integrated control of all larval stages of *Ae. aegypti* and *Ae. albopictus*. The results in this study open the possibility for further investigations of the efficacy of adulticidal and for their repellent properties. Moreover, these results could be useful in the research for selecting newer, more selective, larvicidal compounds. The growing epidemics all over the globe suggest that new larvicides should be explored to control Chikungunya and Dengue in specific environment.

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References

1. World Health Organization. Dengue and serve dengue. Fact Sheet. 2014.
2. Knols BG, Bukhari T, Farenhorst M. Entomopathogenic fungi as the next-generation control agents against malaria mosquitoes. Future Microbiol. 2010; 5: 339-341.
3. Scholte EJ, Takken W, Knols BG. Infection of adult Aedes aegypti and Ae. albopictus mosquitoes with the entomopathogenic fungus Metarhizium anisopliae. Acta Trop. 2007; 102: 151-158.
4. Paula AR, Brito ES, Pereira CR, Carrera MP, Samuels RI. Susceptibility of adult Aedes aegypti (Diptera: Culicidae) to infection by Metarhizium anisopliae and Beauveria bassiana: prospects for Dengue vector control. BiocontSci Tech. 2008; 18: 1017–1025.
5. Silva RO, Silva HHG, Luz C. Effect of Metarhizium anisopliae isolated from soil of the central brazilian cerrado against Aedes aegypti larvae under laboratory conditions. Rev Pat Trop. 2004; 33: 207–216.
6. Luz C, Tai MH, Santos AH, Rocha LF, Albernaz DA, Silva HH. Ovicidal activity of entomopathogenic hyphomycetes on Aedes aegypti (Diptera: Culicidae) under laboratory conditions. J Med Entomol. 2007; 44: 799-804.
7. Santos AH, Tai MHH, Rocha LFN, Silva HHG, Luz C. Dependence of Metarhizium anisopliae on high humidity for ovicidal activity on Aedes aegypti. Biol Control. 2009; 50: 37–42.
8. Leles RN, Sousa NA, Rocha LF, Santos AH, Silva HH, Luz C. Pathogenicity of some hypocrealean fungi to adult Aedes aegypti (Diptera: Culicidae). Parasitol Res. 2010; 107: 1271-1274.
9. Pelizza SA, Scorsetti AC, Tranchida MC. The sublethal effects of the entomopathic fungus Leptolegnia chapmanii on some biological parameters of the dengue vector Aedes aegypti. J Insect Sci. 2013; 13: 22.
10. Luz C, Mnyone LL, Sangusangu R, Lyimo IN, Rocha LF, Humber RA, et al. A new resting trap to sample fungus-infected mosquitoes, and the pathogenicity of Lecanicillium muscarium to culicid adults. Acta Trop. 2010; 116: 105-107.
11. Seye F, Faye O, Ndiaye M, Njie E, Marie Afoutou J. Pathogenicity of the Fungus, Aspergillus clavatus, isolated from the locust, Oedaleus senegalensis, against larvae of the mosquitoes Aedes aegypti, Anopheles gambiae and Culex quinquefasciatus. J Insect Sci. 2009; 9: 1-7.
12. Albernaz DA, Tai MH, Luz C. Enhanced ovicidal activity of an oil formulation of the fungus Metarhizium anisopliae on the mosquito Aedes aegypti. Med Vet Entomol. 2009; 23: 141-147.
13. GereaAL, Katie MB, Jarrod BK, Jianlan Y, Douglas RP, Miller AN, et al.

Secondary metabolites produced by fungi derived from a microbial mat encountered in an iron-rich natural spring. Tetrahedron Lett. 2012; 53: 4202-4205.

14. Kumar CG, Mongolla P, Sujitha P, Joseph J, Babu KS, Suresh G, et al. Metabolite profiling and biological activities of bioactive compounds produced by Chrysosporium lobatum strain BK-3 isolated from Kaziranga National Park, Assam, India. Springer Plus. 2013; 2: 122.

15. Roberts DW. Toxins of entomopathogenic fungi. In: Burges HD, editor. Microbial Control of Pest and Plant Diseases. New York: Academic Press. 1981; 441–460.

16. Samuels RI, Paterson IC. Cuticle degrading proteases from insect moulting fluid and culture filtrates of entomopathogenic fungi. Comp Biochem Physiol B Biochem Mol Biol. 1995; 110: 661-669.

17. Strasser H, Vey A, Butt TM. Are there any risks in using entomopathogenic fungi for pest control, with particular reference to the bioactive metabolites of Metarhizium, Tolypocladium and Beauveria species? Biocontrol Sci Techn. 2000; 10: 717–735.

18. World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. WHO/CDS/WHOPES/GCDPP/2005. 13.

19. Finney D. Probit analysis, 3rd edn. Cambridge University Press, Cambridge1971.

20. Blanford S, Chan BH, Jenkins N, Sim D, Turner RJ, Read AF, et al. Fungal pathogen reduces potential for malaria transmission. Science. 2005; 308: 1638-1641.

21. Singh G, Prakash S. Evaluation of culture filtrates of Culicinomyces clavisporus: Mycoadulticide for Culex quinquefasciatus, Aedes aegypti and Anopheles stephensi. Parasitol Res. 2012; 110: 267-272.

22. Singh G, Prakash S. Efficacy of the Trichophyton ajelloi and Lagenidium giganteum metabolites against mosquitoes after flash chromatography. Parasitol Res. 2012; 110: 2053-2060.

23. Thomas MB, Read AF. Fungal bioinsecticide with a sting. Nat Biotechnol. 2007; 25: 1367-1368.

24. Blanford S, Shi W, Christian R, Marden JH, Koekemoer LL, Brooke BD, et al. Lethal and pre-lethal effects of a fungal biopesticide contribute to substantial and rapid control of malaria vectors. PLoS One. 2011; 6: 23591.

25. Ffrench-Constant RH. Something old, something transgenic, or something fungal for mosquito control? Trends Ecol Evol. 2005; 20: 577-579.

26. Farenhorst M, Mouatcho JC, Kikankie CK, Brooke BD, Hunt RH, Thomas MB, et al. Fungal infection counters insecticide resistance in African malaria mosquitoes. Proc Natl Acad Sci USA. 2009; 106: 17443-17447.

27. Paula AR, Carolino AT, Paula CO, Samuels RI. The combination of entomopathogenic fungus Metarhizium anisopliae with the insecticide Lmidacloprid increase virulence against the dengue vector Aedes aegypti (Diptera: Culicidae). Parasit Vectors. 2011; 4: 8.

28. Leles RN, Sousa NA, Rocha LF, Santos AH, Silva HH, Luz C. Pathogenicity of some hypocrealean fungi to adult Aedes aegypti (Diptera: Culicidae). Parasitol Res. 2010; 107: 1271-1274.

29. Leles RN, D'Alessandro WB, Luz C. Effects of Metarhizium anisopliae conidia mixed with soil against the eggs of Aedes aegypti. Parasitol Res. 2012; 110: 1579-1582.

30. Darbro MJ, Graham RI, Kay BH, Ryan PA, Thomas MB. Evaluation of entomopathogenic fungi as potential biological control agents of the dengue mosquito, Aedes aegypti (Diptera: Culicidae). Biocontrol Sci Techn. 2011; 21: 1027-1047.

31. Paula AR, Carolino AT, Silva CP, Pereira CR, Samuels RI. Testing fungus impregnated cloths for the control of adult Aedes aegypti under natural conditions. Parasit Vectors. 2013; 6: 256.

32. Dong Y, Morton JC, Ramirez JL, Souza-Neto JA, Dimopoulos G. The entomopathogenic fungus Beauveria bassiana activate Toll and JAK-STAT pathway controlled effector genes and anti-dengue activity in Aedes aegypti. Insect Biochem Mol Biol. 2012; 42: 126–132.

33. Pelizza SA, Scorsetti AC, Tranchida MC. The sublethal effects of the entomopathic fungus Leptolegnia chapmanii on some biological parameters of the dengue vector Aedes aegypti. J Insect Sci. 2013; 13: 22.

34. Hamill RL, Higgens GE, Boaz HE, Gorman M. The structure of beauvericin, a new desipeptide antibiotic toxic to Artemiasalina. Tetrahedron Lett. 1969; 49: 4255–4258.

35. Grove JF, Pople M. The insecticidal activity of beauvericin and the enniatin complex. Mycopathol. 1980; 70: 103–105.

36. Jestoi M. Emerging fusarium-mycotoxins fusaproliferin, beauvericin, enniatins, and moniliformin: a review. Crit Rev Food Sci Nutr. 2008; 48: 21-49.

37. Leland JE, McGuire MR, Grace A, Jaronski ST, Ulloa M, Park Y, et al. Strain selection of a fungal entomopathogen, Beauveria bassiana, for control of plant bugs (Lygus spp.) (Heteroptera: Miridae). Biol Control. 2005; 35: 104–114.

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