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Isolation and efficacy of entomopathogenic fungus (*Metarhizium anisopliae*) for the control of *Aedes albopictus* Skuse larvae: suspected dengue vector in Pakistan

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ABSTRACT

Objective: To isolate the entomopathogenic fungus *Metarhizium anisopliae* (*M. anisopliae*) in the local environment, and evaluate its efficacy against the suspected dengue vector *Aedes albopictus* in Pakistan. **Methods:** According to the standard procedure, *M. anisopliae* was isolated from the dead mosquitoes which were collected from the field or dead after the collection. Bioassay was performed to determine its efficacy. **Results:** The results indicated that *M. anisopliae* had larvicidal effect with LC_{50} value 1.09×10^{5} and LC_{90} value 1.90×10^{13} while it took 45.41 h to kill 50% of tested population. **Conclusions:** Taking long time to kill 50% population when compare with the synthetic insecticides, is the only drawback for the use of entomopathogenic fungus but these bio-pesticides are safe for the use.

1. Introduction

The most economical method for mosquitoes control especially dengue vectors lies in eradicating breeding sites and application of environment friendly larvicides through community education[1,2]. *Aedes albopictus* (*Ae. albopictus*) which is an increasingly important disease vector[3] has spread with the urbanization and poor sanitation, thus resulting in its increase[4]. In Pakistan, an outbreak of dengue hemorrhagic fever was first reported in Karachi in 1994[5] and 11 024 confirmed cases of dengue fever including 40 deaths were reported in 2010. Now in 2011 again dengue outbreak occurs in Punjab with 100 confirmed cases daily reported[6].

The adverse effects of synthetic chemical insecticides in the environment have received wide public concern[7]. Over the last five decades, many problems have been resulted due to the misuse of synthetic insecticides in Agriculture and Public Health Programs, *viz.* insecticide resistance, environmental pollution, toxic hazards to human and other non-target organisms[8]. To mitigate these problems, a major emphasis has recently been taken by using entomopathogenic fungus as larvicides which can provide an alternate to synthetic chemical insecticides[9]. In general, mosquitoes show susceptibility towards entomopathogenic fungi and its derived products. They have low toxicity to non-target organisms and using entomopathogenic fungus as larvicides may be a promising approach for biological control of mosquitoes[10] due to their selective toxicity and ready decomposability in the ecosystem. Also unlike the inherent dangers which are associated with the process of production of synthetic insecticides, the process for the manufacture of microbial products is safe and less pollutant[11]. The present study has therefore been taken to explore larvicidal activity of fungus isolate in terms of their cost-effective and environment friendly behavior.

2. Materials and methods

2.1. Collection and rearing of mosquitoes

Mosquito (*Ae. albopictus* Skuse) larvae were collected from different habitats like tires, pots, *etc* with the help of pipette from different localities of Faisalabad (university botanical garden, city park, discarded tire shops) longitude 73°74 East, latitude 30°31.5 North. The larvae were mass reared

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at (28±2 ℃) temperature and (75±5)% humidity in the insectary. The larval population was reared on TetraMin Tropical (Tetra TM). Adults were reared in steel cages and males were provided with 10% sucrose solution, while females were fed on blood of white rats (albino)[12]. Eggs were laid in plastic cups lined with filter paper then they were separated and shifted to rearing trays.

2.2. Isolation of entomopathogenic fungus

Those larvae that died within 5 days of collection and already dead larvae were searched for entomopathogenic fungi. These dead mosquito larvae were placed in the Saboraud dextrose agar (SDA) plates supplemented with chloramphenicol (25 μg/mL) as a bacteriostatic agent according to the method of Paula *et al* with some modification[13]. Incubator was used for incubation of these plates with larvae for 5 days, which was maintained at (24±2 ℃). On emergence of fungi colonies, they were isolated on new SDA plates. Identification of fungus colonies was done in the laboratory by the evaluation of the macro morphological aspects such as color, diameter and mycelial texture while optical microscope was used for the identification of the micro morphological conidial characteristics[14].

2.3. Fungus culture

These entomopathogenic fungus isolates *Metarhizium anisopliae* (*M. anisopliae*) were cultured on potato dextrose agar (PDA) medium which consisted of 20 g glucose, 20 g starch, 20 g agar and 1 000 mL of distilled water. The test tubes containing PDA medium were autoclaved at 121 ℃ (15 Psi) for 15-20 min and incubated at (25±2 ℃), (80±5)% relative humidity and photo phase of 12 h for 16 days after inoculation. The conidia were harvested by scraping the surface of 16-days old culture gently with inoculation needle and were suspended in distilled water containing 0.01% Tween-80. The mixture was stirred with a magnetic stirrer for 10 min. Fine mesh sieve was used to remove the hyphal debris by filtering the mixture. The conidial concentration of final suspension was determined by direct count using haemocytometer. Conidial suspensions of desired concentrations (1×10^4 conidia/mL) were prepared in distilled water containing 0.01% Tween-80 and preserved at 4 ℃ until used in bioassay[15]. Conidial viability was assessed according to Goettel and Inglis[16]. In all tested fungal isolates, spore germination was more than 90%.

2.4. Larvicidal bioassay

Serial dilutions were made of the conidial concentrate, and the appropriate dose was used to give the required concentration expressed as conidia/mL of water in the test beakers. In each bioassay, 20 larvae of the 3rd and 4th instar were added to a 750 mL beaker containing 500 mL of the test concentration of conidia. Each assay was conducted three times. Bioassays were conducted at five different concentrations (10^4, 10^5, 10^6, 10^7 and 10^8 conidia/mL), which were chosen to produce larval mortalities between 20% and 95% for calculating LC_{50} values. Data were obtained after 24 h for three days[17].

2.5. Data analysis

Abbot's formula[18] was used for corrected mortality and the data so obtained were analyzed by Probit analysis[19] by using MANITAB-15[20] software for dose and time mortality regression lines.

3. Results

The efficacy of secondary metabolites of *M. anisopliae* to the 4th instar larvae of *Ae. albopictus* was expressed in terms of LC_{50} and LC_{90}. It showed the effective result as its LC_{50} value was 1.09×10^5 (3.30×10^4-6.80×10^5) and LC_{90} value was 1.90×10^{13} (5.56×10^{11}-8.30×10^{15}) after 3 days of exposure with P=0.39. The slope±SE was (0.11±0.01), regression line was Y=0.11X-2.15 and χ^2 was 2.99.

Fungus isolate took 45.41 h (41.66-49.46) to kill 50% tested population of mosquito larvae with P=0.001. The slope±SE and regression line were (1.49±0.14), Y=1.49X-5.69, respectively while χ^2 was 12.95.

4. Discussion

The present experiment was carried out for the evaluation of natively isolated fungus (*M. anisopliae*) against suspected dengue vector *Ae. albopictus* larvae as immature stage of the vectors which is the most perfect stage for the bio-control agents[21,22], it is also reported to be resistant to all groups of chemicals which are extensively used in the Agriculture Sector of Pakistan[23].

In the present study, fungus isolate was taking time to kill 50% tested population but despite of that few bio-pesticides products have been widely used[24]. Our results also proved by Scholte and Blanford[25,26], that fungus isolates take time to kill different mosquito species but that is depending upon the dose, formulation and fungus strain. The effect of tested fungus is similar to that of *M. anisopliae* and *Beauveria bassiana* against adult and larvae of *Musca domestica* but *M. anisopliae* gave better results than *Beauveria bassiana*[27]. Moreover, metabolites of *Chrysosporiom tropicum*, *Trichophyton ajelloi*, *Chrysosporium lobatum*, *Lagenidium giganteum* have also been reported for their larvicidal potential against *Culex quinquefasciatus*, *Anopheles stephensi* and *Stegomyia aegypti*[28-34]. These results revealed that fungi have some potential against *Ae. albopictus* and these studies can be extended to field conditions.

Conflict of interest statement

We declare that we have no conflict of interest.

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