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Larvicidal Potentiality of Plant-Based Extract Against Mosquito Vector

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ABSTRACT

Mosquitoes are the vectors for the dreadful diseases of mankind. In this study, laboratory experimentation of assessing the mosquito larvicidal potentiality of *Moringa oleifera* plant extract was carried out. Extracting solvents such as hexane, ethyl acetate and methanol were used and the larvicidal activity was assessed against Culex gelidus mosquito larvae under laboratory conditions. The effective larval mortality was found in the methanol extract at concentrations of 500 µg/mL. The study thus provides evidence on the potential of indigenous plant extracts to serve as mosquito larvicides

Introduction

I.1 About Mosquito Menace

One of the major problems faced by human society is mosquito menace. In addition to inflicting a painful bite, mosquitoes can transmit diseases. Mosquitoes require water to breed. Adult mosquitoes lay eggs in stagnant or slow moving water or on moist soil or leaf litter or in areas likely to collect water. Mosquitoes are known to breed profusely in areas such as:

- Drainage systems and catch basins
- Stagnant, standing water
- Small ponds
- Large puddles
- Pool covers etc.

They cause so many diseases like malaria, filaria, chikungunya, Dengue, yellow fever, brain fever, skin irritation, Lyme disease, bullae formation etc. The population of mosquitoes is booming day-by-day due to various reasons. The tropical climate of South India poses a favourable breeding place for mosquitoes[1].

I.2 Mosquito Life Cycle

Mosquitoes lay their eggs in standing water--ponds, lakes, the wet grooves of old tires, birdbaths, a child's wading pool, plugged gutters and drains. Any object that collects water is a potential breeding place for mosquitoes. Eggs develop into larvae, which continue to live in the water until they mature into the next stage, called pupae. After a few days the pupae become adult mosquitoes [2].

The objective of this study to attack the mosquitoes when they are in the larval stage itself. This can be said as a 'Prevention is better than cure' method of curbing the mosquito menace.

II. Literature Review

II.1 Current Remedies Available

There are at present many remedies for mosquito menace such as coils, repellent creams, liquidator etc. But the major problem with all these chemical remedies is that although they reduce the mosquito menace on one hand, they lead to many harmful side-effects on human health on the other hand, especially respiratory problems such as asthma, allergies etc [3].

II.2 Mosquito Control Methodologies

II.2.a Chemical Control

Nowadays chemical mosquito repellants are widely used which are available in the form of liquidator, creams, sprayer, mosquito coils, etc. These mosquito repellents contain major chemicals such as:

Methoprene: It is a man-made chemical which is a copy of a chemical normally found inside mosquito larvae. The long-time usage of this chemical may cause human health problems such as inherent trouble, throat pain and skin allergy.

Pyrethroids: People who have allergies to these chemicals may feel a tight or tingly feeling under their skin, soreness around their eyelids, or a scratchy throat. Pyrethroids can kill fish if they accidentally get into water where fish live.

PiperonylButoxide or PBO: It is a chemical that is added to pyrethrins or pyrethroids to make them work better. A small amounts of PBO in a person or pet could come into contact during mosquito spraying which would not harm them in the short term. But if its amount is high, it could cause health problems and it can kill fish.

Organophosphates: Organophosphates are also used for mosquito control. Organophosphates can be harmful to people who work with them and who do not follow safety rules. People who come into contact with large amounts of these chemicals can have headaches, become dizzy, feel sick to their stomach, or even die [4].

II.2.b Biological Control

The biological control of mosquitoes and other pests involves introducing into the environment, their natural enemies such as parasites, disease organisms and biological 123 predatory animals. They may include insects, viruses, bacteria, protozoa, fungi, plants, nematode worms and fish. The effective use of these agents requires a good understanding of the biology and behavior of the insects to be controlled as well as of local environmental conditions. Such methods may be most effective when used in combination with others, such as environmental manipulation or the

application of larvicides that do not harm the biological control agents [5].

Several organisms have proved effective against mosquito larvae. The most important are:

— fish that eat mosquito larvae (larvivorous fish);

— predatory mosquitoes of the genus Toxorhynchites, the larvae of which feed on other mosquito larvae;

— dragonflies, the larvae of which feed on mosquito larvae;

— cyclopoid copepods, small crustaceans that attack first- and second-instar larvae of mosquitoes;

— nematode worms that are parasites of mosquito larvae;

— fungi that grow in the bodies of mosquito larvae;

— bacteriallarvicides, the toxic products of the bacteria Bacillus thuringiensisH-14 and B. sphaericus;

— extracts of seeds, leaves, barks and roots of certain plants and trees, which have larvicidal properties;

— Azolla, a free-floating fern that can completely cover water surfaces and prevent breeding by mosquitoes. Of these methods only two have become widely employed: the use of larvivorous fish and the use of bacterial larvicides [6].

II.3 About the Product

The chemical based mosquito repellents available in the market contain some harmful chemicals which are likely to cause threat to human health. An attempt has been made to prepare a 100% herbal product, based on traditional practices and rural wisdom. It is effective and cheaper than presently chemical based mosquito repellent. Since it is totally herbal, it has no side effects on inhalation or even on digestion.

This work is aimed at preparing a herbal remedy to prevent the mosquito problem and also destruction of mosquito larvae at the place of their generation. The herbal raw material used is bark of *Moringa oleifera* (drumstick) whose medicinal effects are well known from ancient days. It allows for extraction and then further processes. The final extract is tested against the larvae of mosquito belonging to species *Culex gelidus.*

II.4 About the Mosquito Species targeted

Culex gelidus, the targeted mosquito species is widely populated in India [7]. Its larvae have been found in freshwater ground pools, rivers, marshes and containers and sometimes in dirty water also. It has been reported as a voracious biter of humans and to enter houses [8] while others have reported it as having a preference for larger domestic animals with little preference for humans [9]. *Japanese encephalitis* virus has been isolated from *Culex gelidus* in several countries. It has been found to be one of the most important potential vectors of the disease *Japanese encephalitis* in South East Asia [10, 11].

III. Methodology

III.1 Collection of plant materials

The basic raw material, the barks of *Moringa oleifera* was collected from Melmaruvathur, Kanchipuram district, Tamilnadu, India.

III.2 Mosquito rearing

The larvae of *Culex gelidus* were collected from stagnant water area of Melmaruvathur and identified in Zonal Entomological Research Centre, Vellore, Tamil Nadu. To start the colony, the larvae were kept in plastic and enamel trays containing tap water. They were maintained and reared in the laboratory as per the method of Kamaraj et al [12].

III.3 Preparation of the extract

The barks of *Moringa oleifera* were dried for 25 days in the shade at the environmental temperatures (25-37°C day time).

The shade-dried barks were powdered mechanically using commercial electrical stainless steel blender and the powdered bark 100 g were extracted with 500 mL each of hexane 500 mL, ethyl acetate and methanol in a soxhlet apparatus (boiling point range 60-80°C) for 8 h. The extracts were filtered through a Buchner funnel with Whatman number 1 filter paper. The extract was concentrated under reduced pressure of 22-26 mmHg at 45°C in a rotary vacuum evaporator, and the residue obtained was stored at 4°C. Polysorbate was used as an emulsifier at the concentration of 0.05% in the final test solution.

III.4 Larval bioassay

During preliminary screening with the laboratory trial, the larvae were collected from the insect-rearing cage and identified in Zonal Entomological Research Centre, Vellore. The larval bioassay was determined as per the procedure of WHO [13] and Rahuman et al [14]. Five batches of 25 larvae were used in each bioassay. Five different concentrations (100, 200, 300, 400 and 500 µg/mL) were papered with 100 mL dechlorinated tap water, in a separate plastic container. Polysorbate 80 (Qualigens) was used as an emulsifier at the concentration of 0.05% in the final test solution. The control was set up with acetone, polysorbate 80 and dechlorinated tap water. The numbers of dead larvae were counted after 24 h of exposure and percentage of mortality was reported from the average of five replicates. Control beakers were treated with acetone, polysorbate 80, and distilled water under similar conditions but with no extract added to them. This is used as a comparison between the presence and complete absence of the extract under experimentation.

IV. Results and Discussion

The results showed that larvicidal activity of hexane, ethyl acetate, and methanol extracts of the medicinal plant species *Moringa oleifera* against *Culex gelidus* mosquito vector were varied according to the concentration of the plant extract. The control beaker showed nil mortality while there was significant mortality rates in test beakers. This preliminary screening is good mean to evaluate the potential larvicidal activity. The larvicidal activity of hexane, ethyl acetate and methanol extracts of *Moringa oleifera* were noted. Although all the plant extracts showed moderate toxic effect on the fourth instar larvae after 24h of exposure, the highest mortality was found in bark methanol extract of *Moringa oleifera* against the larvae of *Culex gelidus* with LC50 values of 38.24, 39.31, 66.28, 44.07, 24.90, 21.83, 67.22 µg/mL; LC90 values of 180.36, 189.41, 343.50, 261.45, 123.32, 162.34, 323.50 µg/mL. However, an average mortality rate of 87.6% was obtained proving the potential larvicidal activity of tested methanol extract of Moringa oleifera which showed the maximum effect on the mosquito larvae after 24 h of exposure at 500 µg/mL concentration. Thus, we can establish the potentiality of the barks of Moringa oleifera for utilization as a plant-based alternative to synthetic larvicides for combating the mosquito menace in an eco-friendly way of approach.

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