STUDY OF LARVICIDAL ACTIVITY AND PROTEIN CHANGES INDUCED BY SOME METHANOLIC HERBAL EXTRACTS IN *CULEX QUINQUEFASCIATUS*, FROM NASHIK (MAHARASHTRA)

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Abstract *:* Many dreadful diseases such as Malaria, Dengue, Filariasis, Encephalitis, yellow fever, Chikungunya etc are transmitted by mosquitoes. Use of herbal extract for controlling the mosquitoes is the best alternative method to synthetic pesticides which helps to reduce the pollution. Many plants from India possesses mosquitocidal, larvicidal and repellency activity. Hence there is a need to know the feasibility of using the plant extracts for the control of mosquito larvae. In the present work we used the plants which are easily available and widely distributed in India. The plants *Mentha spicata*, *Tridex procumbens* collected from Nashik **,** Maharashtra. The methanolic extracts of leaves of *Mentha spicata* and *Tridex procumbens* used as a cheap alternative to the conventional synthetic larvicides. Effect of methanolic extract of *Mentha spicata* on 4th instar larvae increased with an increase in concentration .The LC_{50} observed after 24 hrs for 200ppm & 60 ppm and LC_{90} after 24hrs for observed at 360ppm and 100ppm for *Mentha spicata* and *Tridex procumbens* respectively. Protein concentration decreases from 782µg/ml up to 567 µg/ml and 457µg/ml due to the of methanolic extract of leaf of *Mentha spicata* and *Tridex procumbens.*

Key Words- *Culex quinquefasciatus, Mentha spicata, Tridex procumbens*, protein alteration and toxicity.

INTRODUCTION

Many species of mosquitoes are found throughout the world. Two fifth (2/5) of the world population suffer from the diseases transmitted by various mosquito species [1]. The major dreadful diseases like Malaria, Dengue, Filariasis, Encephalitis, yellow fever, Chikungunya, etc are transmitted by mosquitoes. From few decades researchers were carried out various experiments in many fields that deals with biology, physiology, genetics, molecular biology, ecology, behavior & diseases epidemiology etc. of mosquitoes. These work is contributed to integrated mosquito control programme[2]. Because of the development of resistance to synthetic insecticides mosquito control program faces many problems now a days. Since out of many other techniques biological control is one of the method which includes the introduction of parasites, pathogens, predators etc. Pathogens like *Bacillus thuringiensis israelensis* (Bti) and *Bacillus spaericus* [3]. Aquatic predators such as *Gambusia affinis*, *Toxorhynchites sp.* and Cyclopoid copepod ie. *Mesocyclops sp*. used as effective biocontrol agents[4]. Naturally occurring secondary metabolites present in the plants used for control of mosquitoes at different life stages as they have been reported as a substances with mosquitocidal properties [5]. This study mainly focuses on usage of plant based insecticides against mosquito vector.

MATERIALS AND METHODS

Collection and Preparation of methanolic extract:

Fresh leaves of *Mentha spicata* and *Tridex procumbens* were collected from Nashik, Maharashtra. The leaves were rinsed with distilled water, air dried in shadow and crushed using the mixer-grinder. One hundred grams of powder of each plant was extracted with 100 ml of methanol in a soxhalet apparatus separately. The rotary evaporator was used to evaporate the methanol from the plant extracts. The concentrated extracts were preserved at low temperature. The required proportion of extract for calculating the toxicity assay and protein estimation was dissolved in DMSO and distilled water in proper proportion.

Rearing of mosquitoes:

Culex quinquefasciatus larvae were brought from National Chemical Laboratory, Pune. The larvae were reared in glass container in laboratory condition at 28 ±1°C and 70-85 % relative humidity. The adult male mosquitoes were fed on 10% sugar solution and female mosquitoes were fed on blood meal of chick.

Larvicidal bioassay:

Based on the preliminary tests, six concentrations were prepared from all the crude extract. 20 numbers of 4^{th} instars larvae of *Culex quinquefasciatus* were exposed to each concentration in 250 ml glass container. The number of dead larvae were counted after 24 hours of exposure. The larvae were consider *as* dead if they stop movement for a prolong period even after gentle probing with the help of spatula. The LC_{50} and LC_{90} values calculated. Percentage mortality in the treatments was corrected for mortality in the controls by using Abbot's formula [6].

Biochemical analysis:

For the biochemical analysis, control and treated larvae of *Culex* were used. In biochemical analysis, estimation of protein followed by Lowry method. Both the values for control and treated was observed and recorded for herbal extract each.

Protein analysis:

Freshly emerged IV instars larvae were collected from the experimental setup for the protein analysis. 100 mg larvae from each concentration was homogenized in phosphate buffer saline solution. The homogenate was centrifuged at 10,000 rpm for 10–12 min, and the obtained supernatant was used to determine the total protein present in the sample [7].

RESULTS AND DISCUSSIONS

The present study deals with the comparative response of 4th instars larvae of Culex quinquifaciatus to methanolic extracts of leaf of Mentha spicata and Tridex procumbens. Effect of methanolic extract of Mentha spicata on 4th instars larvae increased with an increase in concentration. The LC50 after 24 h was observed at 200ppm & LC90 after 24h was observed at 360ppm. The LC50 after 24 h was observed at 60 ppm & LC90 after 24h was observed at 100ppm. The methanolic extracts of leaf of Mentha spicata and Tridex procumbens, thus, be brought into effect for controlling larval populations. The concentration of protein in control larvae was 782µg/ml which decreases after application of methanolic extract of leaf of Mentha spicata and Tridex procumbens up to 567 µg/ml and 457µg/ml respectively.

Comparative biochemical analysis supports the above assumption, by indicating a dramatic decrease in protein contents of the treated larvae. Protein is required for moulting and metamorphosis [20]. Declining in the above parameter indicates that the irregularities in metabolic activities.

After treatment of *A. indica* leaf extract total protein level was reduced to 0.22mg/g from 0.72mg/g after compared to protein content of control larvae was reported by R. Gnanamani and S. Dhanasekaran[8]. Methanolic extract of *Artemisia annua* L., in north of Iran, was investigated for its toxic effects on: feeding, growth, fecundity, fertility including the biochemical characteristics of elm leaf beetle *Xanthogaleruca luteola* Mull. The LC_{50} observed was 48% and 43.77% of a methanolic leaf extract at 24hr and 48 hr, respectively. After treating 3rd instar larva for 24 hrs with the methanolic extract the levels of glucose, protein, urea, uric acid, a-amylase, alkaline phosphatase, alanine amino transferase and aspartate amino transferase significantly changed[9]. In control larvae of *Aedes aegypti* protein and lipid content were recorded as 210.74 and 94.71 µgm per five larvae which dropped up to 26.53% and 25.5%, respectively, in larvae following treatment with crude alum[10]. The level of sugar, glycogen, lipids, and proteins was

significantly (P<0.05) reduced in larvae treated with *H. benghalensis*. The acetone root bark extracts of *H. benghalensis* is less toxic to adults and repelled laboratory-reared female *A. barbirostris*, *A. albopictus*, and *C. quinquefascitus* with the short median protection times of 57.66–135, 72.41–134.16, and 47.66–93 min, respectively[11].PONNEEM, a novel herbal formulation prepared using the oils of neem (*Azadirachta indica*), karanj (*Pongamia glabra*) and their extracts, was tested for larvicidal, ovicidal and oviposition deterrent activities against *Aedes aegypti* and *Aedes albopictus* at various concentrations like 1, 0.5, 0.3 and 0.1 ppm concentrations. Quantitative analysis of protein was done in the larvae of *Aedes aegypti* and *Aedes albopictus* which were treated with PONNEEM at different concentration (ppm). As the concentration of PONNEEM changes the protein content of larvae reduces as compare to larvae of control. At 1ppm the concentration of protein was 0.177±0.010 & 0.008±0.005 in *Aedes aegypti* and *Aedes albopictus* respectively which was decreased as compared to concentration of protein in control 0.181±0.004 and 0.199±0.010 for *Aedes aegypti* and *Aedes albopictus* respectively. The concentration of protein was reduced from 0.181±0.004 and 0.199±0.010 for *Aedes aegypti* and *Aedes albopictus* respectively to 0.144±0.013 and 0.033±0.007 for *Aedes aegypti* and *Aedes albopictus* respectively when the larvae were treated at 0.5 ppm of PONNEEM. The concentration of protein was reduced from 0.181±0.004 and 0.199±0.010 for *Aedes aegypti* and *Aedes albopictus* respectively to 0.116±0.010 0.070±0.018 for *Aedes aegypti* and *Aedes albopictus* respectively when the larvae were treated at 0.3 ppm of PONNEEM. The concentration of protein was reduced from 0.181±0.004 and 0.199±0.010 for *Aedes aegypti* and *Aedes albopictus* respectively to 0.097±0.007 0.138±0.011 for Aedes aegypti and Aedes albopictus respectively when the larvae were treated at 0.1 ppm of PONNEEM[12].

C. Kamraj and et al. reported the 100% larval mortality of *Culex tritaeniorhynchus* in the ethyl acetate leaf extract of *T. procumbens*[13]. Earlier studies involving the essential oils obtained from various plants, viz. *Ocimum lamiifolium*, *Chenopodium ambrosioides*, *Mentha spicata* and *Eucalyptus globules* recorded LC50 values of 20.9, 17.5, 85.9and 68.3 ppm, respectively against the larvae of the *An. gambiae* mosquito[14].

The essential oil shows a significant toxic effect against early third-stage larvae of *C. quinquefasciatus*, *A. aegypti* and *A. stephensi* with LC_{50} values of 62.62, 56.08, and 49.71 ppm and LC_{90}values of 118.70, 110.28, and 100.99 ppm, respectively. The three major pure constituents extracted from the *M. spicata* leaf essential oil were also tested individually against three mosquito larvae which gave satisfactory results. The LC_{50} values of carvone, cis-carveol, and limonene appeared to be most effective against *A. stephensi* (LC_{50} 19.33, 28.50, and 8.83 ppm) followed by *A. aegypti* (LC_{50} 23.69, 32.88, and 12.01 ppm), and *C. quinquefasciatus* (LC_{50} 25.47, 35.20, and 14.07 ppm)[15]. Aqueous leaf extract of *M. spicata* showed the 100% mortality at 4ml at 72 hrs.[16]. Kamaraj et al. find that leaf and bark extract of *Annona squamosa*, *Chrysanthemum indicum* and *Tridax procumbens* can be developed as ecofriendly larvicides for mosquito control[17]. *Tridax procumbens* petroleum ether extract showed LC_{50} at 219 µg/ml for *Aedes aegypti* [18]. Mentha oil was promising against *Anopheles stephensi* and *Aedes aegypti*, showed LC_{50} values of 39.74 and 46.23 ppm respectively for larvicidal activity. *Tridex procumbens* exhibited high repellency effect against *Anopheles stephensi*[19].

The present results clearly indicate the mortality of larvae was due to the toxic effect of both the plants. The declining concentration of protein indicates the extracts may be creating irregularities in metabolic activities.

CONCLUSIONS

In the present work, we have used the methanolic leaf extract of *Mentha spicata and Tridex procumbens* against larvae of *Culex quinquefaciatus* we noticed satisfactory results as there was declining in protein concentration which hampers the moulting and metamorphosis activity as well as it also showed the mortality at very low concentration. On the basis of above evidences authors are suggested the methanolic leaf extract of *Mentha spicata and Tridex procumbens* can be recommended in field areas and can be effectively used as a herbal larvicidal product in a mosquito control program. However, further studies are needed to determine the active substances and how these substances function and the mechanism of action in the target species.

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