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STUDY ON THE RELATIVE EFFICACY OF INDIGENOUS PLANT EXTRACTS AS LARVICIDE AGAINST THE MOSQUITO *Culex quiquefasciatus* (Say.)

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ABSTRACT

There has been considerable concern for the past few decades about the excessive use of chemical pesticides both in agriculture and for control of vector borne diseases. It has become increasingly difficult to control mosquitoes that serve as vector for various diseases. The present project was undertaken to assess the efficacy of certain plant extracts as larvicides E dipa show your corvette against *Culex quinquefaciatus.* The leaves of *cassia auriculata, eucalyptus sp., Melia azedarach, Vitex negundo and pongamia glabra* were chosen for the study. The leaf extracts were tested against mosquitoes. The results revealed that the effect was greater in the pure extracts of the leaves than in the water extracts. *Pongamia glabra* shows the least LC50 value among tested plants. The crude acetone extract of *Pongamia glabra* was highly effective against the selected species.

Key words: Cow urine distillate, *Culex quinquefaciatus*

INTRODUCTION

Mosquitoes that constitute the most single family of insects are still the world's number one vectors of human and animal and conspicuous nuisance pest as well. WHO has declared the mosquitoes as "public enemy number one"(19). They are potential vectors and spread malaria, filariasis, yellow fever, brain fever, dengue fever, chikungunyaetc. (1) inalmost all tropical and subtropical countries and many other parts of the world(6,20).

The voracious feeding habit, high reproductive, rate dispersal potential and successful exploitation of environment are the cause for their rapid proliferation. To prevent proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential.

A number of insecticides of botanical origin have been reported to have larvicidal, adulticidal or repellent activity (18). Mosquito repellents based on chemicals has a remarkable safety profile, but they are toxic against the skin and nervous system like rashes, swelling, eye irritation and worse problems, though unusual including brain swelling anaphylactic shock and blood pressure (6,11,12,15,17).

Use of synthetic insecticides to control vector mosquito has caused physiological resistance and adverse environmental effects in addition to high operational cost and acceptance (3,18). Further, non biodegradable nature, higher rate of biological magnification through ecosystem, increasing insecticide resistance on a global scale, harmful effects on human health and non target organisms (3) has been lead to Environmental Protection Act 1967 that framed a number of rules and regulations to check the application of chemical control agents in nature (2,13). Considering these, the application of eco friendly alternatives such as biological control ofvectors has become the central focus of the control programme in lieu of the chemical insecticides.

In spite of so many works carried on botanical derivatives there are much more plants with larvicidal and adulticidal properties yet to be explored. This study attempts to identify bioinsecticides that are plant derived, efficient, as well as being suitable and adaptive to ecological conditions which are imperative for continued effective vector control management.

As a preliminary step the present study has been designed to test whether the leaf extracts of the medicinal plants such as *Cassia auriculata, Eucalyptus sp., Melia azedarach, Pongamia glabra and Vitex negundo* are larvicidal and to test their lethal doses.

MATERIALS AND METHODS

PREPARATION OF EXTRACT

Acetone extract

The fresh leaves of the chosen plants were washed, dried in shade and powdered. 20gm of powder was then soxhleted for 10 hours continuously using acetone as solvent (14). The concentrated crude acetone extracts (CAE) obtained after complete evaporation were kept in air tight containers in refrigerator.

Hot water extract

The dried leaves were boiled in water for 15 minutes under pressure in a pressure cooker. The water extract was then decanted and undiluted extract was directly used for testing its larvicidal activity.

EXPERIMENTAL BIOASSAY

The fourth instar larvae of *Culex quinquifasciatus were* used to assess the larvicidal activity of the biologically active compound, present in the extract of the selected plants. Serial dilutions ranging from 3000ppm-46.875ppm were prepared for individual applications. The concentration employed using hot water extract were 1%, 2%, 3%, 5%, 20% and 25%. For every 200ml of the test solution, 20 larvae were selected and delivered. Parallel controls were maintained along with four replicates of experimental tests. Mortality rate was observed and recorded. Percentage mortality was found out and the LC50 values were calculated.

STATISTICAL TREATMENT- PROBIT ANALYSIS

LC50 value was estimated by using Finneys method of Probit analysis (1971) as described by Dhamu(1990).The data obtained were calculated by computer program given in NCPC Technical Bulletin No.1.

From the results (Table 1,3) it is apparent that all the selected plants were found to have larvicidal effect irrespective of being extracted in hot water or acetone. `The insecticidal property is due to the presence of bioactive ingredients that are produced as secondary metabolites such as alkanes, alkenes, alkynes and simple aromatics, lactones essential oils and fatty acids, terpenes,alkaloids, steroids, isoflavonoids, terpenoids, and ligands (1). For instance the selected plants *Pongamia glabra* contain fluoroflavons, *Eucalyptus* contains monoterpenoids, etc.

The susceptibility of the larvae was greater in acetone extracts than in hot water extracts. The is in accordance with Hartzell 1956. The larvicidal potency of the extracts of the same plant may vary depending on solvent used for extraction (1,18). Extraction of different active biochemicals from a plant depends upon the polarity of the solvents used (1). They range from the most non polar solvent (polarity index 0.1 that mainly extracts essential oils) hexane/ petroleum ether to water, the most polar (polarity index 10.2) that extracts biochemicals with higher molecular weights such as proteins, glycans, etc.

Table 2 indicates LC50 value of crude acetone extracts (CAE) in individual application. *Pongamia glabra* shows the least LC50 value, (283.09 ppm) whereas the LC50 value of the *Eucalyptus sp.,* is highest (574.74 ppm). Low LC50 value indicates high efficacy.

The figure (1&2) indicates the plant extracts exhibited a concentration dependant activity against mosquito larvae. In all experimentation 100% survival was noted in the control setup (fig 1&2).

Highest percentage of mortality in CAE was obtained at 3000 ppm. There was no significant variation between them. It seems that all the plant extracts used in this study were almost equally active, if percentage mortality (in CAE) alone is considered. But, the calculated LC50 value of the extracts vary in their activity. Low LC50 refers to high efficacy and vice versa. The CAE of *Pongamia glabra* was found to be highly efficacious as shown by its low LC50 value (283.09) (Fig 1, Tab 2). The active ingredients in *Pongamia glabra* may be more active against the selected mosquito species of mosquito larvae or antilarval biochemical compound is extracted well in acetone extracts.

Among the selected plants, the CAE of *Eucalyptus sp.*, was found to be least effective as shown by its LC50 value (574.74 ppm). In *Eucalyptus sp.*, the active components was found to be monoterpenoids, dihydro flavones etc. Eucalyptus leaves was reported to contain dihydro flavonol, aroma dendrin,7 methyl ether of P-coumaric acid, citriodol, etc. (16). The mortality of the CAE of other selected plants, *Melia azederach*, *Cassia auriculata and Vitex negundo* fall in between *Pongamia* glabra and Eucalyptus *sp.*

The insecticidal property of *Melia azederach* was reported due to the presence of limnoids salanin meliantriol, meliacarpins (4).Limnoids from Meliaceae have attracted considerable interest because of insect control and anti-feedant propeties(1). Melia carpins have been reported in leaves and have shown anti-feedant properties(4).

The larvicidal effect of *Cassia auriculata* and *Vitex negundo* is reported (6). The compounds such as terpenes, camphenol, sequeterpene, glucosides and flavanoids were isolated from the common hedge plant *Vitex negundo* and is responsible for insecticidal property.

Among the hot water extracts of the selected plants *Pongamia glabra* was found to be more effective. Highest mortality of the larvae was seen at 25% in all plant extracts except in *Pongamia glabra* that shows 100% mortality even at the concentration of 20% (Table 3). At high concentrations of the extract the mat formation was found to be more especially in

Pongamia glabra and *Melia azederach* than in other plant extracts. This may affect the respiration of larvae and result in their mortality. *Pongamia glabra* exhibited 100% mortality at 20% whereas *Vitex negundo* exhibited 100% mortality at the concentration of 25%. At 25% concentration *Melia azederach, Eucalyptus sp.* and *Cassia auriculata* exhibited 86%, 90% and 97% mortality respectively. This maybe because of the solubility of the active component in water may differ from plant to plant. Changes in the larvicidal efficacy of the plant extracts may occur due to geographical origin of the plant, variations in the species of the plant, response in the different mosquito species etc. (1,18).

It is interesting to note that in the present work in addition to mortality certain behavioural responses such as movement of the larvae, resting position etc. was found to be altered when compared to the control. They are

1. The active wriggling movement was not present.
2. The larvae at high concentration (375ppm-300ppm) settled at the bottom instead of making an acute angle at the surface of water.
3. Larval-pupal intermediate was observed (7).
4. Abnormality such as tail elongation was also observed.

The sluggish movement and peculiar coiling of treated larvae suggest some neural or muscular disturbance (5,8) by some active principle. The detail lethal effect of compound is more likely to be caused by a disturbance of the endocrine mechanism that regulate moulting and metamorphosis (21). This is an added advantage in a plant derived compounds. Unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise botanical blends of chemical compounds. These active ingredients produce relative non specific effects on a wide range of molecular targets from proteins nucleic acids, bio membranes and other cellular components (11). Thus the current study also insists the most effective alternative approaches under the biological control programme is to explore floral biodiversity and enter the field of using safer insecticides of botanical origin as a simple and a sustainable method of mosquito control. Though the plant extracts were found to be effective, their utility in the field is to be confirmed only after intensive research and field trials. Today environment safety is considered to be paramount importance. An insecticide does not need to cause high mortality on target organisms but should be ecofriendly in nature and the proposed study is a stepping stone.

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Figure 1: Effect of acetone extracts on the fourth instar larvae of *C. quinquefasiciatus*

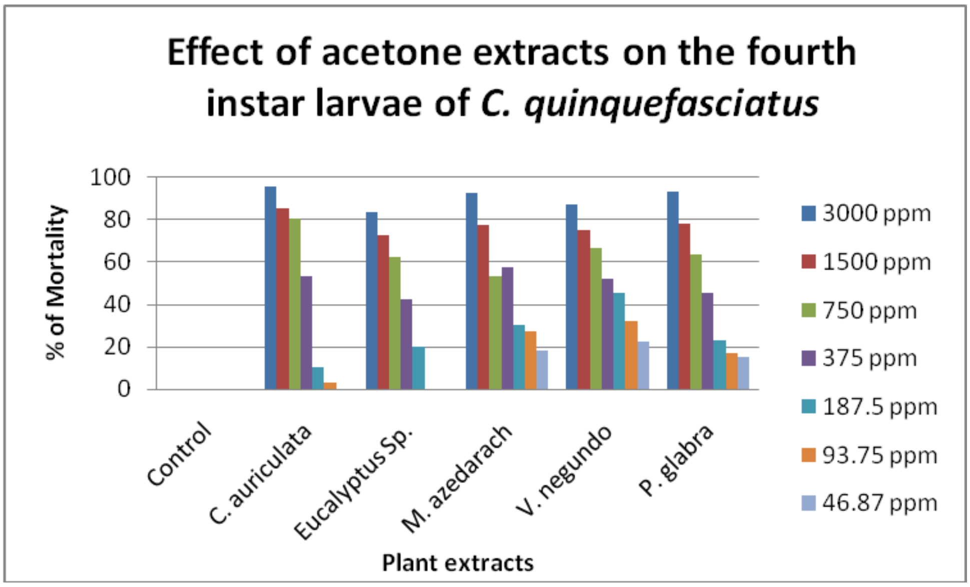

Figure 2: Effect of hotwater extracts of the fourth instar larvae of *C. quinquefasciatus*

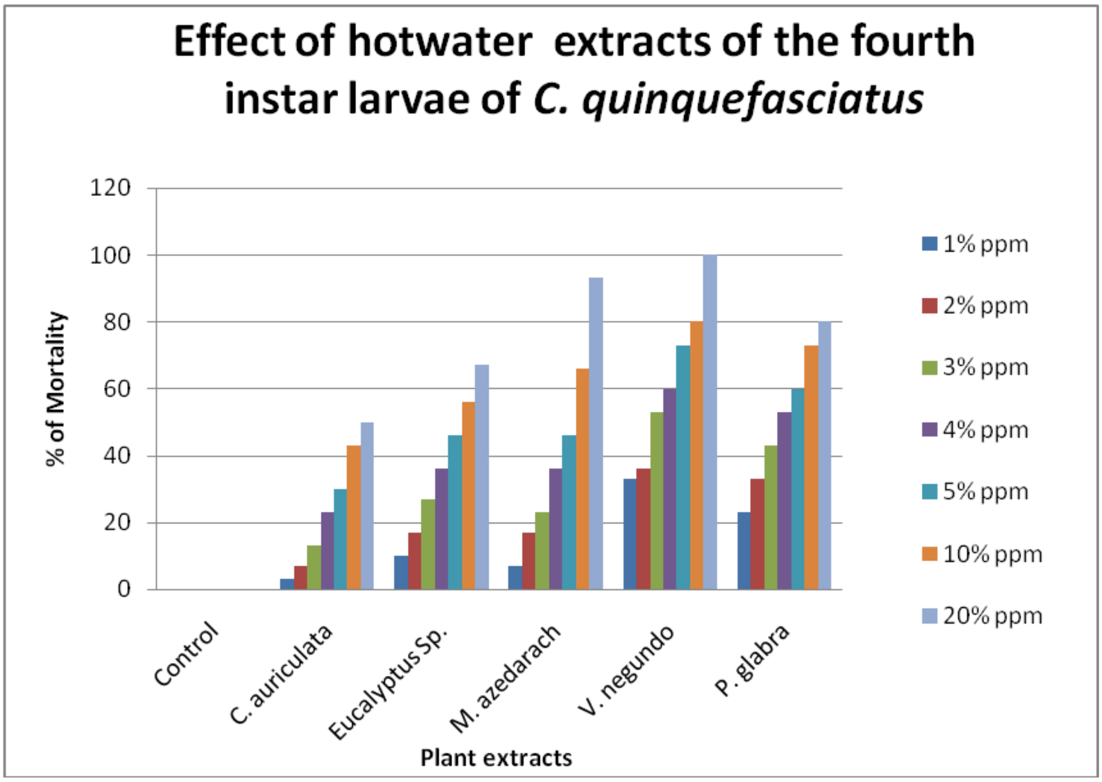

Figure 3: Comparison of LC50 value of the selected plants extracted in acetone