EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 11
278-282
Year: 2017

LARCIVIDAL EFFICACY OF *MUKIA MADERASPATANA* AGAINST *GRYLLOTALPA AFRICANA*

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Article Received on 22/08/2017 **Article Revised on 12/09/2017** **Article Accepted on 03/10/2017**

ABSTRACTS
Gryllotalpa africana is a macroinvertebrates and the characteristics of soil habitat have reciprocal influences on one another. Insects such as mole crickets (Orthoptera: Gryllotalpidae) spend virtually of all their lives in the soil. The application of easily degradable plant compounds is considered to be one of the safest methods to control insect pests and vectors as an alternative source for the synthetic pesticides. The study indicated that essential compounds were the only chemical used for the control of mosquito larvae while extract was used as the control of adult mosquitoes. The essential compounds were extracted by steam distillation and their chemical composition determined by Gas-chromatography coupled to mass spectroscopy. A study was made to monitor the effect of plant extracts on different instars of larvae and pupae of mosquito vector *G. africana*. Bio-assay was made using the solvent acetone to find out the median lethal concentration. *Mukia maderaspatana* which possess insecticidal properties are seemed to be better vector control agents than the synthetic xenobiotics. These results suggest a potential utilization of the extracts of these *Mukia maderaspatana* species for the control of *G. africana*.

KEYWORDS: *Gryllotalpa africana*, insecticide, larvicidal activity, *Mukia maderaspatana.*

INTRODUCTION

Mole crickets are omnivorous, feeding on animal as well as plant material. Studies have indicated that the southern mole cricket, *Scapteriscus borellii* Giglio-Tos, is less damaging than the tawny mole cricket, *Scapteriscus vicinus* Scudder. *Scapteriscus borellii* is a predator and feeds mostly on other insects while *S. vicinus* is mainly herbivorous (Matheny, 1981; Matheny *et al*., 1981; Walker and Dong, 1982). Dissection of field trapped *S. borellii* showed that their gut contents contained 66% animal material and only 2% of plant material, and the rest a combination of plant and animal material. In contrast, 84% of the gut contents of the short-winged mole cricket, Scapteriscus abbreviatus Scudder and 88% of *S. vicinus* contained plant material. Both *S. vicinus* and *S. borellii* are pests of tomato and strawberry fields in Florida (Schuster and Price, 1992), as well as many vegetables, peanut, sugarcane, tobacco and ornamentals such as coleus, chrysanthemum and gypsophila. Among turfgrasses, *S. vicinus* often injures bahiagrass and bermudagrass, whereas *S. abbreviatus* favors St. Augustinegrass and bermudagrass. Mole crickets also feed on weeds such as pigweed and *Amaranthus* spp. (Capinera and Leppla, 2001).

Male mole crickets produce loud songs (50–90 dB) after sunset by rasping a stridulatory file on the forewing. Males use a funnel-shaped opening at the mouth of a subterranean calling chamber, which amplifies the sound of their song. Calling chambers are constructed each evening 10-20 min. before calling. The male mole cricket then tunes these chambers to the frequency of his song (Forrest, 1985) and calling lasts for approximately 1 h after sunset. Songs function to attract flying and walking females. The male’s song intensity varies depending on male size and soil moisture.

Biological control is defined as the action of predators, parasites (parasitoids) and pathogens in maintaining the population of another organism at a lower level than would occur in their absence. The basic concept of biological control is that host-parasite or prey-predators are a part of the food chain and their populations oscillate in a manner that no single species can dominate and all the species exist in mutually balanced density level. The distinct advantages with biocontrol agents of bacterial origin are their ability to kill target species at low doses, safety to non-target organisms, easy application in the field, inexpensive production, lack of infectivity and pathogenicity in mammal including human, and little evidence of resistance development in target mosquitoes. Since the success of biocontrol agents depends upon the interaction of host-parasite or predator-prey, target should be present in the same period and time. Therefore, ecological information of breeding habitats and the ecological niche of target biocontrol regulators are essential. Since the target of most of the potential biocontrol agents are the immature of filarial vectors which breed mostly in vast areas of water bodies, it is envisaged that biocontrol agents will have an important role in the overall strategy of filarial vector control.

Development of natural products like plant extracts are getting an increased attention from the scientists as natural alternative for the mosquito control. Phytochemicals have a major role in the mosquito control programme. *Mukia maderaspatana* (Linn.) Cogn. (Cucurbitaceae), a prostrate annual climber, is widely used in Siddha and Ayurvedic system of medicine. The plant reputedly exhibits anti-inflammatory and anticancer activities and is also used as a hepatotonic (Thabrew *et al*. 1988) and antihepatotoxic agent (Jayatilaka *et al*. 1989). The plant cannot be improved by breeding because of poor seed germination, frequency of seedling death, and environmental challenges such as habitat destruction and illegal and indiscriminate collection. *Melothria maderaspatana* belongs to the family Cucurbitaceae. The plant is a tendril climber/prostrate herb (The Wealth of India, 2003). They can be used as alternatives to synthetic insecticides or along with other insecticides under the integrated vector control programme (Palsson and Janeson, 1999; Hag *et al*., 1999; Saktivadivel and Thilagavathy, 2003). Some herbal products such as pyrethrums from *Chrysanthemum cinerarifolium*, rotenone from flowers of *Derris elliptica* (Ameen *et al*., 1983); azadirachtin from *Azadirachta indica* (Schmutterer, 1981) have been used as natural insecticides before and after the discovery of synthetic organic insecticides. There are more than 2000 plant species, which are used for mosquito control. Plant products can be used, either as insecticides for killing larvae or adult mosquitoes or as repellant for protection against mosquito bites. Plant products obtained either from the whole plant or from a specific part by extraction with different types of solvent such as aqueous, acetone, absolute alcohol, methanol, chloroform, benzene, hexane etc., depending on the polarity of the solvent. Studies carried out so far have shown that some phytochemicals act as general toxicant (insecticide/larvicide) both against adult as well as larval stages of mosquitoes, while others interfere with growth and development (growth inhibitor), reproduction chemosterilant or produce olfactory stimuli thus acting as repellent or attractant. In the present work plant extract of *Mukia maderaspatana* was investigated for potential larvicidal activity.

MATERIALS AND METHODS

For the present study, the medicinal plant *Mukia maderaspatana* belongs to the family Cucurbitaceae was collected from in and around area of Pattukkottai, Tamil Nadu, South India. The plant was identified with the help of flora of presidency, Tamil Nadu and Karnatic flora (Gamble, 1967 and Matthew, 1983) and standard references (Krtikar and Basu, 1935). Preparation of powder of the *Mukia maderaspatana* was collected washed, cut into small pieces and dried at room temperature (27ºC) for two weeks and made into powder by using mixture for further analysis.

Collection and storage of experimental animals

Larvae of *Gryllotalpa africana* were obtained from a permanent colony. The larvae were cultured and maintained in the laboratory at 27 ± 2^0C and 50 - 75% relative humidity. Larval forms were maintained in tray by providing dog biscuit and yeast powder in the ratio 3:1. The eggs of *Gryllotalpa africana* were collected and transfer to the laboratory by using camilin Brush No. 1 and transferred to a cylindrical jar (100 ml. cap.) for hatching.

Test for Larvicidal activity (WHO, 1996)

The laboratory colonies of *Gryllotalpa africana* were used for the larvicidal activity. The instar II and instar IV larvae and pupae of the selected mosquito species were kept in 1 litre glass beaker and different concentration of selected plant extract was added to find out LC_{50}.

Larvicidal bioassay

In order to evaluate the bioefficacy of insecticidal properties of leaves extracts of *Mukia maderaspatana*. 1 g of stored leaves extract is dissolved in 100 ml methanol and this is kept as stock solution. Varied concentrations of extracts of *M. maderaspatana* Viz. 0.025, 0.050, 0.075, 0.100 and 0.125% were prepared by dilution method, using methanol as solvent. These disparate concentrations were used for bioassay experiments. The mosquito larvae were treated with extract by using the method of WHO (1981). Ten larvae of *G. africana* were introduced in different test concentration of both plant extracts along with a set of control containing distilled water without any test solution. After adding the larvae, the glass dishes were kept in laboratory at room temperature. By counting the number of dead larvae at 24hrs of exposure, the mortality rate and the median lethal concentration were obtained. Three replications were maintained for each concentration. Dead larvae were removed as soon as possible in order to prevent decomposition which may cause rapid death of the remaining larvae. The water used for the study was analyzed by using the method of APHA (1996). Mortality was recorded after 24 h of exposure during which no nutritional supplement was added.

The experiments were carried out 27 ± 2^0C. Each test comprised of three replicates with five different concentrations (0.025, 0.050, 0.075, 0.100 and 0.125%). Data were evaluated through regression analysis. From the regression line, the LC_{50} values were read representing the lethal concentration for 50% larval mortality of *G. africana*.

RESULTS AND DISCUSSION

In the present pilot screening of *Mukia maderaspatana* extracts was found to show specific activity against the larval mortality of *G. africana*. The details of activity of methanol extracts of *M. maderaspatana* along with activity profile with standard are tested. The 24h bioassay is a major tool for evaluating the toxicity of phytotoxins and a number of researchers have been applying this method to assess the toxic effect of different plant extraction against mosquitoes (Sakthivadivel and Daniel, 1999). The mosquito larvae exposed under plant extracts showed significant behavioral changes. The changes were observed within 30 minutes of exposure. The most obvious sign of behavioral changes observed in *G. africana* was inability to come on the surface. The larvae also showed restlessness, loss of equlibrium and finally led to death. Remia and Logaswamy (2010) reported that these behavioral effects were more pronounced in case of *Catharanthus roseus* than *Lantana camara* extracts after exposures. These effects may be due the presence of neurotoxic compounds in both the plants. In the present study the behavioral effects were more pronounced in case of *Mukia maderaspatana* extracts after exposures. These effects may be due the presence of neurotoxic compounds in the plant. No such behavioral changes were obtained in control groups.

Results of the experiment conducted for evaluating the larvicidal efficacy of both plants showed that they are toxic to the *G.africana* larvae. Three replicates of each extract and control were performed in order to ascertain the consistency of the results (Tables 1). The corrected percent mortalities were analyzed using Abbott's formula (Abbott, 1925). The mortality data were analyzed using Prism Version 3 from which lethal concentration (LC_{50}) values (24 h) and 95% confidence intervals (CI) were determined. The LC_{50} value of the test extract was compared with that of *M. maderaspatana* reflecting the potencies of the plant.

The crude extract of *M. maderaspatana* was found to be active on the IV instar larvae of *G. africana*. The larvicidal activity varied with the concentration and exposure. The larvicidal activity of *M. maderaspatana* was comparable to that different concentration. The exact active principles in *M. maderaspatana* responsible for the larvicidal effect have been reported to contain sufficient amount of tetranortriterpenoids (Pegel and Rogers, 1990). The observed mosquito larvicidal effects could possibly be due to these compounds (Siddiqui *et al*., 2000).

The results from the *G. africana* larvicidal assay using five different concentration of *M. maderaspatana* are shown in Table 2. The most active essential compounds against third instar larvae of G. africana were those of *M. maderaspatana*. Sukumar *et al*. (1991) reported that *C. citratus* causes significant growth inhibition and mortality in later developmental stages of insect. The analysis of the essential oil of this plant from the state of Ceara, showed that its major components are geranial (60.3%) and neral (39.7%).

The use of vegetable oil presents a better option in comparison to chemical pesticides for the larval mosquito control, as chemicals may cause environmental hazards and proved troublesome in the long run (Ranapukar *et al*., 2001). Extensive research has been carried out on the effect of botanical derivatives of the neem tree and its derivatives (Mulla and Su, 1999).

Mosquito larvicidal property of *Momordica charantia* have already been reported by Manisha Srivastava *et al*. (2007) and Singh *et al*. (2006) and them safe for human health. In conclusion the leaf extract of *Mukia maderaspatana* are highly toxic even at low doses these plants may eventually prove to be useful larvicide. Further analysis is required to isolate the active principles and optimum dosages, responsible for larvicidal and adult emergence inhibition activity in *G. africana*. The product of this plant can be well utilized for preparing phytochemicals from which all the non-target organisms can be rescued from harmful vectors. These plants would be eco-friendly and may serve as suitable alternative to synthetic insecticides as they are relatively safe, inexpensive and are readily available in many areas of the world.

Table 1: Larvicidal effects of methanolic extracts of *Mukia maderaspatana* on larvae of *G. africana* after a 24 h treatment at room temperature.

S. No	Concentration of the extract (mg/ml)	No. of larvae Dead/No. exposed (*Mukia maderaspatana*)	Mortality
1	Control	0/30	0
2	0.010	3/30	10
3	0.020	6/30	20
4	0.025	9/30	30
5	0.050	12/30	40
6	0.075	15/30	50
7	0.100	18/30	60
8	0.150	24/30	70
9	0.200	30/30	80
10	0.300	30/30	100

Table 2: Percentage larval and pupal mortality of *G.africana* for different concentrations of extract of *Mukia maderaspatana* for 24 h exposure.

Stages of exposure	**Parameters**		**Effective concentration**				
Mukia maderaspatana(%)		Control	0.025	0.050	0.075	0.100	0.125
II instar	Larval mortality (%)	Control	25	50	75	100	125
		0	15	22	31	42	55
IV instar	Larval mortality (%)	Control	50	100	150	200	250
		0	14	35	52	63	75
Pupae	Pupal mortality (%)	Control	200	225	250	275	300
		0	29	42	53	66	81

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