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Bio Diversity

Elixir Bio Diver. 42 (2012) 6232-6235

ISSN: 2229-712X

Larvicidal avtivity of stem extract of *Cissus quadrangularis* against the vector mosquitoes

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ARTICLE INFO

Article history:

Received: 26 September 2011;

Received in revised form:

28 December 2011;

Accepted: 10 January 2012;

Keywords

Cissus quadrangularis,
Culex quinquefasciatus,
Aedes aegypti,
Petroleum ether,
Benzene and Methanol.

ABSTRACT

Biological activity of extract of *Cissus quadrangularis* against IV instar larvae and pupae of *Culex quinquefasciatus* and *Aedes aegypti* were studied. The effect of the Petroleum ether, Benzene and Methanol extracts of *C. quadrangularis* are tested against IV instar larvae and pupae of *Culex quinquefasciatus* and *Aedes aegypti*. All kinds extracts showed moderate larvicidal and pupicidal effects at 24h. When the IV instar larvae of *Cx quinquefasciatus* were exposed to the doses of 12.56, 13.83 and 32.91 ppm respectively of petroleum ether, benzene and methanol extracts of 50% mortality was recorded. After 24h of exposure, the same extracts of *C. quadrangularis* at 15.84, 17.50 and 38.94ppm concentrations gave 50% pupal mortality. The LC 50 values for IV instar larvae of *Ae. aegypti* are 15.73, 17.16 and 33.37ppm and the LC50 values of pupae are 22.13, 25.73 and 48.76ppm respectively for Petroleum ether, Benzene and Methanol extracts. The larvicidal and pupicidal effects of plant extracts indicate that *Cx quinquefasciatus* is highly susceptible to the extracts of *C quadrangularis*. The larvae were more susceptible to plant extracts than pupae of both *Cx quinquefasciatus* and *Ae aegypti*. Among the various organic solvent extracts of *C. quadrangularis*, petroleum ether was found to be the most effective larvicidal and pupicidal agent. The efficacy of *Cissus quadrangularis* was in the order of Petroleum ether > Benzene > Methanol

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Introduction

Mosquitoes cause annoyance to man and other animals and affect health for centuries. These are the carriers of malaria, yellow fever, filariasis and encephalitis (Pfadt, 1978). Mosquito problem has become acute in recent years, though many programmes have been launched to control these vectors. Among these carriers, filarial mosquitoes are cosmopolitan and primary vector of filariasis in India and elsewhere (Gopalan *et al.*, 1996) Moreover the distribution of mosquitoes are almost world wide as they range from equator nearly to the poles and from sea level to atleast 7000 feet altitude.

Disease prevention by the control of mosquito has received more attention in medical entomology (Mani, 1982). Therefore, there is a crying need to check the population of vector mosquito in order to reduce vector borne disease using appropriate control measures (Service, 1983). One of the methods of control of mosquitoes is controlling them in their larval stage. Extensive research has been carried out on the control of mosquito larvae (Rajavel *et al.*, 1987).

The common methods available for the control of mosquitoes and agricultural pests are the use of insecticides. Chemical control methods using synthetic insecticides had been favoured so far, because of their speedy action and ease of application. Although, the economic benefits of pesticides are well recognized, a major drawback with the use of chemical insecticides is that they are non-selective and could be harmful to other beneficial organisms, environmental pollution and rising costs. Moreover, many vectors have developed resistance to widely used pesticide. This causes the problem of vector resurgence in many epidemic and endemic zones (Das *et al.*, 1991; Kalyanasundaram and Babu, 1982). widely used pesticide. This causes the problem of vector resurgence in many epidemic and endemic zones (Das *et al.*, 1991; Kalyanasundaram and Babu, 1982).

The pesticides carried through food chain affect the non target organisms. Some of the insecticides are carcinogenic agents and are positively dangerous (Pingale, 1976). Many of these pesticides are known to persist for long period of time and this in turn affects the physiological status of beneficial organisms (Rao and Rao, 1976).

Recently the workers have shifted their focus from synthetic insecticides to botanicals because the plant materials are non-toxic to non-target animals, have no phytotoxic properties and leave no residues in the environment. The plants and their products could be used in the control of insects, offering a safer alternative to conventional pesticides use (Mani, 1982). In recent times, sustained efforts have been made to reduce the population of insect vectors, particularly mosquitoes, by using biological means, which have considerable advantages over chemical pesticides (Rajamohan *et al.*, 1987).

The phytochemicals may be secondary metabolites and are divided into two major groups based on their solubility.Carbohydrates, aminoacid, nucleic acid bases, organic acids and phenolic compounds are polar or water soluble constituents and are extracted with alcohol and ethyl acetate where as lipids, steroids, carotenoid, simple quinone and chlorophylls are non polar or lipid soluble constituents and are extracted with petroleum ether or chloroform. Using a single

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solvent, one cannot extract all the constituents of secondary metabolites and the single solvent gives only partial extraction, which may not be effective against mosquitoes. In order to achieve complete extraction of active plant constituents non polar to polar solvents should be used in extraction. Therefore, in the present investigation, it is aimed to extract all the active principles of plant, *Cissus quadrangularis* using non-polar to polar compounds, viz., Petroleum ether, benzene and methyl alcohol as solvents and to study their larvicidal and pupicidal activity against two mosquito species, *Culex quinquefasciatus* and *Aedes aegypti.*

Materials and methods

Bionomics of the selected mosquito

Mosquitoes are well known biological vectors of many distinct human diseases. Malaria, Filariasis, Japanese 'B' encephalitis, yellow fever and dengue fever are the most important diseases transmitted by the mosquito. In mosquito borne diseases, the pathogen is carried in the salivary secretion which is injected by the mosquito into the organ it bites.

Aedes aegypti

Aedes mosquito belongs to the *Culex* group. The adult mosquito looks in all aspects like *Culex* except that it is black in colour and with prominent white bands on head and thorax. Hence this mosquito is called silver spotted or tiger mosquito. The female bite during day time also. The female lays eggs in small collection of water called artificial containers like broken pots, glass tumblers, tree holes with rain water collections and the like. Eggs are laid separately is moist soil since eggs remain dormant for a few weeks or months and hatch only when they come in contact with water.

Aedes aegypti is the vector for transmitting viral infections like yellow fever, dengue fever and chicken – gunya. Presence of two white horse shoe like markings on the dorsal side of the thorax is a diagnostic feature of *Ae. aegypti* mosquito. *Ae. aegypti* has been proved to be a carrier of dengue fever by Bancrofti of Australia in 1906. The blood of dengue patient is usually effective to the mosquito only during the first three days of the disease. The incubation period for the development of virus in *Ae. aegypti* is 11-14 days when once infected, the infection lasts for the remainder of its life.

Culex quinquefasciatus

The ubiquitous of common vector of filariasis in India is *Cx. quinquefasciatus.* The drains, cesspools, disused wells backyard stagnations are greately favoured breeding places and the larvae and pupae developing in the above type of breeding places have luxuriant growth and the adults are also bigger in size. *Cx. quinquefasciatus* is well known because of its vicious man biting habits and its potentiality in the transmission of filarial nematode *Wuchereria bancrofti* in urban and semi urban areas near seacoast where there is greater humidity and where no underground drainage system is made available. The extract mode of transmission has been described by Bancrofti. It has been observed that the microfilaria before it could reach the infective stage.

Culture of Mosquito larvae in the laboratory:

For the culture of mosquito larvae, the method of Prakash and Ponniah (1978) was followed Eggs of *Ae.aegypti* and *Cx. quinquefasciatus* were obtained from C.R.M.E (Centre for Research in Medical Entomology) a unit of I.C.M.R (Indian Council of Medical Research) and were transported to the laboratory in small petridishes covered with cotton. The hatched larvae of each requires were reared in enamal pans containing two litres of clean water. The water used in the acclimation and in experience was clear and non chlorinated. The water in the rearing trays was changed daily to prevent the mortality of the larvae. The larvae were fed with powdered yeast tablets and dog biscuits in the ratio of 3:2. During the time of changing the water, the larvae were seived by a fine seive. On completion of fourth instar, the pupae were collected in a bowl and placed in a standard cage.

Plants chosen for study:

Many of the south Indian plants were screened for their insecticidal and antifeedant activities. *Cissus quadrangularis* was selected to study their larvicidal and pupicidal activity.

Cissus quadrangularis

C. quadrangularis Linn belongs to the family Vitaceae. It is a climber with stout fleshy quadrangular stem. Plant contains proteins, 12.8; fat and wax, 1; fiber, 15.6; carbohydrates, 36.6; mucilages and pectins, 1.2% dry basis. A yellow wax, tartaric acid and potassium salt are present. The plant is remarkably rich in vitamin C, calcium oxalate crystals account for the irritating action of fresh stems (Joshi, 2000).

Soxhlet extracting method

The stem of *Cissus quadrangularis* were collected, dried under shade at room temperature, powdered in a grinder. 50gms of the powder of plant materials were extracted in a soxhlet apparatus for 8 hours over a mantle heater at 50^{o}C temperature with a range of solvent. (BDH – Boiling point range from 60 to 90^{o}C). The classical procedure for obtaining organic constituents from dried plant tissue is to continuously extract powdered plant material in a soxhlet apparatus with a range of solvents starting in turn with petroleum ether, benzene (to separate lipids & terpenoids) and then using methanol (for more polar compounds). After 8 hours of extraction the extracts were filtered through Whatmann filter paper No.1. and concentrated on water bath. After complete evaporation of the solvent, the residue of the extract was stored in a refrigerator and used when ever needed, after redissolving in acetone.

Experimental design

The standard solution of desired concentrations were prepared is acetone to yield a concentration of 100mg/ml. One hour old IV instar larvae and pupae of *Ae. aegypti* and *Cx. quinquefasciatus* were separated from respective culture trays and placed as groups of 100 in 500ml glass beaker containing 150 ml tap water. Desired concentration of plant extracts were added to the beaker containing test animals to bring the final volume 250ml. A control was kept for each experiment and it received the solvent acetone equal to the highest concentration used in the test in 250ml of tap water containing the test animal. Five different concentrations of plant extracts were employed and replicated three times. Beakers with larvae and pupae were examined for mortality after 24hours. Immobile larvae and pupae were considered as dead. The obtained data were statistically analysed to calculate the LC_{50} and 95% confidential limits using the software described in NCPC technical bulltin No. 1 Finney (1971).

Result and discussion

In the present investigation, the larval and pupal susceptibility of *Culex quinquefasciatus* and *Aedes aegypti* to the petroleum ether, benzene and methanol extracts of the plant *Cissus quadrangularis* were studied and the results are presented.

I. Toxicity of *Cissus quadragularis* against two species of mosquitoes

i) Culex quinquefasciatus

The result of the larval and pupal response to the petroleum ether, benzene and methanol of *Cissus quadrangularis* against *Cx. quinquefasciatus* are given in the table 1. When the fourth instar larvae of *Cx. quinquefasciatus* were exposed to the doses of 12.56, 13.83 and 32.91ppm of respectively petroleum ether, benzene and methanol extracts of *C.quadrangularis* 50% mortality was noticed. After 24 hours of exposure, the same extracts of *C. quadrangularis* at 15.84 17.50, 24.71, 39.47, 43.00 and 38.94 ppm concentrations gave 50% pupal mortality.

ii) *Aedes aegypti*

The effect of three different extracts of *C. quadrangularis* on the larvae and pupae of *Ae. aegypti* are presented in the table 2. For the fourth instar larvae, the LC_{50} values are 15.73, 17.16 and 33.37ppm respectively for petroleum ether, benezene and methanol extracts after 24 hours of exposure. The LC_{50} values for pupae are 22.13, 25.73 and 48.76 ppm of respectively petroleum ether, benzene and methanol extracts.

Now a day, mosquito control is mostly directed against larvae and only against adults when necessary. This is because the fight against adult is temporary, unsatisfactory and pollutes the environment, while larval treatment is more localized in time and space resulting in less-dangerous outcomes. Larval control can be an effective control tool due to the low mobility of larval mosquitoes, especially where the principal breeding habitats are man-made and can be easily identified.

Plant based natural or bio pesticides have distinct advantages over organic synthetic insecticides. They do not leave poisonous residues in food chains, are easily biodegradable and do not pollute the environment (Jacobson 1977; Verma *et al.,* 1980) Pyrithrins from pyrethrum flowers are well known plant insecticides. Pyrethroids, known commonly as "nerve poison" act on the central nervous system of mosquitoes, paralyzing them and knocking them down in a stupor. Acute toxicity studies have shown pyrethrins to be toxic to insects and non-toxic to mammal and higher animals (Feinstein, 1952; Elliott *et al.,* 1976). Rotenoid bearing plants and several flavonoids, organic acids and other related compounds were isolated and assessed for their antifeedant or insecticidal activities. Biologically active plants hold great promise and are therefore being studied for their potential efficiency to minimize the extent of pollution and to reduce the cost.

For basic toxicological investigation and screening the flora for insecticidal activity, acetone is commonly used as it has good solvency for synthetic insecticides based on petro-chemical derivatives (Sawicki and Holbrook 1961) but cannot be used for extraction of all plant products (Singh and Jain, 1987). Other solvents can also be used in extraction. In order to extract fatty and non alkaloidal components, non polar solvents are used (Harborne, 1984).

The larval and pupal susceptibilities for two important species of mosquitoes to petroleum ether, benzene and methanol extracts of *Cissus quadrangularis* are presented in Table 1 and 2. It is found that the plant material extracted in three different solvents behaved differently in bringing out 50% mortality of larvae and pupae. The order of toxicity of the three plant fractions was Petroleum ether > Benzene > Methanol. Data presented in the Tables 1 and 2 clearly indicate that the plant products in petroleum ether is comparatively more toxic to the larvae and pupae that other solvent fractions. Next to petroleum ether fraction benzene followed by ethyl acetate extract also produced satisfactory results. Methanol extracts showed only minimum activity.

In the present investigation it was found that petroleum ether extracts of *C. quadrangularis* were found to be more toxic than other solvent fractions. The minimum does of petroleum ether extract of *C.quadrangularis* (12.56ppm) brought 50% mortality of the fourth instar larvae of *Cx. quinquefasciatus.* The regression equation for these LC_{50} values are Y = .495071 + 4.098839 X. The doses of 15.73 ppm of *C. quadrangularis* in petroleum ether extract are required to kill 50% mortality of the fourth instar larvae of *Ae. aegypti*. The regression equation for calculating the LC_{50} values are Y= 1.02049 + 3.324575 X.

The results of the larval and pupal susceptibility of plant extracts in three different solvents indicate that the maximum larvicidal activity is seen in petroleum ether extract. The petroleum ether extraction may remove certain toxic principles of lipids such as leaf alkanes and steroids and it was insecticidal probably by interfering with the insect endocrine system (Sieber and Rembold, 1983) and such extracts seem to inhibit influence on neurosecretory cells which are responsible for the production of enzymes for the tanning or circular oxidation processes.

Srivastava (1970) reported that petroleum ether extract of *Piper peepuloides* effectively kills larvae of *Ae. aegypti.* Damodar *et al.* (1964) observed the insecticidal action of petroleum ether extract of fungal mat (Macrosporium species) against *Cx. fatigans.* The petroleum ether extract of *Calotropis gigantea* was found to be effective against *An. stephensi* with an LC_{50} of 21.7 ppm (Kalyanasundaram and Das, 1985).

In the present investigation next to petroleum ether, benzene extract was found to be effective in controlling the vector population. Bhaduri *et al.* (1985) observed that the extract of *Tridax procumbans* in petroleum ether was more effective in reducing the egg laying capacity of pulse beetle than other solvents and *Adothoda vasica* extracted in benzene was the next. The petroleum ether extract of some terrestial plants and *Ipomea cornea* diluted in benzene proved very repulsive, a potent oviposition, inhibitor against *Callosobruchus chinensis* (Pandey *et al.,* 1986).

The plant extract of *C. quadrangularis* were bioassayed for their larvicidal and pupicidal activities against two species of mosquitoes. The plant extracts were found to have promising effect on larvae and pupae of two mosquito species. However, *Cissus quadrangularis* was found to be more effective and significantly in checking the vector population.

Observations of the present study suggested that the extracts of *C.quadrangularis* may be used in the management of larvae and pupae of biological vectors such as *Cx. quinquefasiatus* and *Ae. aegypti.*

The results also reveal that the susceptibility was also dependent on the age of life stage of the mosquitoes. Further, the results clearly indicate that the degree of susceptibility decreased in the higher stages (pupae) whereas little variation is found in the lower stages (fourth instar larvae). The toxic effects of plant products in petroleum ether on the larvae were more significant whereas, in the successive higher stages the same extract has lesser activity. Purohit (1983) and Ursula Stein and Klingauf (1990) also reported that earlier instars were found to be highly susceptible when compared to later instars. Here the fourth instar larvae were found to be more susceptible to plant extracts than the pupae.

In the light of present study, it is suggested that petroleum ether and benzene extracts of *C. quadrangularis* can be used as effective larvicidal and pupicidal agents against *Cx.quinquefasciatus* and *Ae. aegypti* instead of synthetic insecticides which produce hazardous effect on the non-target organisms and to which the vectors have developed resistance. The indigenous plants used in this study are perennially available in large quantities in Tamilnadu. Since the cost involved in the preparation of these extracts is also very minimal, their use in insect pest control operation is economical without polluting the environment. It is suggested to use plant origin secondary metabolites in the field of public health to control some important mosquito vectors such as *Cx. quinquefasciatus* and *Ae. aegypti.*

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Table: 1Toxicity of *C.quadrangularis* plant extract against the IV instar larvae and pupae of *Cx. quinquefasciatus*

Larvae			Pupae	
Plant extract	LC_{50} Value (ppm)	Regression equation	LC_{50} Value (ppm)	Regression equation
Petroleum ether	12.56	Y=.495071+4.098839X	15.84	Y=1.121238+3.232312X
Benzene	13.83	Y=1.1245181+3.39658X	17.50	Y=1.310666+2.967517X
Methanol	32.91	Y=2.452269+3.133453X	38.94	Y=.4129462+2.884137X

Table: 2 Toxicity of *C.quadrangularis* plant extract against the IV instar larvae and pupae of *Ae. aegypti*

Larvae			Pupae	
Plant extract	LC_{50} Value (ppm)	Regression equation	LC_{50} Value (ppm)	Regression equation
Petroleum ether	15.73	Y=1.02049+3.324575X	22.13	Y=1.354383+4.724345X
Benzene	17.16	Y=6.549216+4.103079X	25.73	Y=1.320068+4.480788X
Methanol	33.37	Y=1.366711+3.291077X	48.76	Y=3.517958+5.045895X