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Efficacy of natural product, *Clerodendron inerme* against dengue mosquito vector *Aedes aegypti*

Aedes aegypti is on focus worldwide because of its role as a vector of major diseases like dengue haemorrhagic fever and yellow fever. Development of resistance in mosquitoes against currently used synthetic insecticides coupled with their persistence and toxicity to non-target organisms has been widely recognized and has prompted the search for new means of control strategies. One approach to combat these insect vectors has been to make maximum use of the differences which exist between insects and vertebrates. This can be done using agents which will be selective in their action and create physiological imbalance during developmental stages, which are peculiar to insects. During their co-evolution between plants and insects, the former produced several metabolic by-products, which act as repellents, growth disruptors, larvicides, antifeedants and growth regulators against invading insects. Studies have shown that natural plant products could be effectively used against mosquitoes as alternatives to synthetic pesticides[1–4]. In this context, the work of Patterson *et al.*[5] is of significance. Over 2000 extracts of higher plants prepared from 325 different plant species were screened for insecticidal activity against the larvae of *A. aegypti*. There are a good number of reports on the successful use of neem and its by-products against mosquitoes[6–8]. However, these studies have shown that among the extracts prepared from different parts, the neem seed kernel extract obtained with organic solvents demonstrated higher effectiveness than that of aqueous extract against mosquitoes[9]. Literature survey has revealed that invariably organic solvents have been favoured to extract larvicidal factor(s) from the plant tissues against mosquitoes.

Clerodendron inerme Gaertn. (Verbinaceae), commonly known as Kashmir bouquet is a biennial, hardy plant and widely grown as a hedge plant along home gardens. The leaf extract of the plant has been shown to contain insecticidal properties against mosquitoes[10]. Review of the literature revealed that various solvent extracts of plant materials have been tested against mosquitoes. Therefore, it was thought rewarding to investigate the dry powder of leaf material as source of insecticidal properties against the mosquito larvae. In the present investigation, we have examined the effect of sun-dried leaf powder of *Clerodendron inerme* against fourth instar larvae of *A. aegypti*.

For the present study, leaves of *C. inerme* were collected from local areas of Dharwad city. They were washed in tap water and dried under the sun for four days. The leaves were pulverized (60–80 mash) and various quantities of this powder were tested against fourth instar larvae of *A. aegypti*.

Freshly molted fourth instar larvae were obtained from the cyclic colony maintained in the rearing house at temperature 28 ± 2°C, RH 70–75%, photoperiod 14 : 10 (light : dark), and pH of the rearing water 7.4–7.6. Twenty-five larvae were introduced into wide mouth (250 ml capacity) conical flasks containing 100 ml tap water, pH 7.4. After an hour of acclimatization, food in the form of a pellet containing yeast and dog biscuit (1 : 2 w/w) was added to each flask followed by known quantity of test compound. Food was given *ad libitum*. Four replicates were maintained (25 larvae in each) for each treatment tested. Control groups with four replicates were also maintained throughout the experiment. The mouth of the flasks was covered with cheese-cloth. All the experiments were continued till the emergence of adults, if any. Mortality was recorded during larval–pupal moult and pupal stage. In the present experiments both emergence inhibition of adult mosquitoes as well as fourth instar larval mortality were subjected to probit analyses[11] to determine effective 50% emergence inhibition (EI_{50}) and effective powder quantity to kill 50% of larval population (EPQ_{50}).

In the first experiment, the dry powder was tested from 10 to 60 mg against

Table 1. Comparative data on effect of different quantities of *Clerodendron inerme* powder on larval and pupal mortality

Powder added (mg/100 ml)	No. dead as larvae	Per cent larval mortality (mean ± SE)*	No. dead as pupae	Per cent pupal mortality (mean ± SE)*	Total per cent mortality
100	8	8.00 ± 2.31	92	92 ± 2.31	100
120	17	17.00 ± 1.91	83	83.00 ± 1.91	100
140	38	38.00 ± 2.00	46	62.00 ± 2.00	100
160	85	85.00 ± 1.00	15	15.00 ± 1.00	100
180	85	85.00 ± 3.42	15	15.00 ± 3.42	100
200	87	87.00 ± 2.52	13	13.00 ± 2.52	100
Control	0	0	0	0	0

*Results are mean ± SE of four replicates of 25 larvae in each.

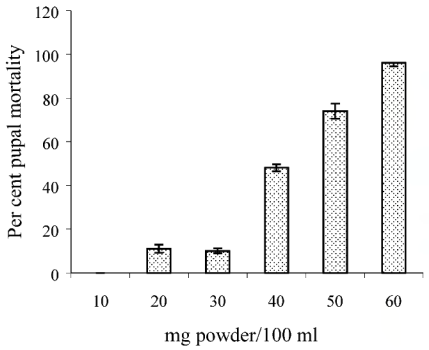

Figure 1. Per cent pupal mortality following addition of different quantities of *Clerodendron inerme* powder against fourth instar larvae.

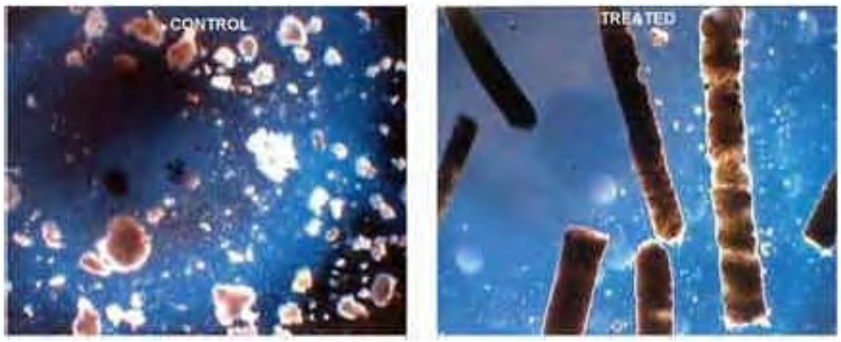

Figure 3. Noncellular peritropic membrane is ejected along with faecal matter in treated larvae, while control demonstrated loose faecal matter.

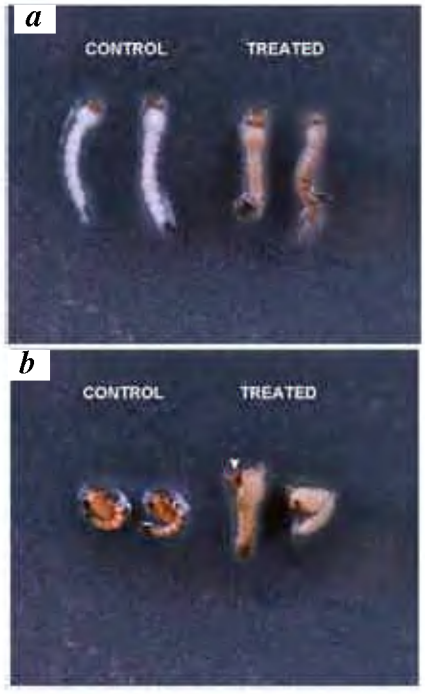

Figure 2. ***a***, Fourth instar treated larvae exhibiting early sclerotization. ***b***, Pupae showing less sclerotization compared to control pupae and attachment of head capsule (arrow mark).

freshly moulted fourth instar larvae. The results revealed that there was no larval mortality in the treated ones and they moulted to pupae after 60 h from the start of the experiment and the process was completed by 72 h. Control larvae also required 60–72 h to pupate. There were no visible behavioural changes in the treated larvae, except for the fact that they were not as active as those of control ones after 24 h of treatment. During pupal stage also, the pupae in treated flasks were not as active as control groups. Flasks containing 40, 50 and 60 mg powder showed pupal mortality after about 18–20 h. At the end of 72 h, the per cent pupal mortality in the same treated groups was 48, 74 and 96 respectively. Flasks containing 20 and 30 mg of powder exhibited less than 10% pupal mortality (Figure 1).

In order to determine the quantity of powder required to cause larval mortality, the quantity of powder was increased from 100 to 200 mg with 20 mg increment between the treatments. The results showed dose-dependent larval mortality (Table 1). As much as 85% larval mortality was seen when the powder quantity was increased to 160 mg. It was further noted that the fourth instar larvae that moulted to pupae died during the early pupal stage. In this experiment, final analysis of results revealed 100% mortality in all the experimental flasks, which included larval as well as pupal mortality.

Microscopic examination of dead larvae revealed that the larval cuticle had started sclerotization (Figure 2 *a*), which appeared to be a characteristic feature of the pupal cuticle. The dead pupae on the other hand, showed less sclerotization of the cuticle compared to untreated ones, and in majority of the pupae, the head capsule remained attached to the pupal head (Figure 2 *b*). These results clearly suggested that the *C. inerme* interfered with developmental processes of the fourth instar larvae and pupae of *A. aegypti*. In this context, the observations that exposure of fourth instar mosquito *Culex quinquefasciatus* to ether extract of *C. inerme* leaves resulted in death at larval–pupal molt and pupal–adult eclosion and suggesting inhibition of the moulting process[12], lend further support to our observations. EI_{50} and EPQ_{50} were found to be 40.8 mg and 144.8 mg respectively.

It is of considerable significance to note that the treated larvae excreted faeces in the form of pellets packed in noncellular membranous structure, whereas

control larvae excreted faeces as loose particles without membranous structure (Figure 3). Microscopic examination revealed that the non-cellular membranous structure was suspected to be the peritropic membrane (PM). It has been documented that the root extract of *Derris* affects peritropic matrix structure of *A. aegypti*[13]. It has been recognized that the PM separates food from epithelial cells of the gut involved in digestion and absorption of nutrients from the gut lumen, and acts as a protective barrier against various chemical, physical and microbial food components[14].

It may be argued from our results that the *C. inerme* powder taken by the larvae along with other food materials caused damage to the PM and subsequently affected the process of digestion and absorption. Disruption of growth of the larvae to pupae observed in this study may be the result of disturbances in the digestive process, which led to inadequate supply of nutrition to the larvae.

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Effects of the 2005 Muzaffarabad (Kashmir) earthquake on built environment

Studying the effects of earthquakes has long been recognized as a necessary step to understand the natural hazard and its risk to the society in the long term. A rapid assessment of general damage survey and documentation of initial important observations, not only help management of emergency response and rehabilitation activities, but also help to assess the need of follow-up areas of research[1,2]. The Muzaffarabad earthquake of 8 October 2005 which caused major devastation on both sides of the Line of Control (LoC) in Kashmir, presented another opportunity to further our understanding of earthquake risk in the region.

The *Mw* 7.6 earthquake on 8 October 2005 was a major earthquake at a depth of 26 km from the surface with its epicentre located at 34.493°N, 73.629°E, 19 km northeast from Muzaffarabad, the capital town of the Pakistan Occupied Kashmir (POK) and 170 km west-northwest of Srinagar, Jammu & Kashmir, India (USGS). The event which was similar in magnitude to the 2001 Gujarat earthquake and the 1935 Quetta earthquake caused widespread destruction in POK, Pakistan's North-West Frontier Province (NWFP), and western and southern parts of the Kashmir on the Indian side of LoC. This earthquake is associated with the known subduction zone of active thrust fault along the Himalayan mountain ranges in the area where the Eurasian and Indian tectonic plates are colliding and moving northward at a rate of 40 mm/yr (Figure 1).

The worst affected major towns on the Indian side of LoC are Tangadhar in Kupwara district and Uri in Baramulla district. Significant damages have also been reported from the Poonch and Rajouri district further south from the epicentre on the Indian side of LoC. During the reconnaissance survey we visited places along National Highway NH1A during 14–19 October 2005 from Srinagar to Uri and along Sopore, Durgwilla, Kupwara, Traigaon on the road to Tangdhar.

Damage to buildings and other structures in general agreed well with the intensity of ground shaking observed at various places, with the maximum of VIII at Uri, VII at Baramulla and Kupwara and V at Srinagar on MSK scale[3]. However, the collapse of stone walls of random rubble types was a surprise even with much lesser shaking. It has been well established that the local soil site and topographical conditions play a significant role in modifying the nature of ground motion which leads to varying degree of response to similar structures. Structures located on ridges and along steep slopes were subjected to a greater degree of damage in comparison to those located in valleys, during this earthquake as well. The affected region lies in the top two high risk seismic zones of IV and V of Indian seismic code IS:1893 (ref. 4) with an expected intensity of IX or more in the zone V and of VIII in the zone IV.

The region affected by the Muzaffarabad earthquake is mountainous terrain where the settlement is dense in valleys and sparse on hill slopes. Major civil engineering projects in the area are highways, bridges, small dams and micro hydro-electric projects and a few RC framed buildings. The housing units are largely