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Screening of the weed plant species, *Croton bonplandianum* Baill, for larvicidal activity of *Aedes aegypti.*

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Abstract
In tropical state of India, mosquitoes are the major problem for the health care of human being. This is because of the pollution free nature of plant derivatives which do not produce any health hazard. Biologically active plant extracts have been well documented for evolving an ecologically sound and environmentally acceptable mosquito control programmes.

Keywords: *Aedes aegypti*, *Croton bonplandianum*, mosquito larvicidal activity

1. Introduction
Emergence of resistance among mosquitoes is a recent problem. Safe and ecofriendly agents from biological origins are the need of the hour. Mosquitoes are well known group of insects, which transmit many dreadful diseases causing serious health problems to human beings. Controlling of these vectors is being achieved for a long time, by using synthetic chemicals. But the chemicals may cause pollution problem and help to develop resistance in mosquito species [3,7]. *Aedes aegypti* has been recorded round the year in different parts of the Madhya Pradesh. Biologically active plant extracts have been well documented for evolving an ecologically sound and environmentally acceptable mosquito control programme. A number of such plant products have been used for insect control for the time immemorial. About 2000 species of plants have been reported for their insecticidal properties (Feinstein, 1952). More than 400 plants have been screened for their mosquito larvicidal activity as per the records. The plant products like azadirachtin, pyrethrins, nicotines, rotenones are being used for pest control. Various studies showed the activity of plant extracts against different species of mosquitoes including *A. aegypti* (Gusmão *et al.*, 2002) [3]. The exotic weed, *C. bonplandianum* (Euphorbiaceae) generally distributed in the wastelands of tropical and subtropical regions of Madhya Pradesh, India are reported to have many medicinal uses including the repellent property against the insects (Nishanta *et al.*, 2002 [12]; Chaudhuri, 2007 [9]; Bhakat and Sen, 2008 [7]; Krebs and Ramiarantsoa, 1997 [6]; Maria *et al.*, 2008) [18]. However, no studies are conducted so far about the repellent property of this species against the mosquito, *A. aegypti.* Hence, the present study was aimed to determine the larvicidal effect of methanolic extracts of *C. bonplandianum* leaves against fourth instar larvae of *A. aegypti.* The control mortality was corrected by Abbott's formula (1925). LC_{50} and LC_{90} regressions and 95% confidence limit were calculated by using probit analysis (Finney, 1971) [17].

2. Materials and Methods
2.1 Plant collection
Plant materials were collected from Jabalpur, Rewa, Mandla, Dindori, Balaghat, Sidhi District; Madhya Pradesh, India Leaves were air dried in a shady place for 10 days to retain their active ingredients intact. Dried materials were powdered by using an electric blender. Powdered plant material (500 g) was soaked in methanol in airtight wide mouth bottle and kept for 7 days. After that, the cold extracts from the bottle along with methanol were filtered and kept in Petri dishes for drying at room temperature (Kongkathip, 1994) [5].

Dried extracts were used larvicidal activity A. *aegypti.*

2.2 Collection of mosquito eggs

The eggs of *A. aegypti* were collected from state forest research institute (SFRI), tropical forest research institute (TFRI), and Madhya Pradesh. The larvae were cultured and maintained in the laboratory at 27±1 ^{0}C and 85% relative humidity. Larval forms were maintained in tray by providing dog biscuit and yeast powder in the ratio of 3:1. Adult mosquitoes were maintained in a net cage (90 X 90 X 90 cm) and continuously supplied with 10 per cent sucrose solution with a cotton wick. For continuous culture selected number of mosquitoes were allowed to feed chicken blood every third day, there after moist filter paper was kept in beaker inside the cage for mosquitoes to lay their eggs. Eggs laid on the filter paper were immersed in larval basins containing water for the maintenance of the colony. Test solutions were prepared with metabolic extracts at different concentrations ranging from 25 to 200 ppm by diluting the stock solution using ethanol. Early fourth instars larvae of the species, *A. aegypti* were used for the study with control solution, with four replication for each concentration (WHO, 1970) [14]. The evaluation of mortality rate was performed 24 hours after the beginning of the experiment, verifying the number of dead larvae. The larvae were considered dead when they did not respond to stimulus with a Pasteur pipette. The environmental temperature and humidity were observed during the experiment, which varied between 27 ºC and 30 ºC.

3. Results and Discussion

The results of the larval susceptibility of *A. aegypti* using methanolic leaf extracts of *C. bonplandianum* are presented in Table 1. The results of the study revealed that the methanolic leaf extract was effective against larvae of mosquito. Furthermore, the effect of larval mortality was observed to be dose dependent The LC_{50} and LC_{90} of fourth instar of *A. aegypti* were determined to be 123.8 ppm and 364.0 ppm respectively. So leaf of this plant at 124 ppm is suggested for better vector control. The larvicidal property of the leaf extract of, *C. bonplandianum* may be due to the presence of phorbol derivatives, the secondary metabolites of diterpenoids category (Chandel *et al*., 2005). Maria *et al*. (2006) reported that the essential oils present in four species of a genus, *Croton* are responsible for their larvicidal activity against the mosquito, *A. aegypti*. Nazer *et al.* (2009) [11] reported that the stem extracts of *C. bonplandianum* was active and significantly lethal against the mosquito, *Culex quinquefasciatus* and he explained that the alkaloids present in the extract had toxic effect on mosquito larvae.

Table 1: Percentage of mortality using methanolic leaf extract of *Croton bonplandianum* against fourth instars larvae of the mosquito *A. aegypti*

Concentrations (ppm)	No. of fourth instars larvae exposed	No. of mortality	Percentage of mortality	LC_{50} (ppm)	LC_{90} (ppm)	Y = a + bx
Control	89	0	0	123.8	364.0	2.73x-.072
25	88	12	13.6			
50	90	38	42.2			
100	84	55	65.80			
150	83	73	88.0			
200	88	85	96.6			

Early studies showed that the essential oils are readily biodegradable and less detrimental to non-target organisms as compared to synthetic pesticides (Baysal, 1997 [2]; Dubey *et al*, 2008) [15]. Plants are rich sources of bioactive organic chemicals and offer an advantage over synthetic pesticides as these are less toxic, less prone to development of resistance. It is known that most of the active compounds of essential oils are specific to particular insect group and not to mammals (Isman, 2000) [4]. It is evident from the present study that herbal extracts from *C. bonplandianum* might have promising larvicidal efficacy. Screening, purification and identification of effective compounds available in this species will certainly bring more success towards the control of mosquitoes. Hence the large biomass of the weed, *C. bonplandianum* available in the wastelands of southern India can be used as a bioresource to commercially produce mosquito (*A. aegypti*) repellent.

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