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Larvicidal efficacy of *Capsicum annum* against *Anopheles stephensi* and *Culex quinquefasciatus*

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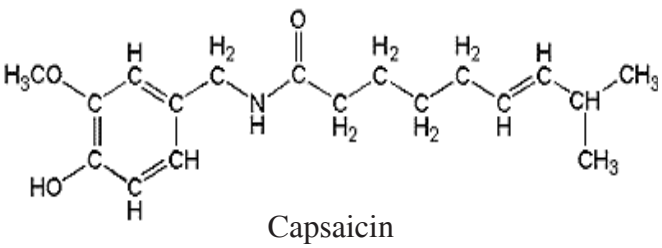
It is estimated that every year at least 500 million people in the world suffer from one or the other tropical diseases that include malaria, lymphatic filariasis, schistosomiasis, dengue, trypanosomiasis and leishmaniasis. Of late chikungunya, a serious mosquito borne epidemic has gained momentum in India. One to two million deaths are reported annually due to malaria worldwide. Lymphatic filariasis affects at least 120 million people in 73 countries in Africa, India, Southeast Asia, and Pacific Islands. These diseases not only cause high levels of morbidity and mortality, but also inflict great economic loss and social disruption on developing countries such as India, China, etc. India alone contributes around 40% of global filariasis burden and the estimated annual economic loss is about 720 corers[1].

Due to the lack of awareness among people, early detection and complete treatment of these diseases are very difficult. Consequently several chemical methods are available for the interruption of their transmission which has been limited by logistic problems, development of resistance, high cost etc. So the most effective and easiest approach to control these diseases requires interruption of the life cycle of the vectors by applying larvicides to their breeding places. Further, toxicity of synthetic insecticides towards non-target animals and environment has been widely observed and recognised. To avoid the propensity of bioaccumulation and induction of malignancy in non-target animals, a safe and more congenial method of vector control by natural and cheaper means of using plants as insecticides became popular[2]. The development of resistance by pests and vectors against botanicals has not been reported so far. Active principles from many plants have been recognised, isolated, purified and formulated as insecticides.

The species of *Capsicum* (Solanacae) is a typical shrub, commonly found in almost all parts of the world. The fruits of *Capsicum annum* contain hot flavour, which is due to the presence of a group of seven closely related compounds called capsaicinoids, among which capsaicin and dihydro capsaicin are responsible for 90% of the pungency[3]. Capsaicin (8-methyl-N-vanillyl-6-nonemide) is an active ingredient responsible for the heat in chilli peppers. It is colourless, pungent crystalline alkaloid, thermoliable and more soluble in alcohols and oils[3].



Capsaicin

It is important to note that capsaicin containing products are primarily used to repel insects rather than to kill them from ancient times. Literature survey has revealed that capsaicin has significant lethal and antifeedent effects on various invertebrates. Capsaicin has been proven as an oleoresin used against cotton pest[4]. Extracts of capsicum were also proven as repellent to some species of stored product beetles such as *Sitophilus zeamais* Motschulsky (Coleoptera: Curculinidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebriouidae)[5]. There are reports of using capsicum as biopesticides against *Alfalfa weevil* larvae[6]. Capsicum has proven its insecticidal activity against rice grain pest, *Sitotroga cerealella* also[7]. In the light of above said information, the present study was conducted to investigate the effect of natural crude extracts of *Capsicum annum* fruits (capsaicinoids) against IV instar larvae of *Anopheles stephensi* and *Cx. quinquefasciatus* (Diptera: Culicidae) as a part of the search for alternative natural products, by screening botanicals on mosquito vectors.

Fresh ripe fruits of *C. annum* (Guntur chilli) were purchased from market, dried and powdered using electric blender as it is unaffected by cold or heat, and extraction was carried out with excess of ethanol. Forty gram of chilli powder was subjected to extraction with 800 ml of absolute alcohol, by continuous stirring for 24 h. The crude extract was filtered through Whatmann No. 1 filter paper and the filtrate was collected in round bottom flask. The excess of alcohol was removed by distillation. The extract residue was poured into a test tube and kept in a boiling water bath till alcohol completely gets evaporated. The thick red crude residue, which contains capsaicinoids, was stored at room temperature (25 ± 2°C). It has been reported that crude extract of Guntur chilli contains 12.5% (w/w) oleoresin, 0.20% capsaicin, 0.6% dihydrocapsaicin and its pungency is recorded as 53250 SHU (Scoville Heat Units)[8]. The crude extracts were prepared at 0.3% concentration in absolute alcohol and diluted using the same solvent for subsequent experiments. Laboratory reared *An. stephensi* and *Cx. quinquefasciatus* larvae were used for the experiments. The experiments were conducted at 26 ± 2°C and relative humidity of 65 ± 5%.

Larval bioassay was conducted according to the standard WHO procedure[9]. The required quantity of chilli fruit extract of different concentrations (0.004–0.3%) in one ml of alcohol was mixed thoroughly with 249 ml of tap water in 500 ml glass beakers. Twenty-five early IV instar larvae were introduced into each beaker and mortality was observed at 24 h period. Three replicates were maintained for each concentration along with control. The experiments were repeated four times and analysis of data was carried out by employing probit analysis[10].

So far no work has been reported to asses the mosquito larvicidal activity of oleoresin present in Capsicum. The ethanol extract of *C. annum* proved to be sufficiently effective on both the species. *Cx. quinquefasciatus* was found to be more susceptible than *An. stephensi* in terms of LC_{50} and LC_{90} values (Table 1 & Fig. 1). The results showed that the LC_{50}

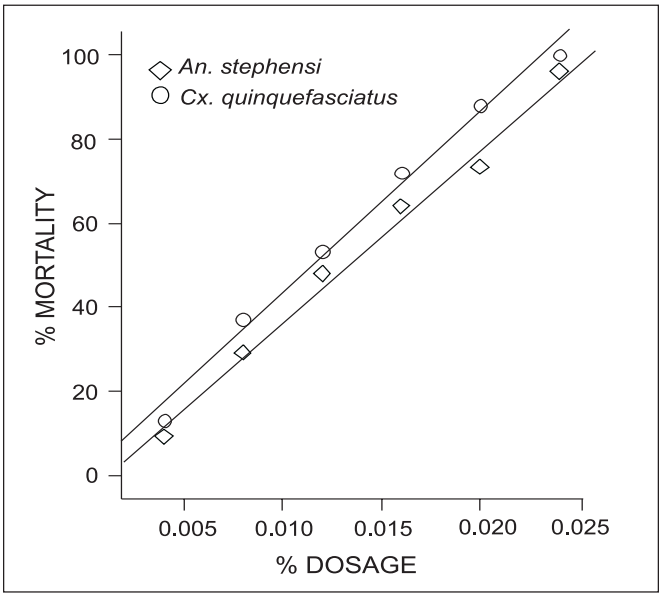


Fig. 1: Effect of *Capsicum annum* ethanol extract on *Cx. quinquefasciatus* and *An. stephensi* larvae

Table 1. Larvicidal activity of ethanol extract of *Capsicum annum* against IV instar larvae of *Cx. quinquefasciatus* and *An. stephensi*

Concentration	Larvicidal activity (95% fiducial limits)		
	LC_{50}	LC_{90}	LC_{99}
Cx. quinquefasciatus	0.0097 (0.007–0.012)	0.022 (0.017–0.037)	0.044 (0.029–0.114)
An. stephensi	0.011 (0.008–0.014)	0.027 (0.020–0.050)	0.057 (0.036–0.167)

Values are in percent.

and LC_{90} values of the extract against *Cx. quinquefasciatus* were 0.0097 and 0.022% and that for *An. stephensi* 0.011 and 0.027% respectively, proving its toxic nature against both the species. The extract of *C. annum* at 0.004% concentration showed no significant mortality ($p > 0.05$), whereas 0.024% at 24 h observations showed 99% mortality in case of *Cx. quinquefasciatus.* A concentration of 0.024% showed 96% mortality in *An. stephensi* larvae. However, larvicidal activity obtained was less when compared to other plant formulations[2].

The results of the present study illustrate the possibility of using *C. annum* for mosquito larval control, as it is less toxic to mammals, where it is quickly metabolised in the liver and excreted in urine within a few hours, even in case of over indulgence[3]. The development of an in-soil repellent for pocket gopher (*Thomomys talpoides*) with capsicum oleoresin suggests its eco-friendly nature[11]. Perusal of literature shows that extracts from *C. frutescens* has biocidal activity against *Bemisia tabaci*, a tomato pest[12].

It was observed that larvae became slowly inactive within 18 h and began to fall towards the bottom of the beaker. Microscopic examination of dead larvae revealed that the extract has penetrated into larval digestive system. The treated larvae showed curling up, agitation, vigorous body movements which are the characteristic of neurotoxicity. Further research regarding the effect of capsaicin on larval nervous system is needed to understand the mechanism of action of capsaicinoids in mosquito larvae. The results suggest for a possible utilisation of the cheap and readily available chilli fruits for possible control of mosquitoes as a part of the integrated vector management programme.

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