Research Article

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TOXICITY OF *EUPATORIUM CANNABINUM* L. AGAINST SECOND AND FOURTH INSTAR LARVAE OF *CULEX QUINQUEFASCIATUS* AND *AEDES AEGYPTI*

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ABSTRACT
The aim of the study was to demonstrate the toxicity of *Eupatorium cannabinum* L. against 2nd and 4th instar larvae of *Culex quinquefasciatus* and *Aedes aegypti.* For growth inhibitory activities early 2nd instar and 4th instar of *Culex* were taken in 500 ml capacitor glass beakers each beaker containing 25 larvae of 2nd and 4th instar separately. The beaker contains 240 ml water and 1 ml of test concentration of *Eupatorium* extract. Four different concentration regions from 20 to 50 ppm concentration were used. Each concentration has three replicates with one control and one untreated group. The following observations were recorded and growth inhibitory activities were photographed. Toxicity of acetone extract of *Eupatorium cannabinum* L. was observed against 2nd and 4th instar larvae of *Aedes aegypti* when four different concentrations were taken along with a control and uncontrolled group in three replicates, it was noticed that larval motility was dose dependent; as the dose concentration increases the mortality also increases. With the view to discourage further aggravation of environmental pollution through the use of synthetic insecticides, it is imperative to explore the abundant natural plant resources and replace the intrinsically hazardous chemicals through natural plant products not only to combat malaria but also precious human life from the ill effects of the synthetic chemicals.
Key words: *Eupatorium cannabinum*, *Culex quinquefasciatus, Aedes aegypti,* 2nd and 4th instar larvae, Insecticidal.

INTRODUCTION

Pharmacological and phytochemical investigations of *Eupatorium* species (including many species now reclassified into other closely related genera) have been carried out by various researchers. Sesquiterpene lactones, flavonoids, phenolic and acetylenic compounds, triterpenes and alkaloids with cytotoxic, antimicrobial, antitumoral, antioxidant and anti-inflammatory activities have been reported[1]. *Eupatorium* species are noted to have anti-inflammatory properties in Argentinean folk medicine. The antinociceptive effect of infusions of *E. laevigatum, E. arnottianum* and *E. subhastatum* was investigated by the same author and the results showed analgesic activity that supported their use in folk medicine. Three South American *Eupatorium* species, *E. buniifolium, E. articulatum and E. glutinosum* were described as inhibitors of herpes viruses[2]. *Eupatorium purpureum* (gravel root and joe pye weed), *E.cannabinum* (hemp agrimony, avicenna) and *E.perfoliatum* (boneset) have a long history of use in traditional medicine and their infusions were used for treatment of fever, cold, flu, arthritic and rheumatic pains by native Americans[3]. *Eupatorium purpureum* has mostly been used as a diuretic and to treat urinary tract infections. More recently, the crude ethanolic extract of *E.purpureum* was further investigated for its significant anti-inflammatory activity[4]. Cistifolin and four benzofurans were isolated and subjected to bioassay guided fractionation using *In-vitro* monocyte endothelial and monocyte-fibronectin adhesions bioassays. Only cistifolin showed strong activity and this result supports its potential use for treatment of inflammation. There have been few studies published on the use of *Eupatorium species* in pest management. The potential molluscicidal activities of aqueous extracts of *E. adenophorum* were recently reported against *Oncomelania hupensis*, the intermediate host snail of *Schistosoma joponicum*[5]. The chloroform extract from the leaves of *E.quadrangulare* showed significant repellency activity against the leafcutter ant (*Atta cephalotes*)[6]. Bioassay guided fractionation of this extract resulted in isolation of five sesquiterpene lactones and two of them (secoeudesmanolide and alloalantolactone) were determined to be the most active repellant compounds. The essential oil of *E.buniifolium* was evaluated against *Varroa* mite (*Varroa destructor*) and showed no attractive or repellent effect on this parasitic mite of honey bees[7]. The ethanolic extract of the aerial parts of *E.amphidictyum, E. bupleurifolium, E.capilare, E. halimifolium, E. kleniodes, E.laevigatum and E.squaliudum* showed no activity against *Aedes Fluviatilis* fourth instar larvae[8].

Criticism of conventional insecticides of the chlorinated hydrocarbons, organophosphorous compounds and carbamate groups always concentrates on the following points.

- The products are considered to be persistent and too wide acting. The characteristics are special danger to ecosystem, in which the insecticides are used.
- The animals are too toxic for man and domestic animals.
- Developing resistance in many insects necessitates the use of new chemicals, and this can further aggravate the toxic situation in the environment.

This criticism gave a fundamental objective of modern research for developing the safest possible insect control products and techniques; and also the need for

environmentally safe, degradable and target specific insecticides. In brief, a new life of interdisciplinary research has sprung into existence with this objective, over the years.

MATERIALS AND METHODS

Collection of Plant Material

Eupatorium cannabinum L. of family Astreaceae is a medicinal herb found in India. It has been extensively used by the natives for the treatment of malaria, fever and flu. The plant grows plentifully on the marshy land. This was identified and authenticated by the taxonomist of Botany department at S.S.L Jain P.G College Vidisha M.P. A voucher specimen bearing specimen number PCADRL-0164 of the plant material was preserved in the herbarium data sheet of the laboratory. The plant was collected in the winter month and leaves and stems were washed thoroughly with water and dried at room temperature.

Extraction and Isolation

The dried and cut plant material approximately 1 kg was extracted with cold methanol/water; 70/30 by cold percolation method[9]. The combined extracts were concentrated to about 1 litre of aqueous solution and filtered to remove the precipitated chlorophyll. Partition between aqueous solution and dichloromethane was used further to remove the lycophyllic substances. The aqueous solution was acidified to pH 2 with 1 N HCL and partitioned with ethyl acetate. After removal of the solvent the residue were lyophilized to yield water soluble fraction and ethyl acetate soluble fraction. Those fractions were further purified by column chromatography using methanol and water; 50:50, followed by Acetone and water; 7:3. These fractions were monitored on TLC on silica gel coated plates using the same solvent system. The purified fractions were kept in small glass vials in a refrigerator.

Collection and Rearing of mosquito larvae

The *Culex* larvae were collected from the foul water reservoirs from the local surroundings of Vidisha by long hand dipper. The larvae were brought to the lab and sorted out instar wise in the animal trays. They were fed dog biscuits and yeast tablets (3:1) ratio. They were reared up to the adult stage. The adult male and females were kept in the insect cages fitted with mosquito net. The males were fed with 10% sucrose solution whereas females were fed with restraining guinea pig/ rabbit in a restraining cage kept overnight inside the insect cage. The *Aedes aegypti* eggs were procured from MRC Delhi and were reared as per procedure adapted for *Culex mosquito* ovitract.

Oviposition and Egg Laying

On day 3rd after blood meal was kept inside the mosquito cage, where the female laid their eggs. The eggs were removed from the cages along with ovitracts and they were reared from egg and generation in the lab. The non aged larvae of different instars were used for experimental bioassay.

Experimental Bioassay Method

For growth inhibitory activities early 2nd instar and 4th instar of *Culex* were taken in 500 ml capacitor glass beakers each beaker containing 25 larvae of 2nd and 4th instar separately. The beaker contains 240 ml water and 1 ml of test concentration of *Eupatorium* extract. Four different concentration regions from 20 to 50 ppm concentration were used. Each concentration has three replicates with one control and one untreated group. The following observations were recorded and growth inhibitory activities were photographed.

Biostatistical method

24 hrs LC_{50} value of each concentration was calculated by Provit analysis. The LC_{50}, LC_{90}, Pudicial limit, Chi-square test, Regression equation and Variance were calculated in this method.

OBSERVATIONS AND RESULTS

Toxicity of acetone extract of *Eupatorium cannabinum* L. was observed against 2nd and 4th instar larvae of *Aedes aegypti* when 4 different concentrations were taken along with a control and uncontrolled group in three replicates, it was noticed that larval motility was dose dependent; as the dose concentration increases the mortality also increases. LC_{50} value and other details as mentioned in table 1 were calculated using provit analysis of Fenny, it gave 24 hr. LC_{50} value at 344 ppm and the chi-square value was 2.498 for 2nd instar larvae. Almost similar trend was noticed when early 4th instar larvae were treated with same concentration. There was more than 4% mortality in control and untreated group. But one thing was very much clear from the observations that 4th instar larvae of *Aedes aegypti* were more susceptible than 2nd instar larvae which is quite clear from the fact that 24 hrs LC_{50} value was much less (150.78 ppm) against 2nd instar LC_{50} value. The results were found to be quite significant ($P < 0.05$).

Table 1: Toxicity of acetone extracts of *Eupatorium cannabinum* on second and fourth instar larvae of *Aedes aegypti*

Treated aquatic stages	Conc. (Ppm)	*Larval mortality (%)	Regression equation (y= a+bx)	°Chi-square [x^2(n-2)]	LC_{50} (ppm)	Log LC_{50} ±S.D	95% FL (ppm)
Second instar	100	26	Y= 2.445+1.006x	2.498	344.90	2.3891±0.07	LL= 182.1
	200	38				3	UL= 574.9
	300	42					
	400	62					
	Control	5					
	untreated	00					
Fourth instar	100	38	Y= 3.647+ 0.621x	0.221	150.78	2.17±0.0685	LL= 63.5
	200	52					UL= 395.5
	300	68					
	400	60					
	Control	6					
	Untreated	4					

FL = Fiducial limit; LL = Lower limit; UL = Upper limit; °= Values are significant, $p<0.01$; * Abott's formula applied.

Table 2: Toxicity of purified fraction of acetone extracts of *Eupatorium cannabinum* L. on second and fourth instar larvae of *Aedes aegypti*

Treated aquatic stages	Conc. (Ppm)	Larval mortality (%)	Regression equation (y= a+bx)	Chi-square [x^2(n-2)]	LC_{50} (ppm)	Log LC_{50} ±S.D	95% FL (ppm)
Second instar	20	32	Y= 2.124 + 1.79x	1.018	40.114	1.6020±0.0	LL= 29.8
	30	40				41	UL= 55.8
	40	48					
	50	56					
	Control	08					
	untreated	00					
Fourth instar	20	36	Y= 2.277 + 1.77x	1.518	34.109	1.5314±0.0	LL= 29.79
	30	44				41	UL= 55.037
	40	52					
	50	64					
	Control	04					
	Untreated	04					

FL = Fiducial limit; LL = Lower limit; UL = Upper limit; °= Values are significant, p<0.01; * Abott's formula applied.

Table 3: Effect of petroleum ether extract of *Eupatorium cannabinum* on the fecundity and fertility of adult of *Aedes aegypti* treated as larvae

Treated group*	% Female dead	% Biting	Oviposition day after blood meal	Total no. of egg-rafts and average number of eggs	% of hatching	% larval mortality	% pupation	% adult emergence	Wing length (mm)
T♀×T♂	33.3	19.45	5th	4, 105	74.3	42.3	57.5	45	3.2
T♀×UT♂	31.2	21.2	5th	4, 126	75	38	62	54	3.6
UT♀×T♂	18.5	36.5	4th	6, 132	87.3	25	75	67	4.2
UT♀×UT♂	13.3	86	3rd	8, 225	98	8	92	88	4.5

T = Treated; UT = Untreated; ♀ = Female; ♂ = Male; * Equal number of adults i-e. 25 were mated in all the different treated groups.

Table 2 depicts the toxicity of purified fraction of acetone extract of *Eupatorium cannabinum* L. against 2nd and 4th instar larvae of *Aedes aegypti*. It was noticed that the purified fraction was much effective even at a low concentrations. 100 ppm and above was found to be toxic sometimes and caused knock-down effect. The LC_{50} value for 2nd and 4th instar larvae of purified compound was noticed to be 40.11 and 34.10 ppm respectively. The chi-square value, regression equation, LC_{90} and 95 % Fiducial limit (both upper and lower) were also calculated in both the cases which have been clearly mentioned in the table.

Table 3 portrayed the effect of crude petroleum ether extract of *Eupatorium cannabinum* L. on fecundity and fertility of adult mosquitoes. For this experiment four groups of insects i.e. treated male and treated female; treated female and untreated male; untreated female and treated male; and untreated female and untreated male were taken. The different parameters which were observed in this set of experiment were the number of eggs per egg raft as well as subsequent metamorphosis up to the adult stage. Wing length was also measured which was almost equal to control group i.e. 4.5 mm. in 4th group i.e. UT female and UT male. Biting activity of the female mosquito got considerably decreased in the treated female and treated male group which were only 19.45%. Female took the blood meal and took 2 days more than the usual 3 days period by the untreated group for Oviposition. % hatching i.e. fertility was also found to be slightly effected with the treatment of *Eupatorium cannabinum* L. leaf extract it was 74.3% in the treated group as compared to the 98% fertility in the untreated group. When metamorphosis was observed it was noticed that only 45% larvae successfully reach to the adult stage whereas in untreated group more than 80% larvae molted into the adult with normal wing venation and other anatomical features.

DISCUSSION

Different parts of the plant have been found to possess insecticidal/larvicidal and insect growth disrupting activities to different types of insect, pests and vectors. Natural pyrethroids and latter synthetic pyrethroids have attracted the attention of research workers all over the world because of their similarity with synthetic pesticides in quick knock down effects, besides having the advantages of plant products.

In order to discourage further aggravation of environmental pollution through the use of synthetic insecticides, it is imperative to explore the abundant natural plant resources and replace the intrinsically hazardous chemicals through natural plant products. Since the higher plants used as the agents for controlling insect pests and vectors for disease are associated largely with their innate quality of being non-phototoxic, systematic and relatively easily bio-decomposable, hence there, judicious exploitation, planned cultivation and appropriate preservation to use at the time of need should be the frontier area of research not only to combat malaria but also precious human life from the ill effects of the synthetic chemicals.

The LC_{50} value observed for *Eupatorium cannabinum* against 2nd and 4th instar larvae in the present study were in agreement to views expressed by pioneer workers in the field[10], who have also expressed similar observation that the 4th instar larvae of culicine mosquito are more susceptible than 2nd instar larvae. A larvicidal compound has been isolated from *Ipomia carnia* against culex quinquefasciatus[11].

A laboratory bioassay of acetone extract of some indigenous plants against 3rd and 4th instar mosquito larvae have been assessed[12]. The 24 h LC_{50} value was 0.95, 0.98 mg/ml this was somehow much higher as compared to the present findings therefore LC_{50} value was little less as reported by these workers. They have further suggested that acetone extract of plants have potential as larvicidal against mosquitoes, this seems to be quite true

with the present findings where alcoholic extract was found to be potent mosquito larvicidal compound. This was quite true in case of *Eupatorium cannabinum* L. extract where if dose dependent mortality was obtained and as the concentration increases the juvenomotic stages were also increased. Since the culicine mosquito used in the present study i.e. *Culex quinquefasciatus* causes phyllariasis but *Aedes aegypti* on the other hand is a vector of yellow / dengue fever. Mosquito repellent activity in plant products have been noticed[13]. They have also mentioned that the bites of mosquito bringing to the genera Anopheles, Aedes and Culex are responsible for the transmission of important tropical diseases such as malaria, dengue / yellow fever and elephantiasis. In the present study also two prominent vectors of *Culicine* i.e. *Aedes* and *Culex quinquefasciatus* were used and a control mechanism of aqueous stages was worked out so that the larvae couldn't emerge into the adult stage successfully. The involvement of chitin inhibitory principles have been reported[14] in the similar way as reported in the present study where chitin was not perfectly synthesized and resulted into Demelanized pupae formation. The antiphyllarial and larvicidal activity of *Typhoneum trilobatum* have been discussed[15]. They have mentioned that the plant extract inhibit the 4th instar larvae of *Culex quinquefasciatus*. Mosquito is responsible for spread of many diseases than any other group of arthropods[16]. Diseases such as malaria, filaraia, dengue fever and chikungunya are still a real challenge to the mankind.

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