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Research Article

EFFICACY OF *ANAMIRTA COCCULUS* (LINN.) WIGHT AND ARN AND *POGOSTEMON PANICULATUS* (WILD) BENTH EXTRACT ON *CULEX PIPIENS*

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ABSTRACT: Acetone extracts of *Anamirta cocculus* fruit and *Pogostemon paniculatus* leaf was tested for its larvicidal activity against different instars of *Culex pipiens*. The data on 24 hr LC50 and LC90 revealed that the acetone extracts of the selected plants have potential larvicidal properties. The LC50 values of the selected plant extracts are dose and time dependent and the data show a three to five fold increase in activity at 72 hr exposure. Total protein content of the different instars of the larvae of *Culex pipiens* are also estimated by modified Lowry method and result shows no significant differences between the control and treated larvae of *Cx. pipiens*. The essential oils of the two selected plant materials were extracted and conducted repellency tests. The study concludes that both acetone extracts and essential oils of the selected plant materials showed their potential to be used as biolarvicides especially to control mosquito populations.

Key words: *Anamirta cocculus, Pogostemon paniculatus* larvicidal activity, *Culex pipiens*

INTRODUCTION

Mosquitoes are dreadful vectors of various diseases and well known for their public health importance. Though chemical insecticides are widely used in their control, the development of resistance among the mosquitoes and the harmful effects of these chemicals upon the environment have caused concern among scientists and this in turn, has created a need to focus on easily degradable alternative insecticides. Most of the mosquito control programmers target the developmental larval stages from their breeding sites with larvicide due to the difficulty of controlling adults which is, always limited to a temporary control and reduction of the mosquito population. Phytochemicals obtained from plants with proven mosquito control potential can be used as an alternative to synthetic insecticides or along with other insecticides under the integrated vector control programmes. Extracts or essential oils from plants may be alternative sources of mosquito control agents as they constitute a rich source of bioactive compounds that are biodegradable into non-toxic products and potentially suitable for the use in control of mosquito larvae. Several workers (Ansari et al., 2005, Amer and Mehlhorn, 2006, Rahman et al., 2008, Rahman and Vekatesan, 2008, Elango et al., 2009) have reported larvicidal and repellent activity of the different extracts and essential oils from plants. In the present study we report larvicidal properties of the acetone extracts of *Anamirta cocculus* fruits and *Pogostemon paniculatus* leaves against the different larval instars of Culex pipiens. We also report the repellent activity of the essential oils extracted from the two selected plant materials against mosquitoes.

MATERIALS AND METHODS

Extraction of Bioactive materials:

The leaves of *Pogostemon paniculatus* (Wild) Benth. (Family: Lamiaceae) and fruits of *Anamirta cocculus* (Linn.) Wight and Arn (Family: Menispermaceae) selected for the present study are collected from in and around the campus of University of Calicut. Fresh leaves/fruits of selected plants were thoroughly washed with water and shade dried at room temperature. The dried material of the selected plant was powdered using a grinder and soxhlet extracted with analytical grade acetone at a temperature of 60˚C. The extraction is fixed for 20 cycles. The extract was then concentrated to dryness and yield is calculated. 1 percent stock solution of the extract was prepared and desired concentrations were made out of it to conduct bioassay tests.

Test Organism

The test organism selected for the present study, *Culex pipiens* Linn, belongs to the order Diptera and family Culicidae. *Cx.pipiens* larvae used for the present study were obtained from the laboratory culture maintained as described in Pushpalatha and Muthukrishnan (1995). The Larvae/Pupae of *Culex pipiens* collected from the field were brought to the laboratory and transferred to plastic trays containing 0.08 percent saline water. The pupae were collected in a small container and kept for emergence in mosquito emergence cage. The freshly emerged adults were maintained at room temperature. The adult females were given blood meal from an immobilized hen and the males were fed with 10 percent sucrose solution. Bowls with 0.08 percent water were provided in the cage to facilitate oviposition. The freshly laid eggs were collected from the cage and transferred in a trough and allowed to hatch. The freshly hatched larvae were fed by fine powder of dog biscuits and yeast in the ratio 3:1. Water was changed in every alternative day and feeding was continued till the larvae transferred into the pupae. The freshly moulted I, II, III and IV instar larvae were selected for the bioassay tests.

Bioassays

The acetone extract of selected plants were tested against I, II, III and IV instar larvae of *Cx. pipiens*. The appropriate volumes of 1 percent stock solution were diluted to 100 ml of 0.08 percent saline water in a disposable tumbler to obtain desired concentration of the test medium. 20 larvae were released to each tumbler containing test medium. A control with 100 ml of 0.08 percent saline water and a control with highest volume of acetone were also set. Triplicates were maintained with each set. Observations were taken after 24 hrs, 48 hrs and 72 hrs, and the mortality of the treated larvae were recorded. LC50 and LC90 were calculated using
a probit programme developed by Finney (1971).
Essential oils from the selected plants were extracted using a Clevenger apparatus. The protection percentage was calculated according to the standard procedure described by Ansari et al (1999) and calculated by using the following formula;

$$\text{Protection percentage} = \frac{C - T}{C} \times 100$$

Where **C** is the number of mosquito bite in control and **T** is the number of mosquito bite in treatment.

RESULTS

Table 1 provides data on percent yield of the plant materials selected for the present study. Percent yield of *A.cocculus* fruit is 13.89 where as that of *P. paniculatus* shows a low yield of 2.9%.

Table 1 : Data on percentage yield of the acetone extract of the selected plants for the present study

Name of Plant	Family	Material Selected	% yield
A.cocculus	Menispermaceae	fruit	13.89
P. paniculatus	Lamiaceae	leaves	2.895

Lethal concentration to kill fifty percent and ninety percent of the treated larvae (LC50 and LC90) of the selected plants were calculated for all the instars of *Cx. pipiens* and provided in table2 and 3. Table 2 presents data on 24 hr LC50 and LC90 (ppm) of *A. cocculus* and *P. paniculatus* concentration for I to IV instar larvae of *Cx. pipiens.* 24 hr LC50 of the acetone extract of the A.cocculus fruit ranged from 17.02 ppm to 86.02 ppm and 98.99 ppm to 141.53ppm for I and III instar respectively.
The range of data on 48 hr and 72 hr LC50 and LC90 (ppm) for the different instars of *Cx. pipiens* tested against the acetone extract of *A. cocculus* fruit and *P.paniculatus* leaves were provided in table 3. The 48 hr LC50 ranges from 11.89 ppm (I instar) to 52.29 ppm (IV instar) for *A. cocculus* and 59.22 ppm (IV instar) and 93.87 ppm (III instar) for *P.paniculatus* whereas 72 hr LC50 ppm of the different extracts tested were showed still lower ranges such as between 8.54 ppm and 34.28 ppm (I and IV instar) and 20.73 ppm to 41.55 ppm (II and III instar) for *A. cocculus* fruit and *P.paniculatus* leaves extracts respectively.
Data on percentage protection of the essential oils extracted from the selected plants from mosquito bite is calculated and provided in table 4. Essential oil of *A. cocculus* fruit has given a protection percentage of 42.8 over control where as essential oil of the leaves of *P.paniculatus* show a maximum protection of 45.45 % over control.

Table 2: 24 hr LC50 and LC90 (ppm) of acetone extract of *A. cocculus* and *P.paniculatus* against different instars of *Cx. pipiens.*

Plant	Instar	LC50 (ppm)	Fiducial limit		LC90 (ppm)	Fiducial limit	
			Lower	Upper		Lower	Upper
A. cocculus	I	17.025	12.68	22.36	39.97	32.21	54.68
	II	22.98	17.60	31.15	54.06	42.31	79.22
	III	86.02	75.08	98.41	180.6	157.40	217.68
	IV	83.59	72.73	94.82	156.25	137.97	185.55
P.paniculatus	I	98.99	90.08	109.66	196.79	176.14	226.12
	II	114.11	103.66	125.57	238.25	214.58	271.76
	III	141.53	125.12	163.54	330.12	281.10	412.55
	IV	111.61	102.07	121.94	223.49	203.13	251.49

Table 3: Data on 48 hr and 72 hr LC50 and LC90 (ppm) of acetone extract of *A. cocculus* and *P. paniculatus* against the different instars of Cx. pipiens.

Plant	Instar	LC50 (ppm)	Fiducial limit		LC90 (ppm)	Fiducial limit	
			Lower	Upper		Lower	Upper
48 hr							
A. cocculus	I	11.89	9.206	14.81	29.91	25.32	37.05
	II	20.47	15.06	28.26	51.40	39.85	76.97
	III	50.941	36.77	71.68	129.59	99.45	198.46
	IV	52.29	42.24	65.37	130.64	107.72	170.47
P.paniculatus	I	72.00	57.48	88.08	157.49	131.41	206.43
	II	62.22	40.76	82.71	158.18	125.36	236.04
	III	93.87	83.14	107.65	219.41	190.55	264.09
	IV	59.22	52.07	67.04	138.36	121.34	164.56
72 hr							
A. cocculus	I	8.54	22.81	27.91	92.01	108.7	66.98
	II	16.74	23.02	8.48	89.67	111.43	76.4
	III	23.92	40.29	4.31	100.84	232.9	71.02
	IV	34.28	47.12	22.28	91.15	144.24	70.46
P.paniculatus	I	33.17	22.69	41.45	122.42	105.85	149.19
	II	20.733	13.04	36.47	127.67	108.37	161.02
	III	41.55	34.16	48.19	114.28	101.39	133.28
	IV	39.29	13.75	56.73	111.98	85.76	190.14

Table 4: Data on repellency test conducted using essential oils of the selected plants

Plant	Day	No. of mosquito bite		% Protection
		Control	Treated	
A. cocculus	1	14	8	42.80
	2	21	13	36.08
	3	20	12	40.00
P. paniculatus	1	11	6	45.45
	2	17	11	35.29
	3	12	7	41.66

DISCUSSION

Vector control is facing a threat due to emergence of resistance in vector mosquitoes to conventional synthetic insecticides. Botanical insecticides may serve as a suitable alternative to synthetic insecticides in future as they are relatively safe and biodegradable. In the present study, it was found that 72 hr LC50 of *Anamirta cocculus* (fruit) extract for the 1st instar larvae is higher than that of other concentrations tested on various instars of *Culex pipiens*.

By comparing the present results with other similar studies shows that acetone extract of *Anamirta cocculus* shows LC50 (ppm) higher activity than *Momordica charantia* (179.66) reported by Lata Batabyal *et al.,* 2007; *Euphorbia hirta* (272.36) reported by Abdul Rahman *et al.,* 2008a and *Argemone mexicana* (313.2) reported by Preeti Sharma *et al*., 2006. The larvicidal activity of the tested extracts increases with increasing time of exposure. However, total protein content of the different instars of the larvae of *Culex pipiens* estimated shows no significant differences between the control and treated larvae of *Cx. pipiens*.

Essential oil extracted from the selected plant shows effective personal protection against mosquitoes and the protection percentage ranges from 36.08 to 42.80 percent and from 35.29 to 45.45 percent for *A. cocculus* and *P. paniculatus* extracts respectively.

The findings of the present study suggests that further purification and isolation of the active principle compounds could lead development of more potent and eco-friendly bio control agents for *Cx. pipiens* and other related mosquito vectors.

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