Studies on Phytochemical Monitoring and Mosquito Larvicidal activity of *Anamirta cocculus* (L.) Wight and Arn

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***Abstract*: Excessive usage of synthetic insecticides has led to the development of insecticide resistance in mosquitoes and it can be reduced to an extent by the use of botanical insecticides. The pharmacological and insecticidal properties of plants have been studied and identified all around the world. The present study aims to find out the larvicidal activities of *Anamirta cocculus* (L.) Wight and Arn against *Culex quinqueafsciatus* Say and *Aedes albopictus* (Skuse), and to isolate the most active compounds. Acetone and Methanol extracts of the *A. cocculus* seeds collected from Calicut University campus were taken using the soxhlet apparatus. Methanol extract is then defatted with equal volume of analytical grade petroleum ether to get defatted methanol extract. Defatted methanol fraction was then subjected to fractionation by using Ethyl acetate and double distilled water. Bioassays were conducted using all these extracts following the protocol of WHO and LC_{50} were calculated Different concentrations of EA fraction were set and the first instar larvae exposed in to it to find out any effect on larval duration. The Phytochemical screening was also done to find out the constituents in seed extract. *A. cocculus* treated larvae exhibited more restlessness, convulsions and sluggishness, and the restless activity is more in *Ae. albopictus* than in *Cx. quinquefasciatus*. *A. cocculus* acetone extracts shown more activity than methanol and defatted methanol extracts. The activity was higher in *Ae. albopictus* as compared with *Cx. quinquefasciatus.* The larvicidal activity is seen increasing with the concentration and time of exposure in every case. There is no prominent change in the duration of larval life span of *A. cocculus* and control population. From the results it has been proved that the plant is having high larvicidal activity. Further purification and experiments are going on to prove the efficacy of the active compounds in the field.**

***Key words: Aedes albopictus, Anamirta cocculus, Culex quinquefasciatus*, larvicidal activity.**

I. INTRODUCTION

The pharmacological and insecticidal properties of herbs have been well established by researchers all around the world, especially in India where the climate is highly conducive for such pesticidal plants. Utilization of plant derived products in the management of insect pests/vectors are not a new concept but renewed recent past due to the development of wide resistance of pests against chemical/synthetic insecticides. The ill effects of synthetic insecticides could be reduced to an extent by the use of botanical insecticides. Plants are the reservoirs of complex phyto-chemicals like alkaloids, flavonoid, saponins, and tannins, which are known to possess medicinal and pesticide properties (Azmathullah *et al*., 2011 and Daniel *et al*., 2013).

The plant-derived metabolites, such as saponine, steroids, isoflavonoids, essential oils, alkaloids and tannins were proved as potent mosquito larvicidal compounds by many researchers in their earlier studies. In addition to natural secondary metabolites, synthetic derivatives also provide alternative source for the management of mosquitoes (Shivakumar *et al.,* 2013). Bioactive organic compounds exerted by plants have a complex mode of action such as growth and oviposition inhibitory, repellent activity, toxic and deterence (Ezeonu *et al.,* 2001; Carlini and Grossi-de-Sa, 2002).

In this research, *Anamirta cocculus* was tested for its efficacy against *Aedes albopictus* Skuse and *Culex quinquefasciatus. Anamirta cocculus* (L.)Wight and Arn.is large woody climbing shrub with vertically furrowed ash colored bark and glabrous young parts. Leaves large, simple, alternate, long petiole, petioles thickened at the base and apex broadly ovate, subcoriaceous, cordate or truncate at the base, tufts of hairs in the axils of the nerves except the basal ones, flowers greenish in long panicles, drooping from the nodes of the old wood, fruits druped kidney shaped turning red on ripening.

II. MATERIALS AND METHODS

Test organism: The mosquito species *Aedes albopictus* Skuse and *Culex quinquefasciatus* Say were used for the study.

Plant used in the Study: *Anamirta cocculus* (L.) Wight and Arn. (Family: Menispermaceae).

Preparation of plant extract: Fresh seeds of *A. cocculus* collected from Calicut University campus were washed thoroughly with distilled water and shade dried ~~in~~ under the room temperature. Then the dried seeds were powdered using a mixer grinder and sifted through a fine mesh of sieve (size: 1). The powder obtained were packed in airtight Ziplog bags (half a kg capacity) and stored at -20ºC. By operating Soxhlet apparatus, extracts were acquired using analytical grade methanol and acetone. The filtrate was taken and concentrated in a rotary vacuum evaporator and transferred in to a pre weighed petridish. The yield of the material was calculated from the dried extract. Stock solutions of 10% was prepared and stored in airtight amber coloured glass containers and kept it in a refrigerator.

Defattation of methanol extracts: Methanol extract is then defatted with equal volume of analytical grade petroleum ether (60 to 80 ^{0}C) in a separating funnel. The distinct layers were separated slowly and collected in to conical flask. After collection, the solution is placed in to pre-weighed petri-plate and allowed to dry. From the dried extract, 1% stock solution was prepared and refrigerated in airtight amber coloured bottle.

Partial purification with Ethyl acetate: Defatted methanol fraction was then subjected to fractionation by using Ethyl acetate and double distilled water.

Bioassay: Conducted using the protocol of WHO (1981). LC_{50} were calculated using a Probit programme developed by Finney (1971).

Effect on larval duration: Different concentrations of EA fraction of the selected plant extracts were set and exposed the first instar larvae in to it to find out any effect on larval duration. Observations were made every day to check moulting into the next stage and/or the death or emergence of the treated larvae.

Phytochemical analysis: Qualitative analysis was done to identify the presence of different secondary metabolites in the leaf extracts of *C. hirtus* according to the methods proposed by Trease and Evans.

III. RESULTS

The percentage yield of acetone and methanol extracts of *A. cocculus* is provided in table 1. The yields of the methanol and acetone extracts of *A. cocculus* are 28 and 30% respectively. The methanol extract appeared in light brown colour, that with acetone was yellowish brown, and both presented a waxy solid appearance. When the extract dissolved in water, it gives a light milky appearance to the water.

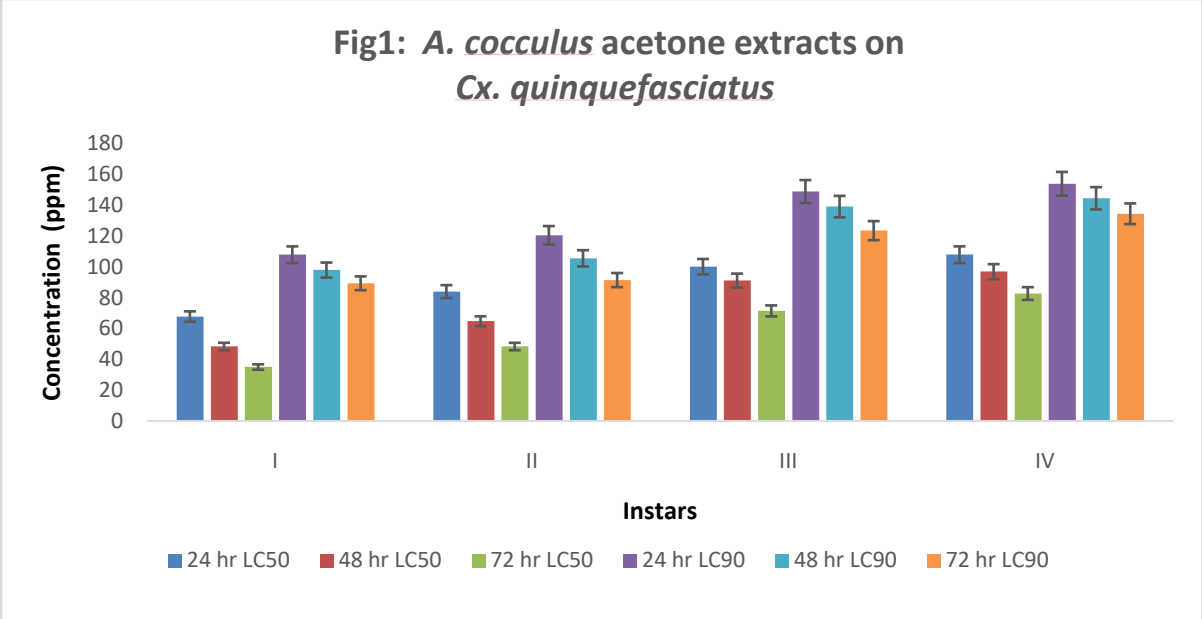
Table 1: Physico-chemical properties of *A. cocculus* seed extracts

Purticulars	Methanol Extract	Acetone Extract
Yield (%)	28	30
Colour	Light brown	Yellowish brown
Consistency	Waxy Solid	Waxy Solid

Critical lethal concentrations:

The data on 24 hr, 48 hr and 72 hr LC_{50} and LC_{90} (ppm) values of the acetone extract of *A. cocculus* seed tested against *Cx. quinquefasciatus* are provided in figure:1.

The values of 24 h LC_{50} for the different instars of the larvae *Cx. quinquefasciatus* ranged from 67.77 ppm to 107.86ppm for all the four larval stages. The 48 and 72 h LC_{50} values of the acetone extract of the *A. cocculus* ranged from 48.36 to 96.89 ppm and 35.06 to 82.7 ppm for all the four larval stages. The LC_{90} values for I, II, III & IV instar larvae were 107.86, 120.41, 148.80, 153.84 ppm for 24 hrs, 97.89, 105.51,139.02,144.43 ppm for 48 hr and 89.33, 91.42, 123.45 and 134.4 ppm for at 72 h respectively.



The data on LC_{50} (ppm) and LC_{90} (ppm) values of acetone extracts of *A.cocculus* tested against *Ae. albopictus* are described in Figure: 2. The 24 h LC_{50} (ppm) values of acetone extracts of *A. cocculus* were 64.71, 81.13, 96.89, 105.57 ppm and LC_{90} (ppm) values were 105.57, 118.48, 139.02, 144.43 ppm for I to IV instar larvae respectively. The 48 h LC_{50} (ppm) and LC_{90} (ppm) values were 44.12, 59.41, 83.95, 94.79 ppm and 96.89, 107.21, 134.41, 139.02 ppm and for 72 h LC_{50} (ppm) and LC_{90} (ppm) values were 31.93, 40.94, 54.51, 74.53 ppm and 87.3, 82.72, 106.86, and 130.36 ppm respectively for the I, II, III and IV instar larvae of *Ae. albopictus.*

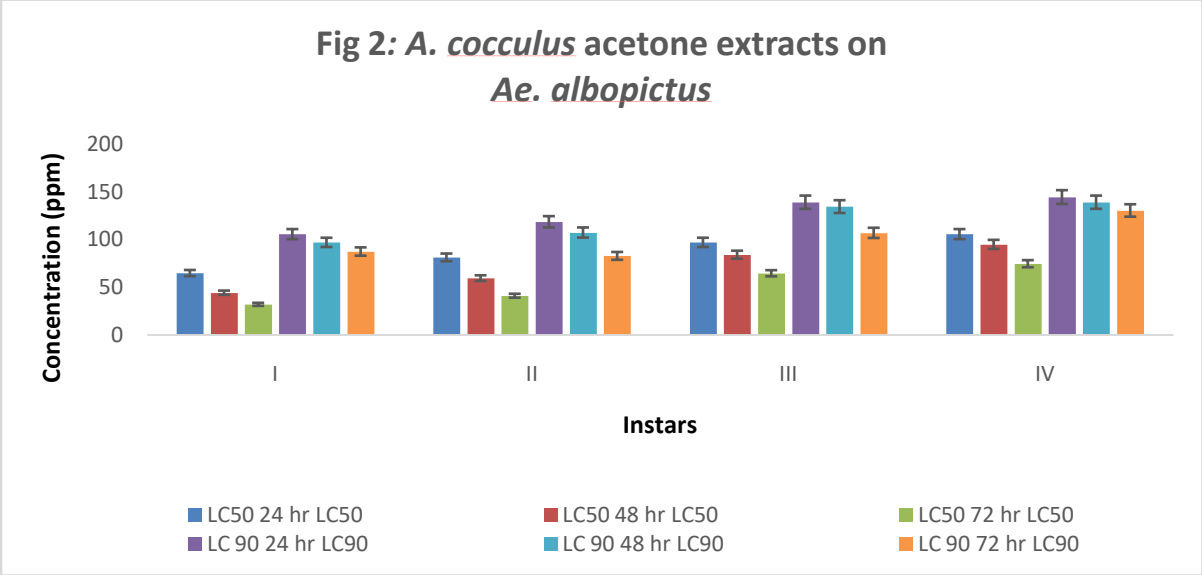


Figure 3, provide the data on LC_{50} (ppm) and LC_{90} (ppm) values of methanol extracts of *A. cocculus* against *Cx. quinquefasciatus.* The LC_{50} (LC_{90}) values for I, II, III & IV instars larvae for 24 hrs were 83.95 (172.64), 96.89 (184.36), 126.37 (197.56) and 132.34 (202.54) and that of 48 and 72hrs were 76.37 (158.84), 87.43 (178.42), 107.86 (173.91), 116.64 (188.44) and 64.71 (136.46), 78.07 (142.54), 91.41 (144.43), 96.89 (159.51) respectively.

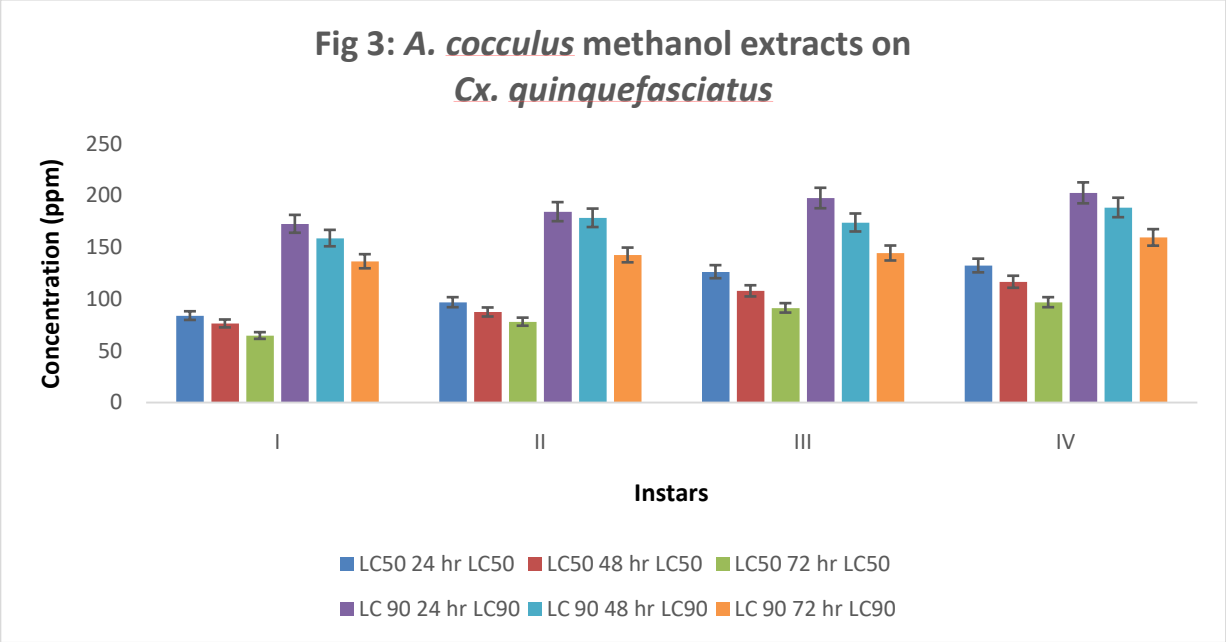
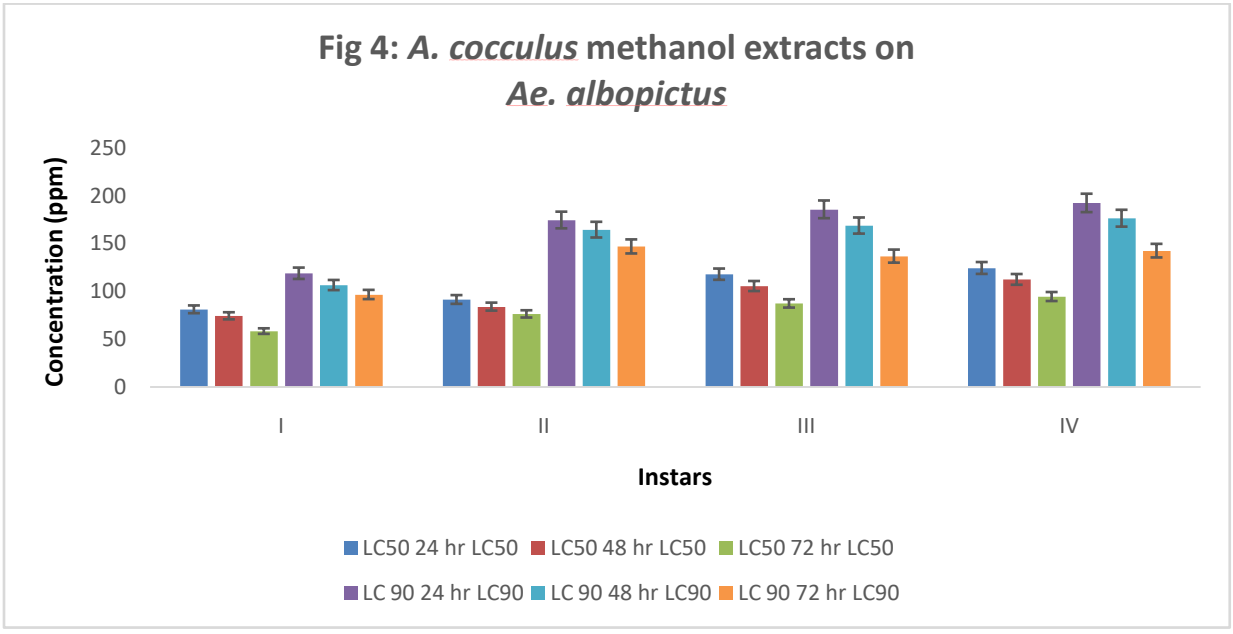


Figure 4, exhibit the data on LC_{50} (ppm) and LC_{90} (ppm) values of methanol extracts of *A. cocculus* tested against different instars of *Ae. albopictus*. The 24 h LC_{50} (ppm) values for I, II, III and IV instars larvae were 81.13, 91.42, 117.93, 124.42 ppm respectively. The 48 and 72 h LC_{50} values were 74.33, 83.94, 105.51, 112.46 ppm and 58.33, 76.33, 87.3, 94.52 ppm for the I to IV instar larvae *Ae. albopictus* respectively. The 24 hr LC_{90} values of I, II, III & IV instar larvae were 118.88, 174.65, 185.82, 192.54ppm and that of 48 hrs were 106.54, 164.54, 168.84, 176.54 ppm and for 72 h were 96.64, 146.98, 136.84 and 142.56ppm respectively.



The data on 24 h, 48 hr and 72 h LC_{50} (ppm) and LC_{90} (ppm) of the defatted methanol fraction of *A. cocculus* tested against different instars of C*x. quinquefasciatus* are presented in figure 5. The 24 h LC_{50} on I, II, III and IV instar larvae of *Cx. quinquefasciatus* were 81.11, 87.33, 102.8 and 112.48 ppm and LC_{90} values were 118.86, 120.24, 132.64 and 144.64 ppm respectively. The 48 and 72 h LC_{50} (LC_{90}) values were 78.65 (106.64), 81.13 (118.42), 91.02(126.42), 105.54 (126.66) ppm (table 16) and 64.95 (98.86), 77.88 (106.66), 86.15(118.98), 98.84 (132.24) ppm for the different instars.

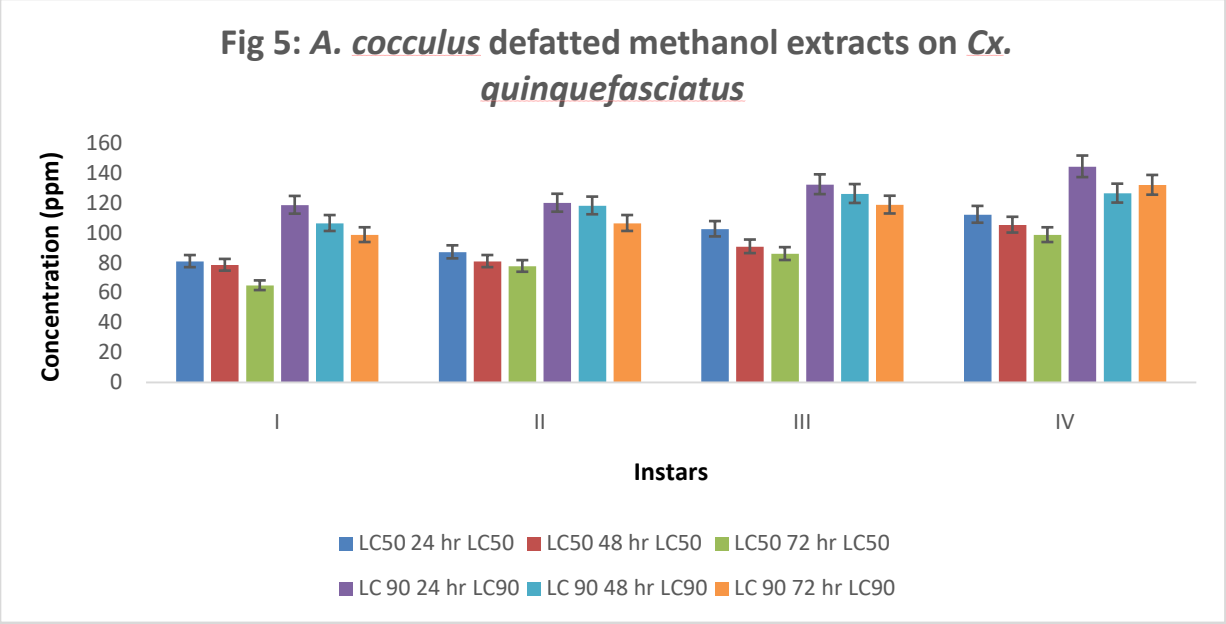
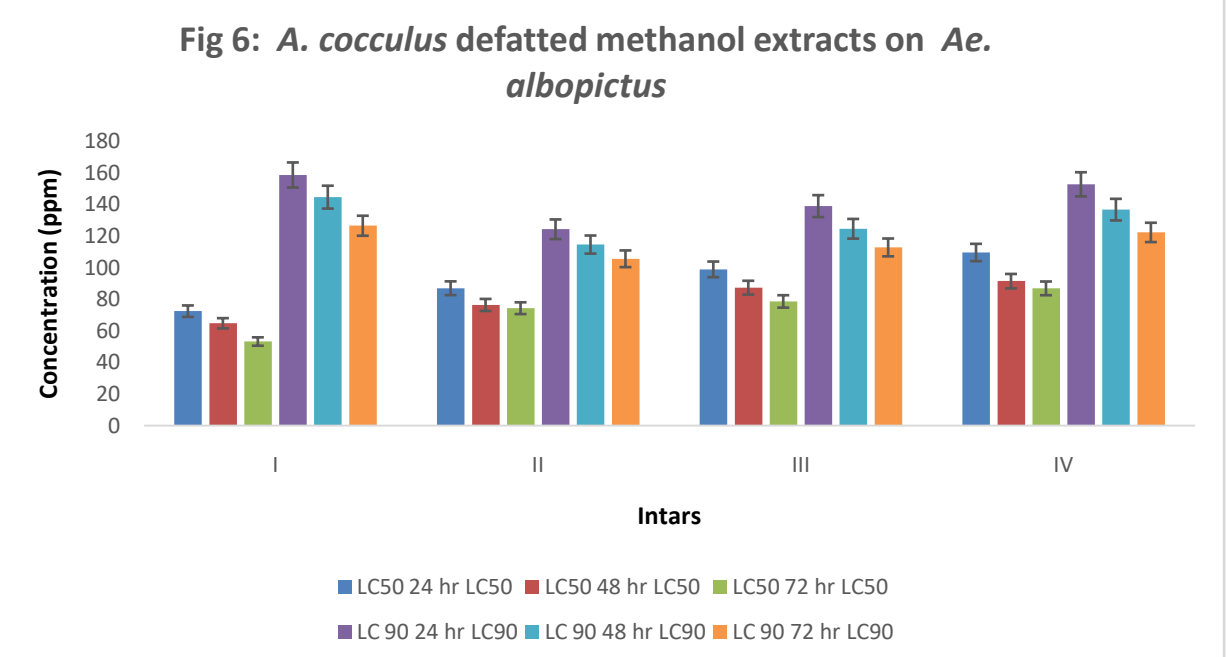


Figure 6, reveal the data on LC_{50} and LC_{90} (ppm) values of defatted methanol fraction of *A. cocculus* tested against different instars of *Ae. albopictus*. The LC_{50} value for I, II, III & IV instars larvae for 24 h were 72.46, 86.95, 98.88 and 109.54 ppm respectively. The 48 and 72 h LC_{50} values were 64.79, 76.37, 87.3, 91.42 ppm and 53.36, 74.34, 78.61, 86.88 ppm for I, II, III and IV instars of *Ae. albopictus* respectively. The LC_{90} values for I, II, III & IV instar larvae at 24 h were 158.54, 124.24, 138.86, 152.65 and that of 48 hrs 144.54, 114.56, 124.54, 136.64 ppm. The LC_{90} for 72 h are 126.46, 105.57, 112.76, 122.24 ppm respectively.



The table 2 presents data on extension of larval duration of *Cx. quinquefasciatus* and *Ae. albopictus* when treated with ethyl acetate fraction of *A. cocculus.* There is no too much change in larval duration while treating with EA fraction of *A. cocculus.*

Table 2: Extension of larval duration after treatment with Ethyl acetate fraction of *A. cocculus* commenced with I instar larvae of *Cx. quinquefasciatus* and *Ae. albopictus*

Mosquito Species	Concent-ration (ppm)	Duration in Days (number of larvae alive)				Total no of days
		I	-	III	IV	
Cx. quinquefasciatus	25	5(6)	4(4)	3(3)	4(2)	16±2
	50	5(4)	4(4)	4(3)	3(1)	15±1
	75	4(5)	4(4)	3(3)	3(2)	14 ±1
	100	4(4)	3(3)	4(3)	3(1)	14±1
Ae. albopictus	25	5(9)	4(8)	4(4)	3(2)	15±1
	50	4 (9)	3 (7)	3(4)	4(2)	14±1
	75	3(7)	3(5)	3(3)	4(2)	13±1
	100	4 (5)	4 (5)	3(4)	3(2)	14±1
Control	-	3(30)	4(30)	3(30)	2(30)	12 ±1

Table 3 provides the data on phytochemicals present in the methnol and acetone extracts of *A.cocculus*. Crabohydrates, sugar, alkaloids, phenolins and oils were present in the acetone extract and sugars, alkaloids, saponins, phenolins and oils were present in the methanol extract.

Table 3: Phyto-chemicals present in the methanol and acetone fractions of *A. cocculus*

Sl.No:	Phytochemicals	Acetone Extract	Methanol Extract
1.	Carbohydrates	+	-
2.	Sugars	+	+
3.	Alkaloids	+	+
4.	Saponins	-	+
5.	Phenolins	+	+
6.	Oils	+	+
7.	Aldehydes	-	-

IV. DISCUSSION

The *A. cocculus* treated larvae exhibited more restlessness, convulsions and sluggishness and the restless activity is more in *Ae. albopictus* than in *Cx. quinquefasciatus*. The sluggish and peculiar coiling movement in treated larvae might be due to the neuronal or muscular disturbances caused by active ingredients released by the extracts in to the water. After exposure to *A. cocculus* extracts, the larvae showed abnormal motions, tremors and convulsions followed by paralysis and finally settle at the bottom of the container. The result is in corroboration with the reports of Sagar and Seghal (1997) against *Cx. quinquefasciatus.*

A. cocculus acetone extracts shows more activity than methanol and defatted methanol extracts. In addition, its activity was higher in *Ae. albopictus* as compared with *Cx. quinquefasciatus.* The larvicidal activity is seen increasing with the concentration and time of exposure in every case. The 72 h LC_{50} value is less as compared with the 24 h LC_{50} value and in every treatment both extracts showed more activity towards the 1st instar larvae. In both cases the acetone extracts showed more activity than methanol and defatted methanol extracts. In all the cases the control did not show any mortality. As the concentration and time of exposure increases the larvicidal effect also increases. There is no much change in the duration of larval life span of *A. cocculus* and control population. The moratlity rate is high in the case of *A. cocculus* treated population.

Both extracts contain alkaloids and phenols. Presence of alkaloids and phenols present in plant extracts are potent and are reported to cause mortality even in ticks. (Kumar *et al*., 2011). So the high mortality rate using the *An.cocculus* seed may be due to the presence of alkaloids and phenols. The presence of oils gives the extract an oily nature. In the present investigation, the overall results from the phytochemical screening of the seed extracts of *A. cocculus* revealed the potentials for anti-larvicidal activity.

The present observation has involved the promising larvicidal property of *A. cocculus* and therefore the study devising active bioinsecticide from *A. cocculus* to control mosquitoes. This study is significant in view of the reports that a large quantity of the chemical insecticides reaches non target species creating environmental problems, decrease biodiversity and soil quality deplete the habitat for birds and can threaten

endangered species (Miller, 2004 and Upadhay, 2014). The larval mortality caused by the *A. cocculus* seed extracts points to the significant relationship between percentage of mortality and concentration of the extracts. The increased concentration of the extracts might facilitate penetration of active moieties into the body of larvae and thereby causing an increase in mortality at higher concentration.

Therefore Further purification and field performance of the active compounds to be needed to recommend *A. cocculus* as an anti-mosquito product to combat and protect from mosquitoes in a control program.

ACKNOWLEDGEMENT

UGC BSR for providing financial support for carry out this project.

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