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RESEARCH ARTICLE

LATENT EFFECT OF CLEOMEDROSERIFOLIA ETHANOLEXTRACT ON THE DEVELOPMENT OFOCHLEROTATUSCASPIUS AND CULEXPIPIENS

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ABSTRACT

Mosquitoes are the most important vectors for serious human and animal diseases causing millions of deaths every year. Among these diseases, malaria, yellow fever, dengue and dengue hemorrhagic fever, filariasis and Rift Valley fever at endemic and epidemic areas in many countries. The ethanolic extract of *Cleomedroserifolia* was evaluated against *Culexpipiens* and *Ochlerotatuscaspius* LC_{50} were 178.61 & 162.63 respectively. The pupation percent was significantly reduces as a result of extract application *Culexpipiens*. The adult emergence was also reduced in *Ochlerotatuscaspius* more than *Culexpipiens*. The gonotrophic cycle was elongated to 8.53 and 7 for *Ochlerotatuscaspius* and *Culexpipiens* respectively. The fecundity and fertility was significantly reduced. *Cleomedroserifolia* extract can be used as larvicide and insect growth regulator agent in the controlling program for mosquitoes.

INTRODUCTION

Mosquitoes are the most important vectors for serious human and animal diseases causing millions of deaths every year. Among these diseases, malaria, yellow fever, dengue and dengue hemorrhagic fever, filariasis and Rift Valley fever at endemic and epidemic areas in many countries.The transmission of diseases can be interrupted by controlling the vectors by different ways (WHO, 1991, Lerdthusnee, *et al.,* 1995 and Madani, *et al.,* 2003). The continuous using of traditional chemical insecticides leads to adverse effects such as developing of resistance, affect the public healthand reducing beneficial non-target biota. Retardation of these effects forced authors to search for new products that are environmentally safe (Abbassy, 1998; Mohamed *et al.,* 2003, Kamel *et al.,* 2005 and Bakr *et al.,* 2008). *Cleome* species leaf extract was evaluated against *Callosobruchusmaculatus* show complete inhibition of embryonic development of eggs exposed for 48h in all botanical treatments (Dabire, 2008). Different fraction extracted from *Thymus vulgaris* were tested for mosquitocidal activity against *Culexquinquefasciatus.*The oils showed highly efficiency with respect to mortality and to the percentage of adult emergence upon short-term exposure in water treated by lethal doses of individual oils. Furthermore, mortality increased significantly in relation to time of exposure, and total mortality of the larvae at the end of their development was about 90%. Such significant mortality was also naturally reflected in the total emergence of adultsPavela (2009).

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The authors do a continuous effort to improve the potency of plant extract as insecticide and using them alternate the traditional insecticides in IVM program.

MATERIALS AND METHODS

Preparation of plant extract

The tested plant (*Cleomedroserifolia*)was washed toavoid dusts and dirt then lift to dry undershade in the laboratory. Dried part of plant was cut into small pieces and ground in anelectric grinder. Hundred grams of theresulting powdered was exhaustively extracted with ethanolabsolute, following the method described by Kamel *et al.,* (2005).

Tested insects

*Culexpipiens*and *Ochlerotatuscaspius* (Culicidae*:* Diptera). Both species wereprovided from Medical Entomology Institute and transferred to the laboratory of Entomology Department – Faculty of Science – Ain Shams University where self-perpetuating colonies were established and maintained during the present study. The maintaining of colonies in laboratory was proceeded according to methods described by (Kamel *et al.* 2005 and Shaalan, *et al*. 2005).

Larvicidal bioassay

The bioassay experiments were carried out according to (Mallick, et al. 2015).The investigation effect of sub-lethal concentration on the pupation, adult emergence and reproductive potential of both studied species were carried out as follow:

The 3rd instar larvae (500 larvae) were exposed to sub lethal dose of *Cleomedroserifolia* extract and Esbiothrin. Survived larvae after twenty-four hours post treatment were gently washed and transferred to labeled pans. Treated larvae and controls were kept under laboratory conditions of 27±2 °C and 75±5 % RH and a photoperiod of 16-8 hrs (L-D). Pupae were collected and placed in breeding cages. Emerged adults were countedand provided with cotton pads soaked with sugar solution (10%). After 2 days provide blood meal to *Culex* sp. Then each female was transferred individually into propylene tubes (4cm in diameter and 7cm depth) one third of which was filled with water and covered with muslin. Adults were observed daily for egg laying. Calculate the duration of the gonotrophic cycle, the females were observed daily for oviposition. The number of eggs laid by each female was counted to calculate the fecundity. The female fertility was calculated as the mean number of hatched eggs / the oviposited eggs per female.

Statistical analysis

Calculate the LC_{50} and LC_{95} values through Log Probit analysis (Finney, 1971). Use Microsoft excel program (Mean ± SD & Z-test).

RESULTS

Ethanolic extract of *Cleomedroserifolia* showed larval mortality against both species used in present study. The LC_{50} and LC_{95} for *Culexpipiens* and *Ochlerotatuscaspius*were represented in table (1) and regression line in (Fig. 1). The result showed that the mortality increased with increasing the concentration of extract. *Ochlerotatuscaspius* is more susceptible to the extract than *Culexpipiens* larvae according to their LC_{50} 162.63 and 178.61 ppm respectively.

Table 1. Larval mortality and LC_{50} & LC_{95} values for the *Cluexpipiens* and *Ochlerotatuscaspius* larvae exposed to*Cleome dorserifolia* ethanolic extract

Mosquito species	Conc. ppm	Mortality %	LC50 (Coefficient limit)	LC95 (Coefficient limit)	Slope
Culexpipiens	80	13.3	178.61	666.13	2.9
	140	41.7	(157.56 – 202.45)	(485.53 – 915.17)	
	200	58.3			
	300	70			
	400	85			
Ochlerotatuscaspius	80	16.7	162.63	540.87	3.2
	140	43.3	(144.28 – 183.29)	(415.73 – 704.42)	
	200	61.7			
	300	73.3			
	400	93.3			

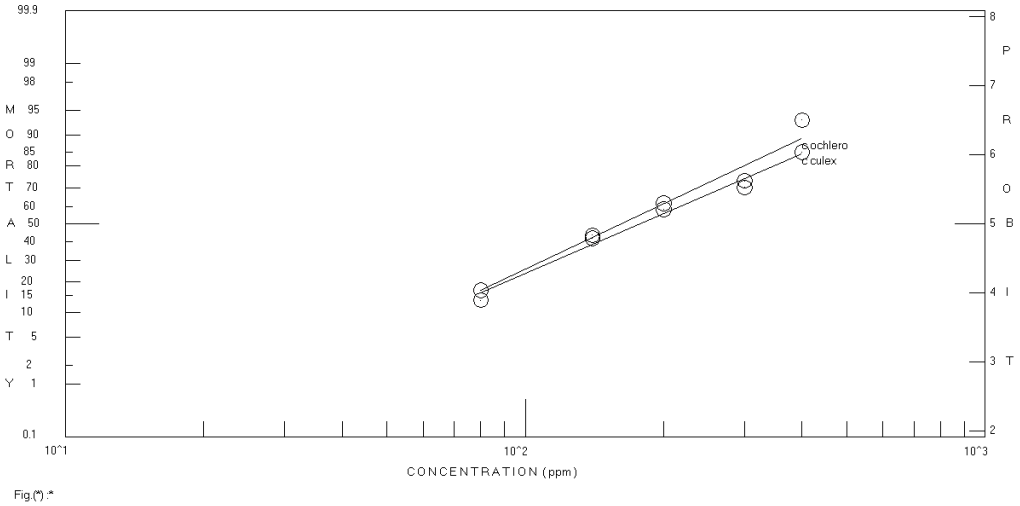

Fig. 1. Susceptibility of *Culexpipiens* and *Ochlerotatuscaspius* larvae to ethanolic extract of *Cleome dorserifolia*

Table 2. Efficiency of *Cleomedorserifolia* on some biological activities of autogenous *Ochlerotatuscaspius* and *Culexpipiens*

Contribution	Control	*Ochlerotatuscaspius*	Control	*Culexpipiens*
Survived larvae % (n)	500	75 (375)	500	75.4 (377)
Pupation % (n)	95.2 (476)	84.27 (316) * 2.96	97 (485)	51.7 (195) *1.00
Emergence % (n)	98.7 (470)	49.37 (156) * 10.21	99.2 (481)	73.8 (144) *1.00
Gonotrophic cycle	3.47±0.52+	$8.53 \pm 0.52^{+}$	3.00 ± .000+	$7 \pm 0.89^{+}$
Fecundity	97.533 ±3.98+	$30.40 \pm 1.92^{+}$	100.80 ± 4.296+	$54.1 \pm 5.68^{+}$
Hatchability (%)	85.40 ± 5.01+	$6.53 \pm 1.19^{+}$ (21.48) *15.31	89.33 ± 5.354+	$17.5 \pm 2.73^{+}$ (32.3) * 1.00

The sub-lethal concentration of ethanolic extract of *Cleomedorserifolia* affect the life cycle of both species from mosquitoes. The results represented in Table (2) showed different variation in life cycle during pupation period and adult emergence also gonotrophic cycle, fecundity and hatchability. There was significantly reduction in pupation for *Culexpipiens* but in *Ochlerotatuscaspius* the reduction during adult emergence. In both species fecundity and hatchability were significantly reduction. Also, the gonotrophic cycle was elongate comparing with the control of both species (autogenus and anatogenus, *Ochlerotatuscaspius* and *Culexpipiens*respectively.

DISCUSSION

Mosquitoes are still the world wide vector of human and animal diseases, and are conspicuous nuisance pests as well, even after massive efforts of control. The extensive uses of chemical insecticides have induced resistance by insect pests, caused contamination of human food, mammalian toxicity, reducing beneficial non-target biota and environmental pollution. Active substances extracted from plants are used as insect repellents or synergists (Thangam and Kathiresan, 1997; El-Gougary, 1998 and Mansour *et al.,* 2000), or effective as insect growth regulator and adulticide (Beehler and Mulla, 1993; Mc Carry, 1996, Kassem *et al.,* 2000 and Nathan *et al.* 2006). Various parts of the plants have been reported by researchers which give insecticidal activity against different species of mosquitoes (Hossain *et al.,* 2011; Singha and Chandra, 2011; Adhikari *et al.,* 2012; Mallick *et al.,* 2014; Singha Ray *et al.,* 2014 and Singha *et al.,* 2015).

The tested extract revealed differences in LC_{50}, LC_{95} values and the slope functions of the regression lines. The *Ochlerotatuscaspius* is more susceptible to the extract than *Culexpipien*. This result may be attributed to the reaction of each species toward the same plant extract.Mallick *et al.,* 2015 reported that different species of mosquitoes appear different degree of susceptibility toward the ethanolic leaf extract of *Annonareticulate*. The sub-lethal concentration of ethanolic extract of *Cleomedroserifolia* showed significant reduction in some biological activities of both species of mosquitoes. The treatment with extract revealed significantly reduces in pupation percent for *Culexpipiens* may be attributed to the high tolerance of *Ochlerotatuscaspius* than *Culexpipiens* during pupal stage. Reduction in the adult emergence of different mosquito species treated with different plant extracts and pyrethroids was previously recorded by several investigators (Pereira and Gurudutt, 1990; Mohsen *et al.,* 1990; Awala *et al.,* 1998; Pushpalatha and Muthukrishnan, 1999; Attiaa, 2002; EL-Bokl, 2003 and Abd El-Baky*et al*, 2005). Also, Shaalan *et al.,* 2005 reported that the duration of developmental stages of *Aedesaegypti* were prolonged when treated with synthetic and botanical insecticides.

In present study, the adult emergence was affected also by treatment and gonotrophic cycle was elongated. The length of the gonotrophic cycle is of crucial importance in determining the vectorial capacity of the mosquitoes in disease transmission. However, the effect of larvicidal treatment on the avidity of the emerged females to acquire blood meals needed to be investigated. Marcard*et al.,* (1986) had indicated that the females of *Ae. aegypti*, *Ae. togoi* and *Cx. quinquefasciatus* developed from treated larvae with methanol extracts of *Ajuga* spp. took smaller blood-meals than those developing from untreated ones.

The reduction of fecundity may be due to lack of proteins required for the formation of yolk granules, small blood meal intake or inhibition of protein synthesis due to the treatment may lead to the reduction the number of deposited eggs. and fertility were significantly reducesmay correlate either from deficiency in the egg protein or the reduction of the sperms viability and the probable presence of sperm abnormalities. These reasons may affect the embryonic development which may lead to reduction of egg hatchability.. All these disorder may correlate with some biochemicalchanges in the tested species which may lead to certain functional and physiological interactions (Teller, 1965; El-Bokl *et al.*,1998; Mohamed and Hafez, 2000; Massoud *et al.,* 2001; Abd El-Baky *et al*, 2005; Helmy *et al*. 2010 and Bakr *et al.* 2010).

Conclusion

From the present study the author can concluded that, the *Cleomedroserifolia*exthanolic extract can be used as larvicide or insect growth regulatorfor mosquito species.

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