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Larvicidal Activity of Selected Xerophytic Plants Against *Culex pipiens* and *Aedes caspius* (Diptera: Culicidae)

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Abstract.- Methanol extracts of different plants namely, *Trichodesma africanum* (Boraginaceae), *Cleome rupicola* (Capparceae) and *Ochradenus baccatus* (Resedaceae), were tested for larvicidal activity against 4th instar larvae of *Aedes caspius* and *Culex pipiens* mosquitoes. All plant extracts tested against *Ae. caspius* showed 100% mortality at 10µg/ml except the stem of *O. baccatus* which showed 90% mortality. However, most of the plant extracts tested against *Cx. pipiens* showed more than 50% mortality at 10µg/ml. *Ae. caspius* reported lower LD_{50} than *Cx. pipiens*. The LD_{50} of the extracts tested ranged between 5.3-0.99. The lowest LD_{50} calculated against *Ae. caspius* was 1.2±0.06 and 0.99±0.16 µg/ml for the stem of *T. africanum and C. rupicola*, respectively. In conclusion, we have documented promising larvicidal potential of xerophytic plants, which could be considered as a potentially alternative source for developing novel larvicides to be used in controlling vectors of mosquito-borne diseases.

Key words: Plant extract, larvicidal activity, *Culex pipiens*, *Aedes caspius*.

INTRODUCTION

Mosquitoes are responsible for the spread of more diseases than any other group of arthropods. Mosquito-borne diseases still remain a major health problem in both human and veterinary sectors. Diseases transmitted by mosquitoes include malaria, dengue hemorrhagic fever, Japanese encephalitis, yellow fever and filariasis (Hotez *et al*., 2004).

In Saudi Arabia, the most common mosquito-borne diseases include dengue (Fakeeh and Zaki, 2001, 2003; Ayyub *et al*., 2006; Khan *et al*., 2008), filarial (Hawking, 1973), malaria (Warrel, 1993; Abdoon, 2004), and Rift valley fever (Jupp *et al*., 2002; Miller *et al*., 2002; Balkhy and Memish, 2003; Al-Hazmi *et al*., 2003; Madani *et al*., 2003). *Ae. caspius* was the most abundant mosquito followed by *Cx. pipiens* in AL-Ahsaa, Saudi Arabia (Ahmed *et al*., 2011). *Ae. caspius* is widely distributed in different regions of Saudi Arabia such as Riyadh district (Al-Khreji, 2005), as well as in the eastern (Mattingly and Knight, 1956; Büttiker, 1981) and southwestern regions (Abdullah and Merdan, 1995). Omar (1996) reported that local *Cx. pipiens* mosquitoes might act as potential vector of introduced Bancroftian filariasis in Saudi Arabia.

The synthetic insecticides are toxic and adversely affect the environment by contaminating soil, water and air. Prolonged exposure to these synthetic insecticides may lead to irritation, severe allergic dermatitis, systemic allergic reactions and large amounts may cause nausea, vomiting, tinnitus, headache and other central nervous system disturbances (Reynolds, 1994). Also, economic and environmental concerns have encouraged a tendency recently towards the use of "soft" pesticides (Awad, 2003). Therefore, there is a need to find out alternatives to these synthetic insecticides. During the last decades various studies on natural plant products against mosquito vectors indicate them as possible alternative to synthetic chemical insecticides (Mittal and Subbarao, 2003; Rajkumar and Jebanesan, 2004). Plants may be an alternative source of mosquito-control agents because they constitute a rich source of bioactive chemicals, which inhibit growth (Sharma and Srivastava, 2006) development and metamorphosis of insects (Mwangi and Rembold, 1986; Sukumar *et*

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al., 1991). The bioactive constituents of these plants could be either a single substance or a mixture of substances.

The present study attempted to investigate the larvicidal efficacy of xerophytic plants against two medically important mosquito species mosquito *Ae. caspius* and *Cx. pipiens* with the purpose of identifying effective indigenous bioproducts to control the vector of mosquito-borne diseases.

MATERIALS AND METHODS

Mosquito culture

Cx. pipiens and *Ae. caspius larvae* were obtained from a colony maintained at Department of Zoology, College of Science, King Saud University. They were reared indoor at 27±2°C, 50±5% relative humidity, a 14:10 light: dark photo-period and they were fed daily with fish feed until become pupae. Pupae were transferred from the trays to a cup containing tap water and were maintained in our insectary. They were moved into mosquito cage where the emergent adults were fed with a 10% glucose solution in a jar with cotton wick. The adult were given a blood meal from a mouse placed in resting cages overnight for blood feeding by females *Cx. pipiens*. Glass Petri dish lined with filter paper with 100 ml tap water kept inside the cage for oviposition.

Collection of plant material

A total of 3 plant species were collected from Riyadh, Kingdom of Saudi Arabia. *Trichodesma africanum* (flowers, leaves and stem), *Cleome rupicola* (leaf, stem and fruit) and *Ochradenus baccatus* (stem) were dried at room temperature and powdered mechanically using electrical stainless steel blender. The plants were identified and a voucher specimen was deposited in the Botany Department, King Saud University, Riyadh.

Extraction of plant material

10 g plant material was extracted with 300 ml methanol using Soxhlet apparatus and the process was continued until clear color was obtained. The extracts were filtered using whatman filter paper No.1 and concentrated under reduced pressure using rotary evaporator and stored at 4 ºC until further use.

Bioassay

One gram of crude extract was dissolved in 10 ml of methanol (stock solution). From stock solution different concentrations ranged between 10-0.313µg/mL were prepared. Each test solution was placed in Multi-Well Plates (12 Well) and left until dried. Later, it was dissolved in one ml of tap water and tested against 10 4th instar larvae (*Cx. pipiens* and *Ae. caspius*). Each experiment was conducted in triplicates and tap water was used as a negative control. The number of dead larvae was counted after 24 h of exposure and the percentage of mortality was reported for the average of three replicates. The LC_{50} and LC_{90} was calculated only for the test extracts that showed 100% mortality of larvae.

The percentage mortality was determined and transformed to arcsine square-root values for analysis of variance (ANOVA). Treatment means were compared and separated by Scheffe test at P = 0.05 (SAS Institute, 1996). Means±SE of untransformed data are reported.

RESULTS

Xerophytic plants are unturned stone of natural products as a larvicidal agent. This is considered the first report of larvicidal activity of *T. africanum*, *C. rupicola* and *O. baccatu*. All the plants parts screened were found to contain some potency against the larvae of the mosquito species tested, with varying degrees of toxicity.

Ae. Caspius showed 100% mortality at 10µg/ml except the stem of *O. baccatus* which showed 90% mortality (Table I). However, most of the plant extracts tested against *Cx. Pipiens* showed more than 50% inhibition at 10µg/ml. The stem of *C. rupicola* was the most toxic, followed by the stem *T. africanum* with LC_{50} values of 0.99±0.16 µg/ml and 1.23±0.06 µg/ml, respectively. Table II presents the LC_{50} and LC_{90} (µg/ml) after 24h of exposure of the methanolic extract of different plant parts treatments used in the experiment.

DISCUSSION

The problem of high cost and development of resistance in many vector mosquito species to

Table I.- Larvicidal activity (% mortality) (Mean±SEM) of different concentrations of crude plant extracts against 4th instar larvae of *Cx. pipiens* and *Ae. caspius*.

Plant species	Tissue used	Concentration (µg/ml)					
		10	5	2.5	1.25	0.625	0.313
Cx. pipiens							
T. africanum	Flower	56.67±6.67b	30.00±0.00b	26.67±3.33abc	3.33±3.33c	3.33±3.33a	0
	Leaf	46.67±8.81b	26.67±3.33b	10.00±0.00d	6.67±3.33bc	3.33±3.33a	0
	Stem	100±0a	43.33±6.67b	16.67±3.33cd	6.67±3.33bc	3.33±3.33a	0
C. rupicola	Leaf	83.33±3.33a	43.33±8.81b	16.67±3.33bcd	6.67±6.67bc	0.00±0a	0
	Stem	100±0a	93.33±3.33a	46.67±6.7a	23.33±8.81a	0.00±0a	0
	Fruit	100±0a	56.67±3.33b	33.33±8.81ab	26.67±8.81a	6.67±3.33a	0
O. baccatus	Stem	50±5.77b	33.33±5.67b	13.33±3.33cd	6.67±3.33bc	0.00±0a	0
Ae. caspius							
T. africanum	Flower	100±0a	73.33±6,67b	50±5.77cd	16.67±8.8bc	0±0c	0±0b
	Leaf	100±0a	100±0a	60±5.77bc	13.33±6.67bc	0±0c	0±0b
	Stem	100±0a	100±0a	100±0a	66.67±8.8a	23.33±5.77b	0±0b
C. rupicola	Leaf	100±0a	100±0a	76.67±3.33ab	23.33±3.33bc	16.67±3.33bc	3.33±3.33b
	Stem	100±0a	100±0a	93.33±6.67a	63.33±3.33a	56.57±3.33a	10±0a
	fruit	100±0a	100±3.33a	56.67±3.33bc	30± 5.77b	23.33±3.33b	0±0
O. baccatus	Stem	90±5.8b	63.3±6.7bc	30±5.77d	0±0c	0±0c	0±0

Control-Nil mortality *Means within a column followed by the same letter are not significantly different (P = 0. 05)

Table II.- LD_{50} and LD_{90} of plant extracts against 4th instar larvae of *Ae. caspius* and *Cx. pipiens*.

Plant species	Insect	Tissue used	LD_{50} ± SE (µg/ml)	LD_{90} ± SE (µg/ml)
T. africanum	*C. pipiens*	Stem	5.35±0.14	8.08±0.14
	Ae. caspius	Flower	4.06±0.10	8.08±0.14
		Leaf	2.63±0.03	4.45±0.06
		Stem	1.23±0.06	2.12±0.06
C. rupicola	*C. pipiens*	Stem	3.61±0.18	7.54±0.10
		Fruit	4.58±0.11	8.69±0.21
	Ae. caspius	Leaf	2.20±0.09	4.11±0.11
		Stem	0.99±0.16	3.85±0.14
		Fruit	2.34±0.05	4.37±0.60

several of the synthetic insecticides have revived interest in exploring the pest control potentials of plants (Grainge and Ahmed, 1988). Many plant extracts possess larvicidal activity against various mosquito species (Berenbaum, 1989; Jacobson, 1989; Miyakado *et al*., 1989; Sukumar *et al*., 1991; Hostettmann and Potterat, 1997). Additionally, some plant-derived materials are found to be highly effective against insecticide resistant insect pests (Amason *et al*., 1989; Sukumar *et al*., 1991; Ahn *et al*., 1997). Prabakar and Jebanesan (2004) reported that the leaf extract of five species of cucurbitaceous plants, *Momordica charntia, Trichosanthes anguina, Luffa acutangula, Benincasa cerifera* and *Citrullus vulgaris* showed larvicidal activity at LC_{50} of 465.85, 567.81, 839.81, 1189.30 and 1636.04 ppm, respectively against the 4th instar larvae of *Cx. quinquefasciatus.* Similarly, it was reported that *Ipomoea cairica* extract possesses remarkable larvicidal properties as it produces 100% mortality

in the larvae of *Cx. tritaeniorhynchus, Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus* mosquitoes at concentrations ranging from 100 to 170 ppm (Sosan *et al*., 2001). Yenesew *et al*. (2003) reported that the chloroform extract of *millettia dura* showed high activity (LC_{50} =3.5 µg/ml at 24 h) against 2nd instar larvae of *Ae. aegypti*. The highest larval mortality was found in methanol extract of *O. canum*, *R. nasutus* and acetone extract *O. sanctum* against the larvae of *Ae. aegypti* (LC_{50} values of 99.42, 94.43 and 81.56 ppm) and against *Cx. quinquefasciatus* (LC_{50} values of 44.54, 73.40 and 38.30 ppm) respectively (Kamaraj *et al*., 2008).The *R. communis* seed extract exhibited larvicidal effects with 100% mortality at the concentration of 32-64 µg/mL, and showed (LC_{50} values of 7.10, 11.64 and 16.84 µg/mL) against the larvae of *Cx. quinquefasciatus, An. stephensi* and *Ae. albopictus*, respectively (Mandal, 2010). Xerophytic plants phytochemicals of *T. africanum, C. rupicola* and *O. baccatus* are untapped source of insect control potentials.

In our study, It was found that *Ae. caspius* is more susceptible than *Cx. pipiens* to the plant extracts tested. This is in accordance with previous studies which attributed these differences to the physiological characteristics of the different species tested (Kim *et al*., 2002; Thekkevilayil *et al*., 2004; Shaalan *et al*., 2005; Abdalla *et al*., 2009). The stem of *T. africanum* and *C. rupicola* showed very high potency at 24 hours with 100% mortality rate. And Mortalities increased with concentration in all the plant extracts tested. This confirms the report of Shadia *et al*. (2007) that there is a positive correlation between concentration and the percentage of the larval mortality.

CONCLUSIONS

It is evident from the present study the xerophytic plant extracts have promising larvicidal efficacy. Plants offer an advantage over synthetic pesticides as these are easily biodegradable and less prone to development of resistance. However, further work is required to isolate the active constituents in order to test them for their larvicidal potentials.

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