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Insecticidal and repellent activities of methanolic extract of *Tribulus terrestris* L. (Zygophyllaceae) against the malarial vector *Anopheles arabiensis* (Diptera: Culicidae).

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ABSTRACT

Methanolic extracts of leaves and seeds from, *Tribulus terrestris* (Zygophyllaceae) was tested against 3rd instar larvae and adults of mosquito, *Anopheles arabiensis* under laboratory condition. The seeds extract showed high insecticidal activity at all concentrations compared to the leaves extract and the Lc_{50} was 36.5 and 123.1ppm for seeds and leaves extract, respectively. All extracts exhibited remarkable effects on the fecundity, fertility and sterility index of adult females resulted from treated larvae, but the seeds extract was more effective than leaves extract. The repellent action of the present plant extracts varied depending on the plant parts and the dose of extract. The seeds extract was more effective in exhibiting the repellent action (100%) against the mosquito tested as compared with the leaves extract (79.5%) at the dose 1.0 and 2.0mg/cm^2, respectively. The seeds extract showed the same repellency percent (100%) of commercial formulation, N. N. diethyl toulamide (DEET) but at the lower dose (1.0mg/cm^2). The extracts in this study showed strong larvicidal, pupicidal, adulticidal, ovicidal, repellency and biting deterrence and these extracts may be used for further integrated mosquito management programs.

Key words: Methanolic extract, Toxicity, Repellent, *Tribulus terrestris*, *Anopheles arabiensis*.

INTRODUCTION

Vector-borne diseases, such as malaria, dengue and hemorrhagic fever (DHF), are still major public health problems in the South of Kingdom Saudi Arabia countries because geographic location next to the Yemen malaria-endemic and their tropical or subtropical climate. High rainfall level, the prevalence of lakes and/or ponds levels, and the belated displacement system in many regions of Saudi Arabia, provided ideal environmental conditions for mosquito breeding and dense mosquito population .

Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations. It has also resulted in the development of resistance (Liu *et al*., 2006), undesirable effects on non-target organisms, and fostered environmental and human health concern that initiates a search for alternative control measures (Hayes and Laws 1991). Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents (Georgewill *et al*. 2010).

Plant products have been used by traditionally human communities in many parts of the world against the vectors and species of insects. The phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents

and ovipositional attractants and have deterrent activities observed by many researchers (Babu and Murugan 1998). Repellents have an important place in protecting man from the bites in insect pests. An effective repellent will be useful in reducing man vector contact and in interrupting disease transmission. A repellent compound should be toxic, non-irritating and long lasting. Amides, imides, esters and other polyfunctional compounds are known to be good repellents (Kalyanasundaram 1982). Plants could be an alternative source for mosquito repellents because they constitute a potential source of bioactive chemicals and typically are free from harmful effects (Isman 1995). The aim of the study was to determine the Insecticidal activity of *T. terrestris* methanolic extract against the malarial vector, *An. arabiensis* and to examine the repellent properties of this plant against mosquito bites.

MATERIALS AND METHODS

1. Mosquito culture

Anopheles arabiensis larvae were collected from stagnant water area during December 2011 through dipping method from the natural sites located in a village called Sanba at a distance of 10 km from Jazan, Kingdom Saudi Arabia to establish the mosquito colony in our laboratory in the Department of Biology faculty of science, Jazan University. Larvae were kept in plastic and enamel trays containing tap water. They were maintained, and all the experiments were carried out, at 27±2ºC and 75–85% relative humidity under 12:12 light and dark cycles. Larvae were fed a diet of brewer's yeast, dog biscuits, and algae collected from ponds in a ratio of 3:1:1, respectively. Pupae were transferred from the trays to a cup containing tap water and were maintained in wooden cages (45×45×40 cm) where adults emerged. Adults were continuously provided with 10% sucrose solution in a jar with a cotton wick. On day five, the adults were given a blood meal from a pigeon placed in resting cages overnight for blood feeding by females. Glass petri dishes with 50 ml of tap water lined with filter paper was kept inside the cage for oviposition. They were maintained and reared in the laboratory as per the method of Rahuman *et al*. (2008).

2. Plant collection and preparation of plant extract

Fully matured *T. terrestris* (Family: Zygophyllaceae) was collected during November 2011 from the Sabia city (Sabia Jazan desert road). The taxonomic identification is made by Dr. Wael Kassem, Ass. Prof., Biology Department, Faculty of Science, Jazan University, and by comparison with the published plant description in flora of Saudi Arabia (Migahed, 1987). A voucher specimen has been deposited in the herbarium of Biology Department, Faculty of Science, Jazan University. The leaves and seeds were washed and dried in the shade at room temperature (27–31 °C) for 10 days till they become brittle, then pulverized to powder in a hammer mill. 100g of powder from each part was extracted three times separately with 300 ml of methanol at room temperature. After 24 h., the supernatants were decanted, filtrated through Whatman filter paper No. 5. and dried in a rotary evaporator at 40 ºC to obtain 12.9g and 9.1g of a semi solid leaves and seeds crude extract, respectively. The dry extracts were kept in deep freezer (- 4ºC) till used for experiments.

3. Larvicidal test

For the larvicidal bioassay, the WHO standard protocols (WHO, 1996) with slight modifications were followed. The different test concentrations were prepared by adding different range of the stock solution (1%) to 200 ml of water in a 500 ml capacity of glass beaker. Then, twenty of third instar larvae were put immediately into plastic cups contained 200 ml of test concentration of extract. Mortality was recorded

daily and the dead larvae and pupae were removed until adult emergence. The experiments were replicated three times and the data were subjected to Probit analysis4. The control experiments were run parallel with each replicate.

Larval mortality percent was estimated using the following equation (Briggs, 1960): larval mortality % = A – B / A × 100 (where: A = number of tested larvae, B = number of tested pupa). Pupation rate was estimated using the following equation: Pupation % = A / B × 100 (where: A = number of pupae, B = number of tested larvae). The pupal mortality percent was estimated using the following equation: Pupal mortality % = A – B / A × 100 (where: A = number of produced pupae, B = number of observed adults). Adult emergence of males and females were counted and calculated using the following equation: Adult emergence % = A / B × 100 (where: A = number of emerged adults, B = number of tested pupae).

4. Reproductive potentiality of females:

Fecundity:

The emerging adult females from the treated 3rd instar larvae were collected and transferred to the wooden cages (20×20×20 cm) using an electric aspirator and fed with 10% sugar solution for three days. These treated adult females were accompanied with some normal adults males. The adult males and females were left for one day without sugar solution. At the 5th day, starved females were allowed to take a blood meal from a pigeon and allowed to lay egg on clean water (oviposition traps) in the cages. The number of eggs was counted using a binocular and then mean value was counted.

Fertility*:*

The fertility egg or hatchability was calculated using the following equation: Egg hatchability % = A / B × 100 where: A = total number. of hatched eggs, B = total number of eggs laid. According to the formula of Toppozada *et al*. (1966), Sterility percentage = 100 – [a×b /A×B×100], Where: a = number of eggs laid / female in treatment, b = percentage of hatched eggs in treatment, A = number of eggs laid / female in control, B = percentage of hatched eggs in control

5. Repellency test

Standard cages (20×20×20cm) were used to test the repellent activity of plant extracts. Different weight from each extract was dissolved in 2ml (70% ethanol) in glass 4×4cm to prepare different concentrations. One ml from each concentration was directly applied onto 5×6cm of ventral surface of pigeon after removed feathers from the abdomen to evaluate the repellency against *An. arabiensis*, compared with commercial repellent Deet (N. N. diethyl toulamide) (Johnson Wax Egypt) as a positive control. After 10 minutes of treatment, the treated pigeons were placed in the cages containing at least 20 *An. arabiensis* starved females 5-7 d-old for 4h. Control tests were carried out alongside with the treatments using ethanol or water. Each test was repeated three times to get a mean value of repellent.

Repellency % was calculated according to Abbott, (1925). Repellency % = [% A - % B / 100 - % B] × 100 Where: A = percent of unfed females in treatment, B = percent of unfed females in control

6. Statistical analysis

Statistical analysis of the data was carried out according to the method of lentner *et al*., (1982). Lc_{50} was calculated using multiple linear regression (Finney, 1971).

RESULTS

Data given in table (1&2) indicated the insecticidal activity of methanolic extract of *T. terrestris* (leaves and seeds), respectively against the 3rd instar larvae of *An. arabiensis*. Complete larval mortality percent (100%) occurred at the highest concentrations (250 and 150ppm), while the lowest mortality percent (18.3 and 23.3%) occurred at the lowest concentrations (50 and 10ppm) for leaves and seeds extract, respectively compared to 5.0% for the control. The lethal effect of leaves and seeds extract was extended to the pupal and adult stages, but the seeds extract was more effective than leaves extract. The highest pupal mortality was 100.0 and 45.0% at 100 and 200ppm for seeds and leaves extract, respectively, vs. 0.0% for the control.

Table 1: Insecticidal activity of *Tribulus terrestris* (leaves) extract against *Anopheles arabiensis*.

Conc. (ppm)	Larval Mortality %	Pupal Mortality %	Adult Mortality %	Total mortality %	Total emergence %	Average development period (days)	Growth index
250	100.0	—	—	—	—	—	—
200	81.7	45.0	33.3	93.3	55.0	12.9±1.37**	4.3±0.45
150	65.0	38.1	30.8	85.0	61.9	12.4±1.43**	5.0±0.55
100	40.0	25.0	14.8	61.7	75.0	11.9±1.65**	6.3±0.43
50	18.3	10.2	9.1	33.3	89.8	10.4±1.79	8.6±0.39
Control	**5.0**	**0.0**	**0.0**	**0.0**	**100.0**	9.2±1.33	10.9±0.51

No. of tested larvae = 60; Conc. = Concentration; ppm = particle per million

Table 2: Insecticidal activity of *Tribulus terrestris* (seeds) extract against *Anopheles arabiensis*.

Conc. ppm.	Larval mortality %	Pupal Mortality %	Adult Mortality %	Total mortality %	Total emergence %	Average development period (days)	Growth index
150	100.0	—	—	—	—	—	—
100	88.3	100.0	—	100.0	0.0	4.7±0.8**	0.0±0.67
50	61.7	73.9	66.7	96.7	26.1	11.7±1.19**	2.2±0.54
25	45.0	60.6	38.5	86.7	39.4	11.1±1.12**	3.5±0.49
10	28.3	48.8	31.8	75.0	51.2	10.3±2.15	5.0±0.33
Control	**5.0**	**0.0**	**0.0**	**0.0**	**100.0**	8.9±1.15	11.2±0.76

No. of tested larvae, Conc., ppm : see footnote of table (1).

A remarkable reduction in the percentage of adult emergence from pupae produced by treated larvae with the seeds and leaves extract was observed. Also, the seeds extract was more effective than leaves extract, the adult emergence percent (0.0 and 55.0%) was occurred at the concentration 100 and 200ppm for seeds and leaves extract, respectively, vs. 0.0% for the control.

From the aforementioned results it is obvious that the toxicity values of the tested methanolic extract of different plant parts of *T. terrestris* based on Lc_{50} values (Table 3) may be arranged in a descending order as follows: seeds > leaves.

Table 3: Lc_{50} values (ppm) of methanolic extract of *Tribulus terrestris* against *Anopheles arabiensis* larvae.

Plant part	Lc_{50} (ppm)	Slop (b)	Correlation coefficient (r)
Leaves	123.1	0.136	0.978
Seeds	36.5	0.119	0.933

As summarized in Table (4&5), the seeds extract exerted a profound reducing effect on the female fecundity (0.0±0.0, 29.8±7.3 and 41.9±7.9 eggs/♀ at

concentrations 50, 25 and 10ppm, respectively, vs. 97.3±6.7 eggs/♀ for the control) more than leaves extract (32.3±4.4, 37.0±4.0, 46.7±5.5 and 63.2±4.8 eggs/♀, respectively, vs. 97.3±6.7 eggs/♀ for the controls). A remarkable increase of the sterility index at all concentrations was observed. The highest sterility index was 100.0 and 91.8% for seeds and leaves extract at 50.0 and 200ppm, respectively compared to 0.0% for the controls. Moreover, Adult fertility was affected by leaves and seeds extract (the lowest fertility was 53.3% at 50 ppm and 31.4% for seeds vs. 97.1% for the controls).

Table 4: Effect of methanolic extract *Tribulus terrestris* (leaves) on fecundity, fertility and sterility index of female *Anopheles arabiensis*.

Conc. ppm	No. of tested females	No. of eggs laid		No. of hatched eggs		Sterility Index (S. I.) %)
		Total	Mean ±SD	Total	%	
200	4	129	32.3±4.4**	31	24.0	91.8
150	6	222	37.0±4.0**	63	28.4	88.8
100	6	280	46.7±5.5**	132	47.1	63.4
50	9	569	63.2±4.8*	303	53.3	64.3
Control	9	786	97.3±6.7	763	97.1	0.0

SD = standard deviation ; ** = highly significant ($p< 0.01$).

Table 5: Effect of methanolic extract *Tribulus terrestris* (seeds) on fecundity, fertility and sterility index of female *Anopheles arabiensis*.

Conc. ppm	No. of tested females	No. of eggs laid		No. of hatched eggs		Sterility Index (S. I.) %)
		Total	Mean ±SD	Total	%	
50	2	0.0	0.0	0.0	0.0	100
25	5	149	29.8±7.3**	21	14.1	95.6
10	7	293	41.9±7.9**	92	31.4	86.7
Control	9	786	97.3±6.7	763	97.1	0.0

SD, **: see footnote of table (4).

The repellent activity of the present extracts against starved *An. arabiensis* females varied according to the plant parts and the doses used (Table 6). At doses 1.0 and 2.0 mg/cm^2, the seeds and leaves extract induced a degree of repellency as calculated in 100.0 and 79.5%, respectively, compared to 100% repellency for Deet at a dose1.5 mg/cm^2.

Table 6: Repellency effect of methanolic extract of *Tribulus terrestris* on *Anopheles arabiensis*.

Plant parts	Dose (mg/cm^2)	No. of tested females	fed No. of	%	No. of unfed	%	% Repellency
Leaves	2.0	60	11	18.3	49	81.7	79.5
	1.5	60	25	41.7	35	58.3	55.3
	1.0	60	34	56.7	26	43.3	39.2
Seeds	1.0	60	0.0	0.0	60	100	100
	0.75	60	3	0.5	57	95.0	94.6
	0.50	60	9	0.15	51	85.0	84.0
	0.25	60	16	26.7	44	73.3	71.4
Deet	1.5	60	0.0	0.0	60	100	100
Control	0	60	56	93.3	4	6.7	0

DISCUSSION

The plant tested in the present study are known to be eco – friendly and are not toxic to vertebrates. Moreover, it is clearly proved that crude or partially purified

plant extracts are less expensive and highly efficacious for the control of mosquitoes rather than the purified compounds or extracts (Jang *et al*., 2002; Cavalcanti *et al*., 2004 and Maurya et *al.* 2009). The present study showed high bioactivity of the different extracts from *T. terrestris* which are grown widely in Saudi Arabia especially Jazan region. Such results may offer an opportunity for developing alternatives to rather expensive and environmentally hazardous organic insecticides.

The present study showed that, the toxicity of the tested plant extracts against 3rd larval instar was varied according to plant part used and concentration of the extract. The larval mortality percent was increased by increasing extract concentration for all plant extracts tested. The toxicity of methanolic extracts based on Lc_{50} was seeds > leaves. These results agree, to some extent, with the previously mentioned suggestions of (Maurya *et al.* 2009 and Kovendan *et al*. 2012). Extracts from several other plant species were tested on different species of mosquitoes by many authors worldwide. The activity of the plant extracts on larval mortality of *An. arabiensis,* in the present study, were in agreement with the results obtained by Coria *et al.* (2008), Maurya *et al.* (2009), Govnidarajan (2010), Patil *et al*. (2010 and 2011) and Kovendan *et al*. (2012). The evaluation of *T. terrestris* Linn (Zygophyllaceae) acetone extract for larvicidal activity against mosquito vector, *An. stephensi* was tested by Singh *et al.* (2008). The LC_{50} values of leaves and seeds acetone extract were 124 and 72ppm, respectively. In the present study, Also, methanolic extract of leaves showed the same effect against *An. arabiensis* with Lc_{50} 123.1ppm but the seeds extract seemed to be more effective than other extract with LC_{50} values of 36.5ppm.

A remarkable decrease in the pupation percent was induced by all plant extracts in the present study. The pupation% decreased as the concentration of the plant extract increased. The present study showed that the toxic effect of methanolic extracts of leaves and seeds had been extended to the pupae. In addition, these extracts induced reductions of the adult emergence. The reduction was found as a concentration–dependent. These results are comparable to earlier results of Sharma *et al.* (2006) using petroleum ether extract of *A. annua* against *An. stephensi* and *C. quinquefasciatus* larvae, respectively, Wiesman and Chapagain (2006) using one fraction obtained from the silica gel column chromatography of the methanol extract against *Ae. aegypti* mosquito larvae, Gunasekaran (2009) using Neem Azal against *Ae. Aegypti,* Patil *et al*. (2011) *Plumbago zeylanica* (Plumbaginaceae) and *Cestrum nocturnum* (Solanaceae) plant extracts against *Ae. aegypti*. and Kovendan *et al*. (2012) using *Carica papaya* (Caricaceae) leaf extract against, *Aedes aegypti*. Gang *et al*. (2000) concluded that, some plants such as *T. terresteis*, *Eupatorium fortunei* and *Datura stramouium* have value for development as botanical insecticides against *C. pipiens*.

Results obtained in the present study indicated that the toxicity of plant extracts against the 3rd instar larvae of *An. arabiensis* was extended to the adults causing mortality reached to 33.3 and 66.7% for leaves and seeds extract at the concentrations 200 and 50ppm, respectively. Similar results were obtained by Jeyabalan *et al*. (2003) using methanol extract of *Pelargonium citrosa* leaf against *An. stephensi*, Nathan *et al*. (2005) using the neem *A. indica* extract against *An. Stephensi*, Nathan *et al*. (2006) using methanolic extracts of leaves and seeds from the chinaberry tree *Melia azedarach* against *An. stephensi* and El-Sheikh *et al*. (2011) using leaf and stem extracts from *Cestrum nocturnum* (solanaceae) against *Culex pipiens*.

Almost all plant extracts tested against the 3rd instar larvae of *An. arabiensis* significantly reduced the female fecundity and induced the sterility % of females. The fecundity and sterility percents depended on the plant part and concentration.

Moreover, a remarkable decrease in the hatchability % of eggs laid by females resulted from treated larvae especially with seeds extract was observed. The hatchability of eggs decreased as the conc. of the extract increased. These results are in consistent with those obtained by many authors using different plant extracts against different mosquito species (Jeyabalan *et al.,* 2003; Nathan *et al*., 2006; Coria *et al.,* 2008 and Pavela, 2009).

Whatever the concentration, the plant extracts used in the present study exhibited some repellency activity against the starved female adults of *An. arabiensis*. The repellent action of the plant extracts tested was varied depending on the plant part used and the dose of the extract. The present study indicate that the seeds extract was more effective in exhibiting the repellent action against the mosquito tested as compared with the leaves extract and showed same repellency percent (100%) but at the lower dose (1.0mg/cm^2) as compared with a commercial formulation, N,N-diethyl-m-methylbenzamide (1.5mg/cm^2).

Many plant extracts and essential oils manifest repellency activity against different mosquito species. The present results are in accordance with such results obtained by Sharma *et al*. (1995) using neem oils against mosquito bites of *Anopheles* spp., *Culex* spp. and *Ae.* spp., Govere *et al*. (2000) using extracts of fever tea (*Lippia javanica*) rose geranium (*Pelargonium reniforme*) and lemon grass (*Cymbopogon excavatus*) against *An. arabiensis,* Yang *et al*. (2004) using methanol extracts from 23 aromatic medicinal plant species against female blood–starved *Ae. aegypti*, Choochote *et al.* (2007) using repellent activity of selected essential oils from ten plant species against *Ae. Aegypti*, Mullai *et al.* (2008) using leaf extract of *Citrullus vulgaris* against the malarial vector, *An. stephensi*, Singh *et al.* (2008) using acetone leaves extract of *T. terrestris* against *An. stephensi,* El-Sheikh *et al*. (2009) using sixteen ethanolic and petroleum ether extracts of 4 indigenous plants as repellent in the field against wild mosquitoes, Elango *et al.* (2011) using methanol extract of *Andrographis paniculata* against *An. Subpictus* and Prabhu *et al.* (2011) using *Moringa oleifera* methanolic extracts against *An. stephensi*. Govnidarajan and Sivakumar (2011) tested the repellent activities of crude hexane, ethyl acetate, benzene, chloroform, and methanol extracts of leaf of *Eclipta alba* and *Andrographis paniculata* at three different concentrations of 1.0, 2.5, and 5.0 mg/cm^2 against important vector mosquitoe *Ae. aegypti* and they suggested that the leaf solvent plant extracts have the potential to be used as an ideal eco-friendly approach for the control of mosquitoes. In the present study the efficacy of seeds extract from *T. terrestris* at the dose (1.0 mg/cm^2) produced the highest protection (100%) during the entire research period of 4h post-treatment.

CONCLUSION

In general, it could be concluded that seeds and leaves methanolic extracts of *T. terrestris* used in the present study act as repellent or antifeedant, ovicidal, larvicidal, pupalcidal, adulticidal and possess emergence inhibiting against the mosquito vector, *An. arabiensis*. Furthermore, the results of the present study may contribute to a reduction in the application of synthetic insecticides, which in turn increases the opportunity for natural control of various medically important pests by botanical pesticides. Further studies on the tested plants including mode of action, synergism with the biocides under field condition are needed.

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ARABIC SUMMARY

النشاط الطارد و السمي لمستخلص الميثانول لنبات أم جريسة على البعوضة الناقلة لمرض الملاريا *أنوفليس أرابينسيس* (ثنائية الأجنحة: كيولسيدى)

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أجريت الدراسة الحالية لتقييم النشاط السمي و الطارد لمستخلص الميثانول لأوراق و بذور نبات أم جريسة (*Tribulus terrestris*) أحد النباتات الصحراوية المحلية في جازان ضد العمر اليرقى الثالث و الطور البالغ لبعوضة *أنوفليس أرابينسيس*. أظهرت النتائج أن مستخلص البذور كان له نشاط ابادى عالي عند كل التركيزات المستخدمة مقارنة بمستخلص الأوراق حيث سجل مستخلص البذور (ت.ن.م 64.6 ج ف م) يليه مستخلص الأوراق (ت.ن.م 173.2 ج ف م). أظهرت نتائج الدراسة الحالية أن كل المستخلصات النباتية المختبرة، أدت إلى حدوث نقصا ملحوظا في الخصوبة و نسبة الفقس و إلى زيادة في نسبة عقم الإناث الناتجة من معاملة اليرقات. و كان من الملاحظ أيضا أن مستخلص البذور كان أقوى تأثيرا من مستخلص الأوراق. سجلت الدراسة أن التأثير الطارد لهذه المستخلصات المختبرة كان يختلف باختلاف جزء النبات المستخدم و كذلك جرعة المستخلص. وبينت الدراسة أيضا أن أعلى نسبة طرد أو منع لتغذية (100%) إناث الطور اليافع للبعوضة قد سجلت باستعمال مستخلص البذور عند جرعة 1.0 مج/سم2 مقارنة بمستخلصي الأوراق عند جرعة 2.0 مج/سم2 . علاوة على ذلك، كان لمستخلص البذور نفس نسبة الطرد (100%) للمركب التجاري ن- ن داى ايثيل- م- مثيلبنزليميد (دييت)، و لكن عند جرعة أقل منه. أظهرت المستخلصات المستخدمة في هذه الدراسة تأثيرا سميا واضحا ضد الطور اليرقى و العذري و الأطوار البالغة الناتجة من معاملة اليرقات و كذلك كان لها تأثيرا طاردا ضد الطور اليافع وعلية فانه يمكن استخدام هذه المستخلصات في برامج المكافحة المتكاملة للبعوض.