Evaluation of the Toxicity Effect of *Tephrosia purpurea* Extracts against Filarial Vector *Culex quinquefasciatus*

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ABSTRACT---- ***Excessive in using insecticides has led to damage to the environment and public health, which led to reconsideration in use the natural pesticides of plant origin in the fight against mosquitoes. Laboratory studies were conducted to evaluate the toxicity effect of methanolic leaves and stems extracts of the plant Tephrosia purpurea (Fabaceae) against the mosquito Culex quinquefasciatus. The efficacy of stems extract against 3rd instar larvae seemed to be less effective with Lc_{50} 2348ppm than leaves extract with Lc_{50} 58.3ppm. Leaves extract showed a remarkable reduction effects on adult emergence, fecundity and fertility more than stems extract. Both leaves and stems extract exerted delayed toxic effect on the pupae and adults resulted from treated larvae. Furthermore, some morphological deformities (pupal-adult intermediate) were observed after treatment with leaves extract. The current results considered promising to move forward in studying the bioactive plants which form an environmentally sound alternative for the synthetic insecticides.***

Keywords--- Methanolic extract, Toxicity, Fecundity, *Tephrosia purpurea*, *Culex quinquefasciatus*.

1. INTRODUCTION

Many diseases, such as malaria, elephantiasis, dengue, Japanese encephalitis and yellow fever transmitted by mosquitoes (Gad *et al*., 1996; Roberts, 2002). In some areas of the world *C. quinquefasciatus* used in this study is a vector of *Wuchereria bancrofti*, Plasmodium (avian malaria), myxomatosis, and other diseases (Goddard *et al*., 2002). Worldwide, nearly 90 million people infected with the lymphatic parasite, *wochereia bancrofti*, (WHO 1985). For that, control of mosquitoes is a major concern for worldwide public health. The effective method to limiting the spread of these diseases is to eliminate and control mosquito vectors, mainly by using insecticides to larval habitant (Corbel *et al*., 2004).

Today Synthetic insecticides one of the important agents for mosquito control. However, control of the mosquitoes become complex because of their resistance to synthetic insecticides, as well the toxic effect of insecticides to other non-target organisms (Rohani *et al*., 2001, Mohan and Ramaswamy, 2007). very important need to develop new materials for controlling mosquitoes in an environmentally safe way, using target-specific insecticides against the mosquitoes.

In many tropical and subtropical countries of the world the plants of genus *Tephrosia* of family Fabaceae are widespread and have been used in popular medicine to treat many of diseases. This plant has some activities such as anticancer, antimicrobial, antioxidant, anti-inflammatory, anti-plasmodial, larvicidal (Touqeer *et al*. 2013).

For that**,** this study was conducted to determine the larvicidal, pupicidal, adulticidal and reproductive effects of leaves and stems methanolic extract of a widespread plant in Jazan region , *T. purpurea* (Fabaceae) on the filarial vector, *C. quinquefasciatus*

2. MATERIALS AND METHODS

2.1.Mosquito culture:

The filarial mosquito used in this study was *C. quinquefasciatus*, (Diptera: Culicidae). The larvae were collected from a swamp within Alebadl area at a distance of 50 km from Jazan city and identified according to Harbach (1985). They were reared for several generations at temperature of 29±3°C, relative humidity 65±5% and 13-11 light-dark. The

adults placed in the cages (40 x 40 x 40 cm) with cotton piece wetted in 10% sugar solution for a time of 2-3 days. Then, a pigeon host placed in the cage in order to the females take a blood meal for laying their eggs. Glass cup (oviposition trap) 15x10cm was placed in the cage containing 200 ml water. The egg masses collected from the surface water of oviposition trap and placed into plastic pans (40 x 30 x 20 cm) contain 4 liters of water. Fish food as a diet was provided daily to the larvae . This food very important for the larval development and a well for female fecundity (Kasap and Demirhan, 1992).

2.2. Plant Collection and extraction

Tephrosia purpurea plant (Family: Fabaceae) were collected from Sabia city field in September 2013. The plant was identified according to (Migahed, 1987). The leaves and stems were washed and dried in an oven at a temperature of 40 ºC untill they become fragile, then grinded to fine powder in the mill. Two hundred grams of leaves and stems powder were extracted separately three times with 400 ml of methanol at room temperature. After 48 h., the supernatant was filtrated and dried by a rotary evaporator to obtain 17.1 g (leaves extract), 8.3 g (stems extract).

2.3. Larvicidal test

To determine the toxicity of leaves and stems extracts, different concentrations of each extract were prepared. Each concentration was performed in 200ml of water contained in 300ml plastic cups. Then, twenty 3rd instar larvae were placed into plastic cups contained different concentrations of extracts. Three replicates were used for each experiment. All experiments were incubated under controlled conditions (29±3°C, RH 65±5% and 13-11 light-dark). Control larvae received only one drop of Tween.80 in 200ml water. Larval and pupal mortality was daily recorded until adult emergence.

2.4. *Reproductive potentiality of females:*

Fecundity:

To test the effect of leaves and stems extracts on the reproductive potential of females, the emerging adult females from the treated larvae were collected, placed into the wooden cages (25×25×25 cm) and fed on 10% sugar solution for two days. The females were accompanied with some normal adults males. The female adults were left for one day without sugar solution. At the 4th day, starved females take a blood meal to lay their eggs on oviposition traps. The number of eggs / mass was counted using a binocular microscope and then mean value was calculated.

2.5.Criteria studied

The mortality of larvae and pupae was indicated by a failure to respond to mechanical stimulation (Williams *et al*. 1986). The percentage of larval and pupal mortality was calculated according to (Briggs, 1960):

larval mortality % = C / D × 100 (C = number of dead larvae, D = total number of tested larvae).

Pupal mortality % = C / D × 100 (C = number of dead pupae, D = total number of produced pupae).

Adult emergence % = A / B × 100 (A = number of emerged adults, B = number of tested pupae).

Egg hatchability % = A / B × 100 (A = total number of hatched eggs, B = total number of eggs laid)

2.6. Statistical analysis

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

3. RESULTS

The present results include the biological activity (larvicidal, pupal rate, pupicidal, adult emergence and reproductive potential) of methanolic leaves and stems extracts of *T. purpurea* on the 3rd larval instar of *C. quinquefasciatus*.

3.1. The toxic effect on larvae, pupae and adult

Leaves extract

Data in table (1) indicated the biological activity of leaves extract of *T. purpurea* on the 3rd instar larvae of *C. quinquefasciatus*. Complete mortality for larvae (100.0%) was caused at the highest concentrations (300ppm). Meanwhile, the larval mortality % decreased to 20.0 % at the lowest concentrations (25ppm) (compared to 1.7% for the

untreated larvae).

Table (1): Mortality percent of different stages of *Culex quinquefasciatus* after treatment with methanolic extract of *Tephrosia purpurea* (leaves).

Conc. ppm	Larval mortality %	Pupation %	Pupal Mortality %	Larval and pupal Mortality %	Adult Emergence %	Pupal / adult Intermediate %	Adult Mortality %
300	**100.0**	—	—	—	—	—	—
200	**91.6**	**8.4**	**40.0**	**95.0**	**0.0**	**60.0**	—
100	**80.0**	**20.0**	**25.0**	**85.0**	**0.0**	**75.0**	—
75	**68.3**	**31.7**	**21.1**	**75.0**	**0.0**	**78.9**	—
50	**35.0**	**65.0**	**12.8**	**43.3**	**23.1**	**64.1**	**77.7**
25	**20.0**	**80.0**	**8.3**	**26.7**	**43.8**	**47.9**	**57.1**
Control	**1.7**	**98.3**	**0.0**	**1.7**	**100.0**	**0.0**	**0.0**

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

The lethal effect of leaves extract was extended to the pupal stage at all the concentrations 200, 100,75, 50 and 75ppm where the pupal mortality recorded 40.0, 25.0, 21.1, 18.2 and 8.3%, respectively. A remarkable reduction in the percentage of adult emergence from pupae produced by treated larvae was observed. The adult emergence percent (0.0%) was occurred at the concentrations 200, 100 and 75ppm, meanwhile the percent increased to 23.1, and 43.8% at the concentrations 50and 25ppm compared to 100.0% at the control group.

As shown from the results (table 1) the toxicity of leaves extract extended to the adult stage, where the adult mortality percent was 77.7, and 57.1% at 50 and 25ppm, compared to 0.0% at the control group. The results recorded that, the leaves extract induced highly % of malformation(pupal-adult intermediate) at all concentrations used. The malformation percent was 60.0% at the concentration 200ppm and 43.8% at 25ppm compared to 0.0% for the control group.

Stems extract

Arranged data in table (2) indicated a biological activity of *T. purpurea* (Stems) against the 3rd larval instar of *C. quinquefasciatus*. As shown from the data, the highest percentage of dead larvae (100%) was recorded at the concentration (3500ppm) and the lowest mortality percent (18.3%) was observed at the lowest concentrations (1500ppm) compared to 1.7% for the controls.

Table (2): Mortality percent of different stages of *Culex quinquefasciatus* after treatment with methanolic extract of *Tephrosia purpurea* (stems).

Conc. ppm.	Larval mortality %	Pupation %	Pupal Mortality %	Larval and pupal Mortality %	Adult Emergence %	Adult Mortality %
3500	**100.0**	—	—	—	—	—
3000	**83.3**	**16.7**	**60.0**	**93.3**	**40.0**	**15.0**
2500	**68.3**	**31.7**	**36.8**	**80.0**	**63.2**	**0.0**
2000	**40.0**	**60.0**	**13.9**	**48.3**	**86.1**	**0.0**
1500	**18.3**	**81.7**	**9.1**	**16.7**	**90.9**	**0.0**
Control	**1.7**	**98.3**	**0.0**	**1.7**	**100.0**	**0.0**

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

The lethal effect of petroleum ether extract was extended to the pupal stage at the all concentrations used, 3000,2500, 2000 and 1500ppm, the pupal mortality percents were 60.0, 36.8, 13.9 and 9.1%, respectively, vs. 0.0% for the control.

A reduction in the percentage of adult emergence from pupae produced by treated larvae with leaves extract was observed especially at the highest concentration. The adult emergence percent (40.0 and 63.2%) was occurred at the

concentration 3000 and 2500ppm, respectively compared to 100.0% at the control group.

From the aforementioned results it is obvious that the leaves extract was more efficient than stems extract. In general, the toxicity values of tested materials of *T. purpurea* based on Lc_{50} values (Tables 3) may be arranged in a descending order as follows: leaves extract > stems extract.

Table (3): Lc_{50} values (ppm) of methanolic leaves and stems extract of *Tephrosia purpurea* against *C. quinquefasciatus* larvae.

Plant extract	Lc_{50} (ppm)	Slope (b)	Correlation coefficient (r)
Leaves extract	58.3	0.428	0.733
Stems extract	2348	0.041	0.986

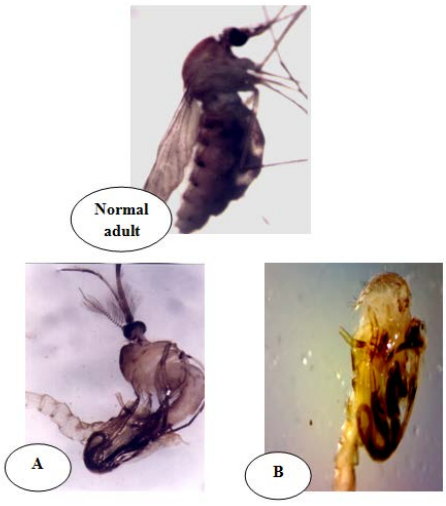

Fig. (1): Deformities effects in *C. quinquefasciatus* as induced by methanolic leaves extract of *Tephrosia purpurea* (160 X). A) not complete emerged adult with the abdominal part, wings and legs attached to the pupal skin B) Failure adult to exit from pupal skin.

2. Reproductive potentiality:

As summarized in Table (4), leaves extract of *T. purpurea* exerted a profound reducing effect on the female fecundity (61.0±21.0 and 87.0±23.2 eggs/♀ at the concentrations 50 and 25ppm, respectively, vs. 121.0±17.9 eggs/♀ for the control). Moreover, there was a significantly decrease in the hatching percent of eggs laid by females resulted from treated larvae (38.4 and 62.1% at 50 and 25ppm; respectively).

Table (4): Effect of methanolic extract *Tephrosia purpurea* (leaves) on fecundity and fertility of female *Culex quinquefasciatus*.

Conc. ppm	No. of tested females	No. of eggs laid		No. of hatched eggs	
		Total	Mean ±SD	Total	%
50	**3**	**185**	**61.0±21.0****	**71**	**38.4**
25	**5**	**435**	**87.0±23.2***	**270**	**62.1**
Control	**10**	**1211**	**121.0±17.9**	**1170**	**96.6**

SD : Standard deviation; * = significant (p> 0.01) .

Table (5): Effect of methanolic extract *Tephrosia purpurea* (stem) on fecundity and fertility of female *Culex quinquefasciatus*.

Conc. ppm	No. of tested females	No. of eggs laid		No. of hatched eggs	
		Total	Mean ±SD	Total	%
2000	6	654	109.0±14.7 ^{ns}	389	59.5
1500	8	857	107.0±20.3 ^{ns}	689	80.4
1000	9	1118	124.2±21.1^{ns}	1037	92.8
Control	10	1211	121.0±17.9	1170	96.6

ns = not significant.

Data in table (5) showed insignificantly effect of methanolic extract of *T. purpurea* (leaves) on fecundity, where the average number of eggs/♀ was 109.0±14.7, 107.0±20.3 and 124.2±21.1 at 2000, 1500 and 1000ppm vs. 121.0±17.9 eggs/♀ for the control. A remarkable reduction in the hatchability percent (59.3%) at the highest concentration (2000ppm) was observed, this percent increased to 80.4% at the concentration 1500ppm. compared to 96.6% for control group.

4. DISCUSSION

Tephrosia purpurea used in this study environmentally safe and nontoxic to human and other animals but used as a medicinal plant. In Saudi Arabia in Jazan region, *T. purpurea* is grow widely as herbal plant. Moreover, Maurya *et al.* (2009) and Kovendan *et al*. (2012) reported that crude plant extracts are less costly and highly effective for mosquitoes control instead of purified compounds.

In this study the toxic effect of the tested plant extracts was dependent on the plant part and extract concentration used. The larval mortality percent increased as extract concentration increased for all plant extracts. The LC_{50} value for leaves extract was (58.3ppm) less than the LC_{50} value (2348ppm) for stems extract. These results consistent to some extent with the results already referred to Maurya *et al.* (2009), Kovendan *et al*. (2012), Ilahi and Ullah (2013) and Govindarajan and Rajeswary (2014).

Extracts from several other plant species were tested on different species of mosquitoes by many authors worldwide. Kumar *et al.* (2012), tested the toxic effect of *T. purpurea* (whole plant) petroleum ether and ethyl acetate extract against the third larval instar of mosquito *C. quinquefasiciatus*was, they reported that, the LC_{50} values were 250 and 300ppm, respectively. Also, Bansal (2012) tested methanolic extract of *T. purpurea* against late 3rd instar larvae of *Ae. Aegypti* and they found that the Lc_{50} was 249.6ppm. In the present study, the methanolic leaves extract showed more effective against *C. quinquefasiciatus* with Lc_{50} 58.3ppm. Sakthivadivel *et al.* (2012) recorded that the toxic effects of petroleum ether leaf extracts of plants *Argemone mexicana* against vector of lymphatic filariasis, *C. quinquefasciatus* showed maximum larvicidal activity with an LC_{50} value of 48.89ppm. Kolli *et al*. (2013) found that both methanol and hydroalcohol extracts of *Pongamia pinnata* against *C. quinquefasciatus* with LC_{50} values of 84.8 and 118.2ppm respectively. Raveen *et al*. (2014) tested the larvicidal activity of *Nerium oleander* L. (Apocynaceae) flower extracts against *C. quinquefasciatus* Say, they observed that, hexane flower extract exhibited highest larvicidal activity with a LC_{50} value of 102.54ppm and 61.1ppm after 24 and 48 hours respectively. The differences in toxic effect of phytochemical components on target insect is due to the plant part used in the extraction (Sukumar *et al,*. 1991). In addition, Jeyabalan *et al*. (2003) noted that other differences were due to responses by species of insect and growth stages of species to the specific extract, type of solvent used in the extraction and plant environment.

The present study showed that the toxic effect of metanolic leaves and stems extract had been extended to the pupae. Moreover, leaves and stems extract caused reduction in the percentages of adult emergence. The reduction in the adult emergence was depend on the concentration. These data are compatible to earlier findings of some authors. Sharma *et al.* (2006) tested petroleum ether extract of *A. annua* on *C. quinquefasciatus* larvae, Patil *et al*. (2011) tested *Cestru nocturnum* plant extracts on *Aedes aegypti* and Kovendan *et al*. (2012) tested *Carica papaya* leaf extract against, *Ae. aegypti*.

Results obtained in this study indicated that the toxic effect of methanolic leaves and stems extract was extended to the adults causing mortality reached to 77.7% at the concentration 50ppm. Similar results were obtained by Jeyabalan *et al*. (2003) using methanolic leaf extract of *Pelargonium citrosa* against *An. stephensi*, Nathan *et al*. (2006) using

methanolic leaves and seeds extracts of *Melia azedarach* against *An. Stephensi*, ElSheikh *et al*. (2011) tested the effect of leaf and stem extracts of *Cestrum nocturnum* (solanaceae) against *Culex. pipiens* and Chalannavar *et al*. (2013) using *Psidium guajava* methanolic extract against *C. quinquefasciatus*.

In the present study methanolic leaves extract induced some morphological abnormalities (pupal – adult intermediates). Also, the percentage of malformation was the concentration-dependent. Similar observations were obtained by different plant extracts against different mosquito species in earlier studies. Abahussain (1999) studied the toxic effect of *Calotropis procera* extract on *C. pipiens* and *An. Multicolor*, he reported some deformations among larvae, pupae and adults. Sharma *et al*.(2006) using petroleum ether leaves extract of *Artemisia annua* against *C. autnauetesctetus* observed some deformities on the pupae and adult. Abnormalities produced in pupae reduced the adult emergence, which may be due to abnormalities in normal molting hormone or interruption in chitin synthesis.

In this study, Methanolic leaves extract significantly reduced the female fecundity. The fecundity percent 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 leaves extract used was observed. The hatchability of eggs decreased as the concentration 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; Pavela, 2009).

5. CONCLUSION

In general, it could be concluded that, plant extracts used in the present study act as larvicidal, pupicidal and possess growth emergence inhibiting activities against the mosquito vector, *C. quinquefasciatus*. Furthermore, the results of the present study may share in decrease the uses of synthetic pesticides. More studies on the tested plant in the future to determine the active ingredients.

6. REFERENCES

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