Larvicidal Activities of Different Parts of *Melia azedarach* Linn. against *Culex quinquefasciatus* Say. (Diptera: Culicidae)

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Abstract: *Melia azedarach* is a perennial tree, belongs to the family Meliaceae, and locally known as Tora Shandi in Swat and Dir regions of Khyber Pukhtunkhwa (K.P.K). The present study was aimed to evaluate the larvicidal activity of aqueous extracts of different parts of *Melia azedrach* against *Culex quinquefasciatus*. Various concentrations (50, 100, 500, 1000, 1500 and 2000 ppm) of aqueous extracts of leaves, fruits and bark of *Melia azedarach* were tested for larvicidal activity against *Culex quinquefasciatus*. There occurred a continuous increase in mortality of 3rd and 4th instar larvae with increase in concentration of the extracts. At 50ppm, the fruit extract caused 1.6 ± 2.2 % mortality, leaves extract 17.60 ± 6.0 % and bark extract caused 17.60 ± 7.3 % mortality. The LC50 value for fruits extract was 2035.13ppm, leaves extract 612.250ppm and for bark extract 368.3ppm. Among the aqueous extracts of fruits, leaves and bark, the aqueous extract of bark was proved to be more toxic and an efficient larvicidal against *Culex quinquefasciatus*. The findings of the present study indicate that the aqueous extract of the bark of *Melia azedarach* of Dir region of khyber pakhtunkhwa can serve as a good source of preparations for pest control especially mosquito control.

Keywords: Aqueous extracts, *Culex quinquefasciatus*, Mortality, LC50.

INTRODUCTION

Melia azedarach Linn. is one of the medicinal plants commonly known as "Persian Lilac", "Bakain" or "think", grows wild throughout the Sub-Himalayan belt. It is cultivated in India and Pakistan for both ornamental and medicinal purposes [1]. It is a perennial tree and belongs to the family Meliaceae, its English name is china berry and locally known as Tora Shandi in Swat and Dir regions of Khyber Pakhtunkhwa (K.P.K).

Various preparations of *Melia azedarach* are being used for the treatment of several diseases [1, 2]. There are several reports on the analgesic, anticancer, antiviral, anti malarial, antibacterial, antifungal and anti-fertility activity of this plant [3]. The use of *M.azedarach* in stomach ache, intestinal disorders, uterine illnesses, cystitis, diuretic and febrifuge has been reported [4]. The powder of the dried fruits of this plant is claimed to be an effective therapy for the treatment of diabetes [5]. In the field of veterinary medicine, the ovicidal and larvicidal activity of *M. azedarach* extracts on the helmenthus *Haemonchus contortus* has been reported [6]. This plant has also been studied for its antimalaria [7, 8] as well as spermicidal [9]) and antifungal activity [10].

The family Meliaceae to which *Melia azedarach* belongs contains 45 genus and over 750 species [11]. The members of Meliaceae plant family are known to contain a variety of compounds, which show insecticidal, antifeedant, growth regulating and development modifying properties [12, 13]. Fruit extracts of *Melia azedarach* and *Azadirachta indica* elicit a variety of effects in insects such as antifeedant, growth retardation, reduced fecundity, moulting disorders, morphogenetic defects, and changes of behavior [7, 14-17]. Many plants of Meliaceae family possess insecticidal properties against fecundity and fertility of mosquito vector and are at the same time very eco-friendly [18-19].

Control of mosquito is essential as many species of mosquitoes serve as vectors for spreading of malaria, filariasis, dengue and many other viral diseases and they cause unbearable biting irritations [20-22]. A more efficient and attractive approach in mosquito control programs is to target the larval stage in their breeding sites with larvicides [23-24]. Various organophosphates (Temiphos and Fenthion) and insect growth regulators (Diflubenzuron and Methoprene) are widely used as larvicides for the control of mosquitoes [25]. These synthetic pesticides have caused the development of resistant insect strains, ecological imbalance and harm to mammals including humans [26]. The best alternative for avoiding these problems could be the use of plant-based insecticides, which are quickly degradable in the environment and their source is renewable [27].

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In the present study *Melia azedarach* Linn. was investigated for its larvicidal activity against mosquito *Culex quinquefasciatus* Say. *Culex quinquefasciatus* is a vector of widely distributed tropical disease "lymphatic filariasis" [28] and also causes unbearable biting irritations. The insecticidal efficacy of extracts of *M. azedarach* has been evaluated against ticks [29], Ae. *aegypti* [8, 30] and *An.stephensi* [31]. There are also some reports on the evaluation of larvicidal activity of *Melia azedarach* against *Culex quinquefasciatus* [32].

The aim of the present study was to evaluate the larvicidal activity of aqueous extracts of different parts of *Melia azedrach* against *Culex quinquefasciatus*.

MATERIALS AND METHODS

Plant Material

The fruits, leaves and bark of *Melia azedarach* Linn. were collected from the campus of University of Malakand, Khyber Pakhtunkhwa, Pakistan during June, 2011. The plant was identified by Mr. Ilyas Iqbal, Assistant Professor, Departmenty of Botany, University of Malakand. A voucher specimen was deposited in the Herbarium of University of Malakand.

Extraction

Fruits, leaves and bark of *Melia azedarach* were cleaned and shade dried. One kilogram each of fruits, leaves and bark were coarsely ground and then soaked in 3000 ml of distilled water for 7 days with occasional shaking. Each soaked material was filtered through a muslin cloth, each filtrate was placed in separate beaker. The filtrates were dried under reduced pressure using rotary evaporator (Heidolph Laborta 4000 efficient). The dried crude extracts were stored in refrigerator at 4ºC.

Test Organism

Before the conduction of larvicidal bioassay, 100 samples of 3^{rd} and 4^{th} instars larvae of mosquitoes were collected with the help of a glass dropper from a ditch of stagnant water at the campus of University of Malakand. The immature stages were reared in plastic jars. To each jar 200 ml of water was added from the collection spot to ensure food supply. The jar openings were closed with net cloth. The larvae were reared to adulthood in the laboratory. The adult mosquitoes were captured with the help of aspirator and killed with a cotton swab of chloroform in an airtight container. The adults were placed in test tubes containing silica gel. In addition to the study of the adults, permanent slides of 4^{th} instar larvae were also prepared. The adults and larvae were studied for taxonomic characters by using binocular microscope and identification was made up to species level with the help of taxonomic keys provided in the literatures [33-34]. All the specimens were belonging to the species *Culex quinquefasciatus*. So it was confirmed that the ditch harbors only *Culex quinquefasciatus*.

Larvicidal Bioassay

For conduction of larvicidal activity, larvae were collected from the same site in a large plastic jar and brought to the laboratory. A stock solution of 10,000ppm of each extract was prepared in distilled water. From the stock solution of each extract, 100 ml each of 2000, 1500, 1000, 500, 100 and 50 ppm were prepared in different 500ml plastic cups. The cups were labeled accordingly. For bioassay of each extract, 25 larvae of late 3^{rd} and early 4^{th} instar were transferred to each plastic cup with the help of a large mouthed glass dropper. Larvae were fed with a diet of finely ground brewer's yeast and dog biscuits in 3:2. A control was also maintained by adding only larval food (yeast and dog biscuit in 3:2). All the experiments were carried out at 30 $\pm$ 2°C and 70-75 relative humidity. The numbers of dead larvae were counted after 24 h of exposure. Dead larvae were identified when they failed to move after prodding with a needle in the cervical or siphon region. Five replicates of the experiment were made. After recording the number of dead larvae, all the larvae (dead and live if present) were identified to confirm the species.

Statistical Analysis

The dose-response data was subjected to Linear regression analysis to point out the relationship between the increase in extract concentration and larval mortality. For the calculation of LC_{50} values the data was subjected to probit regression analysis. To compare the aqueous extracts of fruits, leaves and bark, the data was subjected to Duncan Test of Post Hoc Multiple Comparisons in One Way Anova. For all these analysis computer software SPSS 16.0 was used.

RESULTS

The larvicdal activities of various concentrations of aqueous extracts of different parts of *Melia azedarach*

were studied against *Culex quinquefasciatus*. There occurred a continuous increase in mortality of 3rd and 4th instar larvae with increase in concentration of the extracts. The mortality caused by each of the fruits, leaves and bark aqueous extract was subjected to linear regression analysis. In each case the value of R square was about 0.9 at P = 0.05 significance (Table **1**). These results of linear regression analysis clearly indicate the relationship between the increase in concentration of extract and the increased in larvicidal activity. The percent mortalities caused by various concentrations of aqueous extracts of different parts of *Melia azedarach* are shown in Table **2**. The LC50 values of different parts of *Melia azedarach* with 95 % confidence intervals are shown in Table **3**. During the study the lowest dose of fruit extract (50ppm) caused 1.6 ± 2.2 % mortality and the highest concentration (2000 ppm) caused 61.60 ± 7.3 % mortality. The LC50 value for fruit extract was 2035.13ppm. The lowest concentration (50ppm) of leaf extract caused 17.60 ± 6.0 % mortality and the highest concentration caused 81.60 ± 4.6 % mortality. The LC50 value for leaves extract was 612.250ppm. The lowest concentration (50ppm) of bark extract caused 17.60 ± 7.3 % mortality and the highest concentration caused 88.0 ± 6.3 % mortality. The LC50 value for bark extract was 368.3ppm.

The larvicidal activities of fruits, leaves and bark extracts were compared statistically (Table **4** and Figure **1**). At the concentration of 50ppm the extract of fruits caused less mortality as compared to the bark and leaves extracts (P< 0.05). Each of both leaves and fruits extracts caused the same 17.60 % mortality. At 100ppm fruit extract caused 10.40 ± 7.3 % mortality, and leaves and bark extract caused 24.0 ± 5.7 % and

Table 1: Linear Regression Analysis of Larvicidal Activity of Aqueous Extracts Of Different Parts of *Melia azedarach* Against *Culex quinquefasciatus* After 24 Hours Exposure

Model	R	R square	Significance
Fruit	0.996	0.973	0.000
Leaves	0.998	0.976	0.000
Bark	0.949	0.900	0.001

Table 2: Larvicidal Effects of Various Concentrations of Aqueous Extract of Different Parts of *Melia azedarach* Against 3rd and 4th Instar Larvae of *Culex quinquefasciatus* After 24 Hours Exposure Period

Concentration (ppm)	Plant part used		
	Fruits (Mortality %)	Leaves (Mortality %)	Bark (Mortality %)
(Control)	0.80 ± 1.78	0 ± 0	0 ± 0
50 ppm	1.60 ± 2.2	17. 60 ± 6.0	17.60 ± 7.3
100 pmm	10.40 ± 7.3	24.0 ± 5.7	31.20 ± 5.2
500 ppm	20.0 ± 10.9	36.80 ± 4.4	48 ± 4.0
1000 ppm	24.0 ± 5.7	50.40 ± 4.6	60.80 ± 7.2
1500 ppm	44.0 ± 10.2	64 ± 4	77.20 ± 6.3
2000 ppm	61.6 ± 7.3	81.60 ± 4.6	88.0 ± 6.3

Values are means and standard deviations of means of five replicates

Table 3. LC 50 Values of Aqueous Extracts of Different Parts of *Melia azedarach* Against 3rd and 4th Instar Larvae of *Culex quinquefasciatus* after 24 Hours Exposure Period

Plant Part	LC 50 (ppm)	95 Confidence interval		df	X^2
		Lower bound	Upper bound		
Fruits	2035.13	1002.8	17393.4	4	24.968
Leaves	612.250	291.6	1484.3	4	18.276
Bark	368.3	94.9	639.8	4	13.730

LC 50: Extract concentration that kills 50% of *Culex quinquefasciatus* larvae.

Table 4. Evaluation of Aqueous Extracts of Different Parts of *Melia azedarach* for Larvicidal Activity at Various Doses Against *Culex quinquefasciatus* After 24 Exposure

Plant Parts	Concentration					
	50 ppm	100 ppm	500 ppm	1000 ppm	1500 ppm	2000 ppm
Fruit	1.60 ± 2.2[a]	10.40 ± 7.3[a]	20 0 ± 10.9[a]	24.0± 5.7[a]	44.0 ±10.2[a]	61.60 ± 7.3[a]
Leaves	17.60 ±6.06[b]	24.0 ± 5.7[b]	36.80 ± 4.4[b]	50.40 ±4.6[b]	64 ± 4[b]	81.60 ±4.6[b]
Bark	17. 60 ± 7.3[b]	31.20 ± 5.2[b]	48 ± 4.0[c]	60.80 ±7.2[c]	75.20 ± 6.3[c]	88.0 ± 6.3[b]

Values are mean and standard deviations of five replicates. The alphabetical order is according to increasing mean values.
Means sharing no letter in common are significantly different at P< 0.05.
Means sharing a letter in common are not significantly different at P< 0.05.

31.20 % mortality respectively. At this concentration leaves and fruit extracts showed similar (P > 0.05) larvicidal activity. Also at the concentrations of 500, 1000, 1500 and 2000 ppm the fruit extract showed less mortality compared to the leaves and bark extracts (P< 0.05). The leaves and bark extracts showed efficient larvicidal activity. The comparison of larvicidal activities of leaves and bark extracts at 500, 1000 and 15000 ppm showed that bark extract is more larvicidal compared to leaves extract (P < 0.05). At 2000 ppm concentration the leaves and bark extracts caused 81.60 ± 4.6 % and 88.0 ± 6.3 % mortality respectively. At this dose the leaves and bark extracts showed homogeneity (P> 0.05) in larvicidal activity.

DISCUSSION

In the present study the aqueous extracts of the leaves, fruits and bark of *Melia azedarach* L. were studied for larvicidal activity against *Culex quinquefasciatus* Say. The aqueous extract of each part showed dose dependent increase in larvicidal activity. Compounds isolated from *Melia azedarach* have received particular attention from applied entomologists because of their excellent insecticidal action [35]. The insecticidal effect of *Melia azedarach* could be attributed to the presence of limonoids [36-38]. The lemonoids isolated from *Melia azedarach* include melianoninol, melianone, melianol, meliandiol, meliantriol, toosendanin, trichilins, Salannin, nimbin, salannal, meliacarpinin, azedarachin and lignanes [39]. Most of these limonoids posses antifeedent property that lead to the killing of insects. The antifeeding action is caused by the destruction of the function of chemoreceptors or stimulating the deterrent receptors located in the medial or lateral sensillum styloconicum [35]. Limonoids alo posses poisonous effect on insects. They destroy the structure of integument and the alimentary canal. Limonoids cause the disorganization of the extracellular membrane layers and the basal portion of the epithelial cells of the stomach [40].

During the present study the aqueous extract of bark showed significantly higher larvicidal activity as compared to leaves and fruit. This can be attributed to the presence of larger quantity of limonoids in the bark part of *Melia azedarach* of Dir region. The insecticidal efficacy of a plant part may vary with the region. Many plants although non toxic under one set of conditions

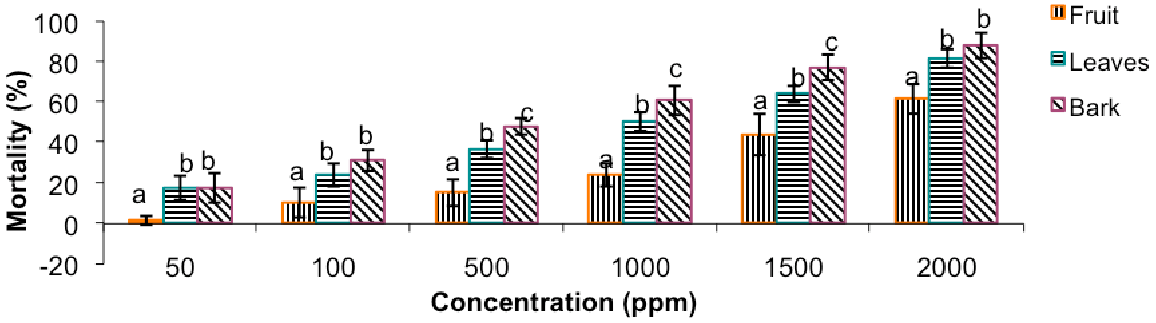

Figure 1: Comparison of larvicidal activities of aqueous extracts of different parts of *Melia azadarach* against *Culex quinquefesciatus.*

The alphabetical order is according to increasing mean values.
Means sharing no letter in common are significantly different at P< 0.05.
Means sharing a letter in common are not significantly different at P< 0.05.

can prove to be most toxic and noxious under another set of conditions [41].

The various solvent extracts of different parts of *Melia azedarach* are very effective against various pests [42]. The hexane extract of fruits of *Melia azedarach* has been reported to be strongly larvicidal against malaria vector *Anopheles stephensi* [43]. The methanol extracts of leaves and seeds of *Melia azedarach* have proved to be potent larvicidal against *Anopheles stephensi* [31]. The ethanol leaf extract of *Melia azedarach* has killed the larvae of dengue fever vector *Aedes aegypti* [30].

The larvicidal effects of *Melia azedarach* against *Culex quinquefasciatus* have been reported [32] but the present study differs from the previous studies in solvent and parts of the plant used for extract preparation.

The pesticidal action of *Melia azedarach* have also been tested against *Spodotera littoralis* (order Lepidoptera, family Noctuidae) [14] and *Boophilus microplus* (Acari: Ixodidae) [29].

Our findings, and those of earlier studies indicate that the plant *Melia azedarach* can serve as a good source of preparations for pest control especially mosquito control. In some areas of Swat and Dir, Khyber Pakhtunkhwa, the cattle occasionally feed on twigs of *Melia azedarach*. The plant is not toxic to vertebrates and is eco-friendly.

CONCLUSION

Among the aqueous extracts of fruits, leaves and bark, the aqueous extract of bark is the most efficient larvicidal against *Culex quinquefasciatus*.

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