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

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ABSTRACT: The plant *Artemisia vulgaris* is a perennial weed, belongs to the family Asteraceae, and locally known as Tarkha in Dir and Swat regions of Khyber Pakhtunkhwa (K.P.K), Pakistan. The present study aimed to evaluate the larvicidal activity of methanol extracts of roots, stem and leaves of *Artemisia vulgaris* against *Culex quinquefasciatus*. The 3rd and 4th instars larvae of *Culex quinquefasciatus* were exposed for 24 hours to various concentrations (50, 100, 500, 1000, and 1500 ppm) of methanol extracts of different parts of *Artemisia vulgaris*. The leaves extract resulted in significantly higher ($P<0.05$) mortality when compared to the mortality caused by the root and stem extracts. The LC_{50} value for roots extract was 9141.0 ppm, stem extract 2224.2 ppm and leaves extract 803.2 ppm. The findings of the present study presented the methanol extract of the leaves of *Artemisia vulgaris* as a good source of preparations for pest control especially mosquito control.

KEYWORDS: Methanol extracts, Mortality, LC_{50}, 3rd instars, 4th instars.

1 INTRODUCTION

The mosquito, *Culex quinquefasciatus* Say. is a painful and persistent biter and acts as a vector of *Wuchereria bancrofti* which causes lymphatic filariasis [1]. Lymphatic filariasis, commonly known as elephantiasis is a painful and profoundly disfiguring disease. According to WHO (2010) [2], 120 million people in tropical and subtropical areas of the world suffered from lymphatic filariasis. Among these infected people, about 25 million men suffered with genital disease most commonly hydrocele, and 15 million, mostly women, suffered with lymphoedema or elephantiasis of leg [2]. In Pakistan, imported cases of human filariasis were reported from Sindh province during 1969 by Wolf and Aslamkhan [3]. In 2001, confirmed cases of tropical pulmonary eosinophilia (TPE) were reported in indigenous patients; however the disease is rare in Pakistan [4]. Other species of mosquitos are also constant threats to man by manifesting different diseases including malaria, dengue, yellow fever and certain other mosquito- borne viral diseases.

The increasing incidence of mosquito-borne diseases is due to the availability of breeding places and the increasing resistance of mosquitoes to current commercial insecticides [5]. For the control of mosquito-borne diseases, especially the vectors (mosquitoes) should be eradicated. In mosquito control program, a more efficient and attractive approach is to kill them in larval stages [6]-[7]. Various synthetic insecticides are widely used for controlling adult and larval mosquito populations. Larvicides are used for controlling mosquito population in standing water. There are three main groups of mosquito larvicides; natural organic, synthetic organic and inorganic, which are further classified on the basis of the way in which the chemical compounds enter into the body of the insect. The larvicides that are ingested and absorbed through alimentary tract are called stomach poison e.g Paris green; the larvicides that penetrate into the body wall are called contact poisons e.g Pyrethrum, and those which enter through the spiracles are called respiratory poison such as Dichlorvos, a fumigant [8].

The constant use of chemical insecticides leads to the disruption of natural biological control systems and outbreaks of insect species [9]-[11]. This constant use of synthetic insecticides also leads to insect resistance, environmental pollution and

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undesirable effects on humans, mammals, and other non-target organisms [9]-[10]. Therefore there is increasing inclination towards alternative and sustainable methods of mosquito control which is eco-friendly [11].

Plant based insecticide are gaining attraction. Botanical based pesticides are effective, eco-friendly, easily degradable and also inexpensive [12]. Many plant species are known to have insecticidal properties [13]-[16]. Various types of plant preparations such as powder, solvent extracts and essential oils have been reported for their larvicidal activity against insect pests including mosquitoes. These plant preparations act as insect growth regulators, anti-feedants and insect repellents [17]-[19]. Several plants are known for their larvicidal activities, for example *Artemisia annua* Linn. [20], *Ageratina adenophora* (Spreng). king & H. Rob [21], *Artemisia parviflora* Buch. [22], *Sterculia guttata* Linn. [23], *Solanum xanthocarpum* Linn. [24] and *Melia azedarach* Linn. [25]-[30].

In the present study, the plant *Artemisia vulgaris* Linn. was selected for the larvicidal activity against *Culex quinquefasciatus*. The plant *Artemisia vulgaris* (mugwort or common wormwood) is a perennial weed, belongs to the family Asteraceae, growing wild and abundantly in temperate and cold-temperature zones [31]. The *Artemisia vulgaris* is locally known as Tarkha in Dir and Swat regions of Khyber Pakhtunkhwa, Pakistan. Different parts of *Ar. vulgaris* have also been reported for their antibacterial and anti-viral activities [32]. This plant is also known for its insecticidal and insect repellent property [33]. Its essential oils have shown insecticidal activity against *Aedes aegypti* [34]. The branches and leaves of *Artemisia vulgaris* have been reported for repelling insects and rats from granaries [35]. Pugazhvendan et al. (2012) [36] studied the insecticidal and repellent activity of *Artemisia vulgaris* against the stored grain pest, *Tribolium castaneum.*

1.1 AIMS

The present study aimed to point out the efficient and potent larvicidal part of *Artemisia vulgaris* by evaluating the larvicidal activities of methanol extracts of its root, stem and leaves, against a mosquito, *Culex quinquefasciatus*. The objective of the study was to contribute further to the knowledge of use of eco-friends, the herbs, against the most frequently occurring mosquito, *Culex quinquefasciatus*, which is a persistent bitter and vector of filariasis.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

The leaves, roots and stem of *Artemisia vulgaris* (L) were collected from the area of Bandagi Talash, Dir (L), Khyber Pakhtunkhwa, Pakistan, during May, 2012. The plant was identified by Dr. Nasurallah Khan, Assistant professor, Department of Botany, University of Malakand, Khyber Pakhtunkhwa (K.P.K), Pakistan.

2.2 EXTRACTION

The root, stem and leaves of *Artemisia vulgaris* were cleaned and shade dried. The stem, roots and leaves of the plant were separately ground and their powder forms were obtained. The powdered forms of roots, stem and leaves in amount of 64, 47 and 55gm respectively were socked in methanol for seven days. The volume of methanol used was 300ml for roots powder, 560 ml for stem powder and 300 ml for leaves powder. The socked materials were separately filtered through muslin cloth. Each filtrates was then separately evaporated on a rotary evaporator (Heidolph Laborata efficient), under reduced pressure at 40^0C and a thick solution of each filtrate was obtained. Each solution was then transferred to a separate petridish and the remaining solvent was evaporated by placing the petri dishes under running fan in shade. The dry weight of stem extract was 1.42g (3.02 %), roots extract 1.12g (2.03 %) and leaves extract was 5.12g (8 %). The extracts were stored in refrigerator.

2.3 TEST ORGANISM AND LARVICIDAL BIOASSAY

The 3^{rd} and 4^{th} instars larvae of Culex mosquito were collected in a large plastic jar with the help of a glass dropper from a ditch of stagnant water at the campus of University of Malakand, and brought to the laboratory. A stock solution of 5000 ppm of each extract was prepared in distilled water. From the stock solution of each extract, 100 ml each of 50, 100, 500, 1000 and 1500 ppm, were prepared in different 400ml plastic cups, which were labeled accordingly. For larvicidal activity of each extract, 20 larvae of late 3^{rd} and early 4^{th} instars were transferred to each plastic cup with the help of a large mouthed glass dropper. Larval food consisting of finely ground brewer's yeast and dog biscuit in 3:2 was added to each cup. A control was also maintained by adding only larval food. All the experiments were carried out at 35 ± 3˚C and 65-70 % relative humidity. The larvae were exposed to the extract solution for 24 hours. After 24 hours, the dead 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 [37]-[38]. The larvae that remained alive were killed by transferring to petridish containing hot water and then identified up to species. All the larvae were belonging to the mosquito species, *Culex quinquefasciatus.*

2.4 STATISTICAL ANALYSIS

The larvicidal activities of extracts were presented as percent mortality of larvae by using the following formula; percent mortality = Number of dead larvae ⁄ Total exposed larvae X 100.The results were presented as mean ± standard deviation of means of three replicates. The dose-response data were 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 Larvicidal activities of methanol extracts of leaves, root and stem, 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.

3 RESULTS

The larvicidal activity of various concentration of methanol extracts of different parts of *Artemisia vulgaris* were studied against *Culex quinquefasciatus.* There occurred a continuous increase in mortality of 3^{rd} and 4^{th} instars larvae with increase in concentration of the extracts. The mortality caused by each of the roots, stem and leaves methanol extract, was subjected to linear regression analysis. The value of R square for roots, stem and leaves was 69.9, 72.5 and 96.2 respectively (Table 3.1). The results of linear regression analysis clearly indicated the relationship between the increase in concentration of extracts and the increase in larvicidal activity. Maximum linearity (R square = 96.2) was shown by the leaves extract. The LC_{50} values of different parts of *Artemisia vulgaris* with 95 % confidence interval are shown in table 3.2. The LC_{50} values of roots, stem and leaves extracts were 9141.0ppm, 2224.2ppm and 803.2ppm respectively. These results indicated the leaves extract as efficient larvicidal against *Culex quinquefasciatus.*

The larvicidal activities of roots, stem and leaves extracts were compared statistically (Table 3.3). At the concentrations of 50 and 100ppm, the leaves extract caused non-significantly higher (P>0.05) mortality. At 500, 1000 and 1500 ppm, the larvicidal activity of leaves extract was significantly higher (P<0.05) as compared to the larvicidal activities of roots and stem extracts.

Table 3.1.Linear regression analysis of larvicidal activities of methanol extracts of Artemisia vulgaris against Culex quinquefasciatus after 24 hours exposure

Model	R	R square	Significance
Root	69.9	48.9	0.122
Steam	72.5	52.6	0.103
Leaves	96.2	92.6	0.002

Table 3.2.The LC_{50} values of methanol extracts of different parts of Artemisia vulgaris against* 3^{rd} and 4^{th} *instar larvae of Culex quinquefasciatus after 24 hours exposure period

Plant Parts	LC_{50} (ppm)	95 Confidence interval		df	X^2
		Lower bound	Upper bound		
Root	9141.0	3457	78179.6	3	0.129
Stem	2224.2	1461.12	8320.60	3	0.554
Leaves	803.2	602.9	1146.03	3	2.728

LC_{50} -extract concentration that kills 50 % of *Culex quinquefasciatus* larvae.

4 DISCUSSION

In the present study the larvicidal potentials of methanol extracts of roots, stem and leaves of the plant *Artemisia vulgaris* (Linn) were evaluated against a culicine mosquito, *Culex quinquefasciatus* Say. The extract of each part showed dose dependent increase in larvicidal activity. At higher doses, the leaves extract caused a significantly higher mortality against the 3rd and 4th instar larvae of *Culex quinquefasciatus.*

Documented reports are available about the insecticidal potential of *Artemisia vulgaris.* Lavor et al. (2012) [39] studied the larvicidal activities of essential oils from the leaves of *Artemisia vulgaris*, *Cymbopogon flexuosus* (Nees ex Steud.) Wats. and *Piper tuberculatum* Jacq. against *Aedes aegypti*. The essential oil of *P. tuberculqatum* had the lowest LC50 value (106.3 +/- 2.2 microg/mL), followed by *A. vulgaris* (114.1 +/- 1.7 microg/mL) and *C. flexuosus* (121.6 +/- 0.8 micro/mL). Thujone, an active component of essential oils, extracted from *A. vulgaris* and other plants of the genus *Artemisia*, is a cyclic ketone and insecticidal [40]-[41]. Pugazhvendan et al. (2012) [36] studied the insecticidal and repellent actions of *A. vulgaris* and some other plant extracts against stored grain pest, *Tribolium castaneum* (Herbst). It was suggested that the plant extract contain actives toxic substances acting after consumption or topical application. The insecticidal activity of Artemisia *vulgaris* extract has also been reported against *Sarcoptes scabiei* [42]. Jogar et al. (2006) [43] studied the effects of *A. vulgaris*, *A. absinthum* and some other plants extracts, on the development and physiological state of Lepidoterous insects. The respiration and transpiration systems of the insects were the vulnerable targets for extracts [43]. The plant extracts caused a loss in discontinuous gas exchange cycles (DGCs) which resulted in water loss and death of insect [43].

Other plants of the genus *Artemisia* have also been reported for their larvicidal activity. Abd-Elhady et al. (2012) [44] reported the insecticidal properties of essential oil derived from aerial parts of *Artemisia judaica* (Linn), against the cowpea weevil, *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae). Sharifian et al. (2012) [45] reported the insecticidal activity of *A. herba-alba* oil against *Tribolium castaneum* (Herbst), *Callosobruchus maculatus* (F.) and *Rhyzopertha dominica* (F). Panneerselvam et al. (2012) [46] reported the larvicidal, pupicidal, adulticidal, and repellent activity of *Artemisia nilagirica* against the mosquitoes, *Anopheles stephensi* and *Aedesa egypti,* and suggested the leaf extract as a potent larvicidal.

Artemisia extracts contain secondary metabolites mainly monoterpenoids such as vulgarole, spathulenol, vulgarin, triterpenoide: α-amyrin, α-amryin acetate and fernenol [47]-[48]. The insecticidal properties of *Artemisia vulgaris* and other plants of the genus Artemisia have been attributed to the presence of these secondary metabolites. Huwang et al. (1985) [49] isolated and identified mosquito repellent compounds in *Artemisia vulgaris* against *Aedes aegypti*. The compounds isolated were mainly monoterpenoids such as linalool, camphor, isoborneol, borneol, terpinen-4-ol, isobornyl, Nonanone-3, (α +β)-thujone, bornyl acetate, β-Pinene, myrcene, α-terpinene, limonene, and cineote [49].

Table 3.3.Comparison of larvicidal activities of methanol extracts of different parts of Artemisia vulgaris against Culex quinquefasciatus after 24 hours exposure

Concentration (ppm)	Root (Mortality %)	Stem (Mortality %)	Leaves (Mortality %)	F Value
50 ppm	9.30 ± 2.85[a]	7.5 ± 2.85[a]	11.65 ± 2.85[a]	0.333
100 ppm	13 ± 10[a]	11 ± 5[a]	17 ± 5[a]	0.500
500 ppm	22 ± 8.65[a]	17.23 ± 10[a]	38.3 ± 7.85[b]	4.32
1000 ppm	28.3 ± 7.635[a]	21.65 ± 11.545[a]	49.65 ± 23.6[b]	10.81
1500 ppm	33.3 ± 10.4[a]	28.3 ± 7.6[a]	66.3 ± 8[b]	44.33

Values are mean and standard deviation of means of three replicates. Means sharing no letter are significantly different at $P<0.05$. Means sharing a letter in common are not significantly different at $P<0.05$.

During the present study, the larvicidal activity of the methanol extracts of roots, stem and leaves of *Artemisia vulgaris* was studied against the mosquito *Culex quinquefasciatus*. Many other plants have also been reported for their larvicidal activity against *Culex quinquefasciatus*. Ilahi et al. (2012) [30] studied the larvicidal activity of aqueous extracts of bark, fruits and leaves of *Melia azedarach* (Linn) against *Culex quinquefasciatus* at the concentrations of 50, 100, 500, 1000, 1500 and 2000 ppm. Among these extracts, the bark extract caused significantly higher mortality of 3rd and 4th instar larvae of Culex *quinquefasciatus*. Tennyson et al. (2012) [50]reported the larvicidal activity of *Aegle marmelos* (Linn), *Alstomia scholaris* (Linn), *Aristolochia indica* (Linn), Cassia fistula (Linn), *Cinnamomum zeylanicum* (Breyn), *Cleistanthus collinus* (Roxb) and several other plants against *Culex quinquefasciatus*. Sakthivadivel et al. (2012) [51] reported the larvicidal activity of *Argemone mexicana* (Linn), *Clausena dentate* (Wild) M. Roem, *Sepadessa baccifer* (Roth), *Dodonaea angustifolia* (Linn) and *Melia dubia* (Cav) against *Culex quinquefasciatus*. Other plants that possess larvicidal activity against *Culex quinquefasciatus* include *Citrus aurancium* (Linn) [52], *Jatropha curcas* (Linn), *Euphorbia hirta* (Linn) [53], *Aloe barbandensis, Cannabis sativa* [54], *Ocimum basilicium* (Linn) [55], *Solanum nigram* (Linn) [56] and *Feronia limonia* (Linn) [57].

During the present study, the methanol extract of leaves of *Artemisia vulgaris* showed significantly higher larvicidal activity against *Culex quinquefasciatus*. The methanol extracts of other parts of this plant were weak larvicidal. The higher insecticidal potential of leaves extract presents the *Artemisia vulgaris* leaves as the rich source of toxic metabolites.

4.1 Conclusion and Future Recommendations

From the findings of the present research it was concluded that the methanol extract of *Artemisia vulgaris* leaves is the most efficient larvicidal against the mosquitoes and eco-friendly. Further research is needed on activity oriented fractionation, qualitative and quantitative screening of insect repellent, insect growth regulators and antifeedent metabolites which may contribute to the enhanced quality measurements of humanity against mosquitoes.

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