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EVALUATION OF MOSQUITOCIDAL ACTIVITY OF WATER EXTRACT OF *MORINGA OLEIFERA* SEEDS AGAINST *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE) IN PAKISTAN

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ABSTRACT

The water extract of *Moringa oleifera* seeds was evaluated for larvicidal, pupicidal and repellent effect against the *Culex quinquefasciatus* (Diptera: Culicidae). Larvicidal effect of the treatments was assessed at different concentrations (0, 30, 60, 90 and 120 mg/L) after 6, 12, 18 and 24 h of the exposure against 2nd and 4th instar larvae, following the procedure of WHO with some modifications. The pupicidal activity was checked at same dose rates after 48 h of the treatments. The repellent activity of the extract was performed with human volunteers and dose-response method was used to estimate the percentage protection. The results indicated significant effect of the treatments and the effectiveness increased with extended exposure interval and enhanced dose rates. The findings of the present study clearly revealed that water extract of *M. oleifera* seeds is an effective agent for the control of *C. quinquefasciatus* at its larval and pupal stage and exhibits good repellent action against adults, however, the efficacy depends upon dose rates and the exposure interval.

Keywords: *Culex quinquefasciatus*, Mosquito, *Moringa oleifera*, Seed extract, Mortality

INTRODUCTION

Moringa oleifera is the most widely cultivated plant species worldwide (Santos *et al.*, 2009) originating from western and sub-Himalayan region, Pakistan, India, Africa, Asia minor and Arabia (Mughal *et al.*, 1999). Since then, it is distributed in Cambodia, Philippines, Caribbean Islands and Central North and South America (Morton, 1991). It is important in regards to health point of view, for instance, the seeds exhibit anti-inflammatory, anti-tumor (Cáceres *et al.*, 1992), antimicrobial (Chuang *et al*., 2007), antispasmodic and diuretic properties (Babu and Murugan, 1998).

The plant extracts exhibit properties vis ovipositional, larvicidal, repellents, deterrents and insect growth regulators, (Coelho *et al.*, 2009; Freitas *et al.*, 2010; Ravikumar *et al.*, 2011). Extract of *M. oleifera* seed has showed a great potential as larvicidal and pupicidal agent against mosquitoes (Leite *et al*., 2005; Prabhu and Murugan, 2011). Similarly Several lectins extracted from *M. oleifera* seeds have showed efficacy against different life stages of several insect orders such as Coleoptera (Sauvion *et al*., 2004; Macedo *et al*., 2007), Hemiptera (Sá *et al*., 2009), Diptera (Bandyopadhyay *et al*., 2001), Homoptera (Couty *et al*., 2001), Isoptera (Silva *et al*., 2009), Hymenoptera (Coelho *et al*., 2007), and Lepidoptera (Phasomkusolsil and Soonwera, 2010). Essential oils of a number of plants exhibit mosquito larvicidal properties (Bilal *et al*., 2012) and are used to treat many diseases.

Mosquitoes are important vector of various diseases of economic importance in humans causing nuisance, local skin and systemic reaction (Govindarajan, 2010) and death in acute cases. *C. quinquefasciatus* serves as a vector of filariasis which is fastest spreading disease of man in Tropical region resulting about 146 million affected people every year (WHO, 1992). In Pakistan, during summer months, a high level of population buildup of *C. quinquefasciatus* has been recorded in various cities (Tahir *et al*., 2009). Pakistan is at high risk of large epidemics because of over crowded cities and unhygienic conditions. The misuse of insecticides in agriculture and public health programs raised many health and environment related problems like insecticide resistance, resurgence of pest species, toxic hazards to humans and other non-target organisms and environmental pollution (Junwei *et al*., 2006). In order to overcome these hazards, researcher should innovate biological or plant based products that can provide an effective alternative approach to synthetic insecticides (Olaifa *et al*., 2003). Keeping in view all of these facts, present study was planned to investigate the insecticidal efficacy of *M. oleifera* extract against *C. quinquefasciatus*.

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The experiment was aimed to evaluate the larvicidal, pupicidal and repellent activity of the water extract of *M. oleifera* seeds against *C. quinquefasciatus*.

MATERIALS AND METHODS

Preparation of seed extracts

Seeds of *M. oleifera* were collected from the University of Agriculture, Faisalabad, Pakistan. Healthy seeds, excluding the seeds with visual damage, were selected for the preparation of extracts. The collected seeds were dried under shade, dehulled and made as the powder using pestle and mortar. The powder was placed in desiccators for later use in stock solution preparation. Stock solutions of *M. oleifera* seed powder was prepared by addition of 1 g of powder in 1 liter of distilled water (WHO, 1996). The mixture was stirred for 45 minutes at 25°C with the help of magnetic stirrer followed by filtration through Whatman No. 1 filter paper.

Mosquito culture

The larvae of *C. quinquefasciatus* were collected from different breeding sites with in Faisalabad, Pakistan. Culture was maintained in the laboratory of Department of Agri. Entomology, University of Agriculture, Faisalabad (Pakistan). Larvae were reared in plastic trays (30×24×5 cm) containing tap water and the rearing water was changed daily. The larvae were fed with powdered yeast and the colony was kept at 25±2°C with 80-90% r.h under a photoperiod of 12:12 h. until the formation of pupae. The pupae were shifted to cups containing tap water and placed in wire gauze cages (35×25×20 cm dimension) where the adults emerged. After 72 h, the ovitraps were kept in the cages; the eggs were collected and transferred to plastic trays.

Larvicidal effect

The insecticidal effect of *M. oleifera* seed extract against 2nd and 4th instar larvae of *C. quinquefasciatus* was evaluated following WHO method (1996) with some modifications. Twenty five (25) larvae for each instar were released into 500 ml beaker containing 250 ml water. The seed extract was applied at the rate of 0, 30, 60, 90 and 120 mg/L and each treatment was replicated five times. The data for dead larvae was taken after 6, 12, 18 and 24 h of exposure intervals.

Pupicidal effect

For pupicidal effect, 15 freshly emerged larvae were shifted into sterilized plastic cups of 100 ml capacity, holding 75 ml water. The water extract of *M. oleifera* seeds was added at the concentrations of 0, 30, 60, 90 and 120 mg/L. Each treatment was replicated five times and the number of emerged adults for each replication was recorded after 48 h.

Repellent effect

The repellent effect of *M. oleifera* seeds extract was observed with human volunteers and percent protection to dose-response method (Ringu-perez *et al*., 1997) was followed for this purpose. Fifty, 3-4 days old starved female mosquitoes were kept in net cages and the arms of volunteers were cleaned with ethanol. The cleaned arms were air dried and only 25 cm^2 of the dorsal side of the skin was exposed for each arm. The arms treated with seeds extract (0.3, 0.6, 0.9 and 1.2 mg/cm^2) were exposed simultaneously into the cages and the total number of bites was counted over 5 min after every 30 min, from 06:00 to 08:30 am. The experiment was conducted for four times and the percentage protection (repellent activity) was calculated by using following formula with modification (Prabhu and Murugan, 2011).

$$\%\text{Protection} = \frac{\text{Bites on control arm} - \text{Bites on treated arms}}{\text{No. of bites received by control arm}} \times 100$$

Statistical analysis

The observed mortality was corrected by Abbot formula (Abbott, 1925) and data for mortality were analyzed through one way analysis of variance for each developmental stage with dose as main effect. Means were compared using LSD test at 5% level of significance.

RESULTS

Larvicidal activity

The insecticidal nature of seed extracts of *M. oleifera* showed greater impacts on the development of second and fourth instar larvae of *C. quinquefasciatus* and hence displays significant mortality. Severe toxic effects were observed showing an increasing trend towards higher concentration in dose dependent manner. A complete control over the larvae was recorded in case of higher dose of extract and overall larval mortality ranged from 9.36 to 98.89% for second instar which was significantly higher in all treatments (F=8.01, $P \leq$ 0.00 at 6h, F=23.4; $P \leq 0.00$ at 12h, F=23.9; $P \leq 0.00$ at 18h and F=17.5; $P \leq 0.00$ at 24h) when compared with control. The highest dose rate of water extract of *M. oleifera* seeds (120 mg/L) caused highest mortality at all intervals and showed 25.94±4.15, 50.31±2.83 and 71.66±1.90% larvicidal effect after 6, 12 and 18 h, respectively which approached to 98.89±0.54% after 24 h of treatment (Figure 1).

Development of 4th instar larvae of *C. quinquefasciatus* was also deterred and similar insecticidal nature of seed extracts of *M. oleifera* was observed. A significant level of mortality was recorded in case of 4th instar larvae of *C. quinquefasciatus* (F=7.78; $P \leq 0.00$ at 6h, F=24.3; $P \leq 0.00$ at 12h, F=14.6; $P \leq 0.00$ at 18h and F=12.3; $P \leq 0.00$ at 24h) when water extract of *M. oleifera* seeds was used but range of mortality (3.60 to 83.98%) was lower than for 2nd instar larvae. Minimum level of larvicidal activity (3.60±1.33%) was recorded with application of lowest dose (30 mg/L) after 6 h and the highest level (83.98±5.94%) was encountered after 24 h at 120 mg/L of water extract of *M. oleifera* seeds (Figure 2).

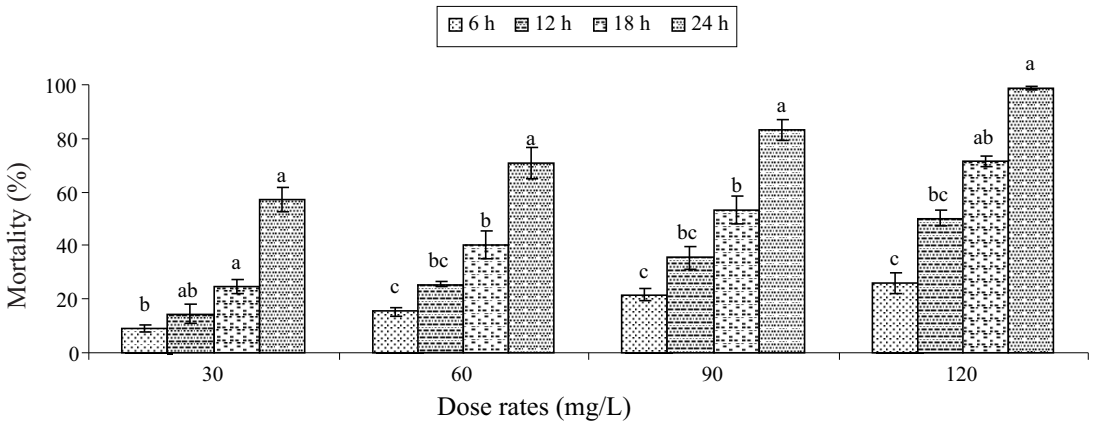

Fig. 1
Larvicidal activity (%mortality ± SE) of water extract of *M. oleifera* seeds after 6, 12, 18 and 24 h of treatment against second instar larvae of *Culex quinquefasciatus*; within each column, means followed by the same letter are not statistically different.

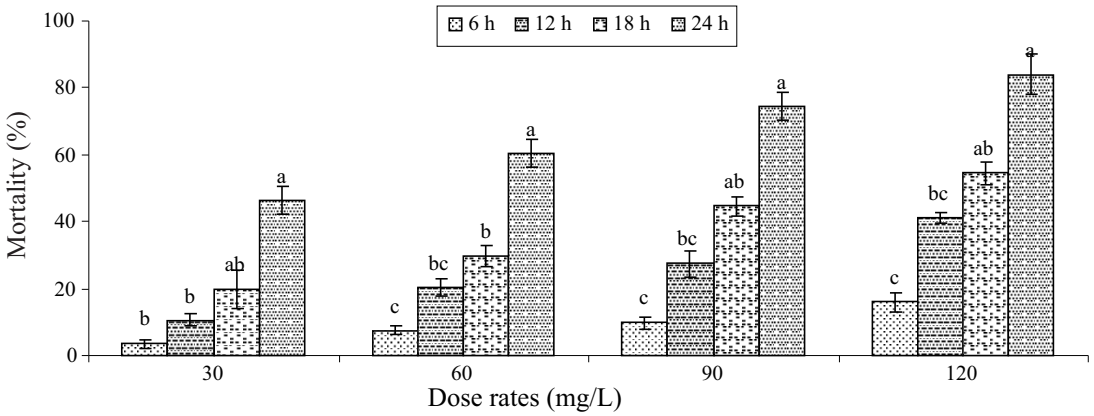

Fig. 2
Larvicidal activity (%mortality ± SE) of water extract of *M. oleifera* seeds after 6, 12, 18 and 24 h of treatment against fourth instar larvae of *Culex quinquefasciatus*; within each column, means followed by the same letter are not statistically different.

Pupicidal activity

A significant level of pupicidal effect was encountered when water extract of *M. oleifera* seeds was used against pupae of *C. quinquefasciatus.* Emergence of the adults from pupae was greatly affected and appreciable level of reduction in pupal survival (F=48.9; $P \leq 0.00$) was recorded using different levels of seed extracts. Minimum percentage of pupal survival was observed at highest dose rate (120 mg/L) of water extract of *M. oleifera* seeds where only 30.62±3.16% of pupae were able to survive and to grow into adults. A dose dependent response was observed and minimum level of pupal survival was found in case of the lowest dose rate (30 mg/L) where percentage of pupal survival (78.07±4.53%) was found higher than the remaining dose rates (Figure 3).

Repellent action

Appreciable level of repellency was recorded in adults of *C. quinquefasciatus* which ensures presence of compounds that render the adults away. This repellency was also found to be dose dependent and successful action was recorded for many hours. Up to 150 minutes after application, a successful level of repellence was observed. Repellency was found to be 100% after 30 minutes of application which gradually decreased with time (F=20.1; $P \leq 0.00$ at 60 min, F=15.1; $P \leq 0.00$ at 90 min, F=14.0; $P \leq 0.00$ at 120 min and F=13.4; $P \leq 0.00$ at 150 min). A 100% repellency was recorded at the start which gradually decreased and lasted for 90.33±2.35% at highest dose rate (1.2 mg/L) up to last observation in the study (Table 1) which highlights the importance of water extract of *M. oleifera* seeds that can be used successfully as a repellent against mosquitoes for many hours without losing its efficacy.

DISCUSSION

The findings of the present study confirm the presence of active compounds which are responsible for insecticidal characteristics of seed extract of *M. oleifera*. The level of larvicidal activity and hence the mortality encountered was strictly found to be dose dependent. Second instar larvae of *C. quinquefasciatus* were relatively more susceptible than fourth instar larvae as higher level of mortality was documented in present study against second instar larvae. Similar findings of

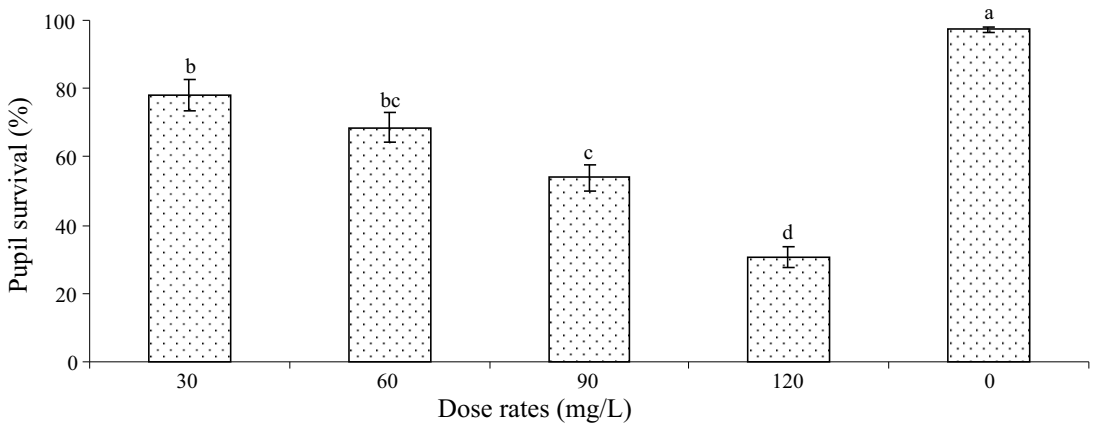

Fig. 3
Pupicidal effect (%pupal survival ± SE) of water extract of *M. oleifera* seeds at different dose rates (0, 30, 60, 90 and 120 mg/L) against pupae of *Culex quinquefasciatus*; means followed by same letters are not statistically different.

Table 1
Repellent effect (% repellence) of water extract of *M. oleifera* seeds against *Culex quinquefasciatus.*

Dose rate (mg/L)	Exposure interval				
	30 min	60 min	90 min	120 min	150 min
0.3	100.00±0.00a	80.57±3.00b	73.71±3.93c	58.85±7.07c	45.07±4.58c
0.6	100.00±0.00a	87.52±2.79b	82.60±3.87bc	70.95±5.48bc	56.91±8.63bc
0.9	100.00±0.00a	98.74±0.63a	91.99±1.99ab	86.71±3.05ab	73.77±3.91ab
1.2	100.00±0.00a	100.00±0.00a	100.00±0.00a	99.37±0.63a	90.33±2.35a
df	3,11	3,11	3,11	3,11	3,11
F	-	20.1	15.1	14	13.4
P	-	0.0004	0.0012	0.0015	0.0017

Within each column, means followed by the same letter are not significantly different.

increased mortality with increased dose rate were also reported by Ferreira *et al*. (2009) who found 99.2±2.9% larval mortality within 24 h at 5200 µg/mL of water extract of *M. oleifera* at the highest application rate. Parallel to present study are the findings of Prabhu and Murugan (2011) who reported that LC_{50} and LC_{90} increased from 1st to 4th instar which ensured that susceptibility decreased from 1st to 4th larval instar and hence mortality also decreased. Maheswaran and Ignacimuthu (2011) used different herbal plant extracts against *Ae. aegypti* and *Ae. albopictus* and found 100% larvicidal effects of the test substances which confirmed the findings of the present study. The larval mortality obtained in the present study may be contributed by certain active chemical compounds present in *M. oleifera* like Quercetin and kaempferol are flavonoids, which are the compounds of phenolic hydroxyl groups, antioxidant in action and of potential therapeutic uses (Pace-Asciak *et al*., 1995).

A significant reduction in survival of *C. quinquefasciatus* pupae was caused by all application rates of water extract of *M. oleifera* seeds, which is according to the findings of Prabhu and Murugan (2011) who observed a significant pupicidal effect of plant extracts of *M. oleifera* against *Anopheles stephensi* and pupal mortality of greater than 70% was encountered. The pupae of *Ae. aegypti* were found greatly susceptible to higher dose (230 ppm) of plant extract *Catharanthus roseus* which caused mortality of 79% (Remia and Logaswamy, 2010) confirms the findings of the present study. The repellency (78.66%) was found against *Ae. aegypti* using crude seed extract of celery, *Apium graveolens* and a protection period of about 3 hours (Choochote *et al*., 2004). The similar results were reported using *Solanum tribolium* leaf extract against blood starved adult female mosquitoes (Venkatachalam and Jebanesan, 2001; Rajkumar and Jebanesan, 2004).

The repellent action of plant extracts depends on the dose rate of extract and nature of solvent used (Mandal, 2012). This is supported by many researches who have demonstrated the improved repellency of plant derived topical repellent products after formulation with some bases or fixative materials such as liquid paraffin (Oyedele *et al*., 2002), vanillin (Tawatsin *et al*., 2001) and mustard and coconut oils (Das *et al*., 1999). The better protection from mosquitoes is evidently demonstrated using *Zanthoxylum piperitum* oil after the addition of vanillin (Kamsuk *et al*., 2007).

The findings of the present study revealed that seed extract of *M. oleifera* exhibited excellent larvicidal, pupicidal and repelling action against *C. quinquefasciatus* and can effectively be used to over the awe of dengue fever vector

which now a days is an epidemic disease causing number of casualties in Pakistan. Further studies about effective mode of action, discovery of procedures to enhance and stabilize the toxic contents, techniques required to prepare a proper formulation may, in future, help to root out this havoc.

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