International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 7 No. 4 Aug. 2014, pp. 1633-1641

http://www.ijias.issr-journals.org/

Phytotoxicological assessment of Moringa oleifera Lam. against larvae of important human malaria vector Anopheles *stephensi* Liston (Insecta:Diptera:Culicidae)

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ABSTRACT: Application of plant extracts have become an available alternative in sustainable vector control strategy due to their less toxic, easily available and non persistant nature. In the present study, leaves, flowers and seeds of *Moringa oleifera* Lam. are evaluated for their larvicidal activities against *Anopheles stephensi* (L). The larval mortality was observed at 24hrs and 48 hrs of time exposure. Highest larval percent mortality was observed to be found in the seed extract of *Moringa oleifera* Lam. plant with 100% mortality at 100 ppm of concentration. LC_{50} value was calculated against different concentrations. The lowest LC_{50} value was observed in the *M. oleifera* Lam. seeds followed by flowers and leaves i.e. 15.17 ppm, 23.99 ppm and 38.99 ppm respectively after 24 hrs of exposure time and 12.62 ppm, 20.46 ppm and 25.11 ppm. respectively at 48 hrs of exposure time. The plant extracts also exhibited some developmental deformities in larvae and pupae. The obtained data indicates that phytochemical derived from *M. oleifera* Lam. seed extracts are effective mosquito vector control agent.

KEYWORDS: *Moringa oleifera* Lam., *Anopheles stephensi*, Insecticidal, Phytochemicals.

1 INTRODUCTION

Vector and vector-borne diseases have become a challenging problem to public health in these days as it has social and economical impact. Insect-transmitted diseases remain a major cause of illness and death worldwide (Pavela 2009)). Mosquitoes are vectors of several diseases affecting humans and domestic animals around the world and are the major vectors for the transmission of malaria, dengue, yellow fever, filariasis, Japanese encephalitis, *etc*., causing millions of deaths every year (James 1992). In the last 50 years, insect pests have mainly been controlled with synthetic Insecticides. However, one major drawback with the use of these chemical insecticides is that these are non-selective and could be harmful and adversely affect the other organisms in the environment (Omena et al. 2007). Plant products have been used traditionally by human communities in many parts of the world against the vectors and the different species of insects**.** Phytochemicals explored from plant sources can act as larvicides and can be responsible for the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community level (Govindrajan et al. 2008)**.**

Moringa oleifera Lam. (drumstick tree, horseradish tree) is indigenous to northwestern India. The tree is valued mainly for the tender pods, which are esteemed as a vegetable (Ramachandran et al. 1980). A paste of the leaves is used as an application for wounds. Moreover, the leaves are a good source of essential amino acids such as methionine, cystine, tryptophan, and lysine with a high content of proteins (Makkar and Becker 1997). Decoctions and extracts made from these leaves are also variously employed in traditional medicine (Morton 1991). Pal et al., 1995 have reported that the methanol fraction of *Moringa* leaf extract possesses antiulcer activity against induced gastric lesions in rats. On the other hand, pressed juice of the fresh leaves shows strong antibacterial activity. Nonetheless, the flowers of *M. oleifera* are considered to possess

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medicinal value as a stimulant, diuretic, and cholagogue, and they have been also reported to contain flavonoid pigments such as quercetin, kaempferol, rhamnetin, isoquercitrin, and kaempferitrin (Nair et al. 1962). One of the best uses in the west of moringa is to flocculate contaminants and purify drinking water with its powered seeds. The seeds possess antimicrobial properties (Ali et al. 2004), ovicidal and larvicidal effects on *Ae. aegypti* L. (Paulo et al. 2009). Njom et al., 2011 reported the toxicity and growth regulatory effects of Moringa oleifera seed extracts were assessed on third larval instars of Anopheles gambiae. Biological effects of the water extract of *Moringa oleifera* seeds (WEMOS) were assessed on eggs and 3rd instar larvae of *Aedes aegypti* by Paulo et al. 2009.

Information on effects of *Moringa oleifera* Lam. on the malaria vector *Anopheles stephensi* (L) have been limited in this part of the world despite the havoc the vector is causing. This research amongst others intends to assess the toxicity and growth regulatory effects of *Moringa oleifera* Lam. on the larvae of *Anopheles stephensi* (L.) (Diptera: Culicidae). The present study was conducted to evaluate the mosquito larvicidal activity of leaves, flowers and seeds of *Moringa oleifera* Lam. against human malaria vector *Anopheles stephensi* (L).

2 MATERIALS AND METHODS

2.1 COLLECTION OF PLANTS

Fully developed leaves, flowers and seeds of the *Moringa oleifera* Lam. were collected from in and around the Mohan lal Sukahadia University campus, Udaipur, Rajasthan, India. The dried plant materials were powdered by an electrical blender.

2.2 EXTRACTION

The plant parts were washed with tap water, shade-dried, and finely ground. The finely ground plant material powder (10g/ solvent) was loaded in Soxhlet apparatus (Vogel 1978). The solvents from the extracts were removed using a rotary vacuum evaporator to collect the crude extract. Standard stock solutions were prepared at 1% by dissolving the residues in methanol. From this stock solution, different concentrations were prepared and these solutions were used for larvicidal bioassays.

2.3 TEST ORGANISMS

The mosquitoes *Anopheles stephensi* (L), were reared in the Insect Microbial and Herbal Control laboratory, Department of Zoology, Mohan Lal Sukhadia University. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and rabbit for blood meal. Mosquitoes were held at 28±2°C, 70–85% relative humidity, with a photo period of 14-h light and 10-h dark.

2.4 LARVICIDAL BIOASSAY

The larvicidal activity of the plants crude extracts were evaluated as per the method recommended by World Health Organization (2005). Batches of 25 third instar larvae were transferred to small disposable test beakers, each containing 100 ml of water. The appropriate volume of dilution was added to 100 ml water in the beakers to obtain the desired target dosage, starting with the lowest concentration. Five replicates were set up for each concentration, and an equal number of controls were set up simultaneously using tap water. The control mortalities were corrected by using Abbott's formula (Abbott 1925). The LC_{50} and LC_{90} were calculated after 24 and 48 h by Probit analysis (Finney 1979).

$$\text{Percentage mortality} = \frac{\text{Number of dead larvaes}}{\text{Number of larvaes introduced}} \times 100$$

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

2.5 STATISTICAL ANALYSIS

The mortality observed (ppm) was corrected using Abbott's formula during the observation of the larvicidal potentiality of the plant extracts. Statistical analysis of the experimental data was performed with MS EXCEL 2003 to find the LC_{50}, regression equations (Y = mortality; X = concentrations) and regression coefficient values.

3 RESULTS

The extracts of different plant materials of *Moringa oleifera* Lam. has been studied for use as ecofriendly insecticide instead of eco enemy synthetic insecticide. The five different concentrations 20, 40, 60, 80, 100 ppm of leaves, flowers and seeds were tested against malarial vector *Anopheles stephensi* (L). The effect of larvicidal activity was demonstrated in the present study; confirm their potential for control of larval population. Effect of *Moringa oleifera* Lam. on the third instar larvae increased with the increase in the concentration. Larval mortality and LC_{50} value were observed. Regression analysis showed a concentration dependant significant correlation of the plant extract with larval mortality. Figure 1 shows the toxicity evaluation assay of *Moringa oleifera* Lam. leaves, flowers and seeds and their effect on *Anopheles stephensi* (L) larvaes. With regard to morphological abnormalities larval aberrations reached the highest values when treated with flower extracts. In the meanwhile, the morphological abnormalities were more pronounced with the seed extracts as compared to normal larvae. Several forms of morphological malformations resulted from the treatment of larvae. The apodous larvae (Fig1E & F) show several types of morphological malformations: deformed mouth brushes, melanized and scleoritized cuticle, light yellowish albino coloured abdominal structures with lack of peritrophic membrane outline compared to control (Fig 1D) shows prominent mouth brushes and healthy cuticle and digestive tract structure shows clear peritrophic membrane outline. Most of the treated larvae died as highly pigmented forms and some other died with peritrophic membrane disrupted forms.

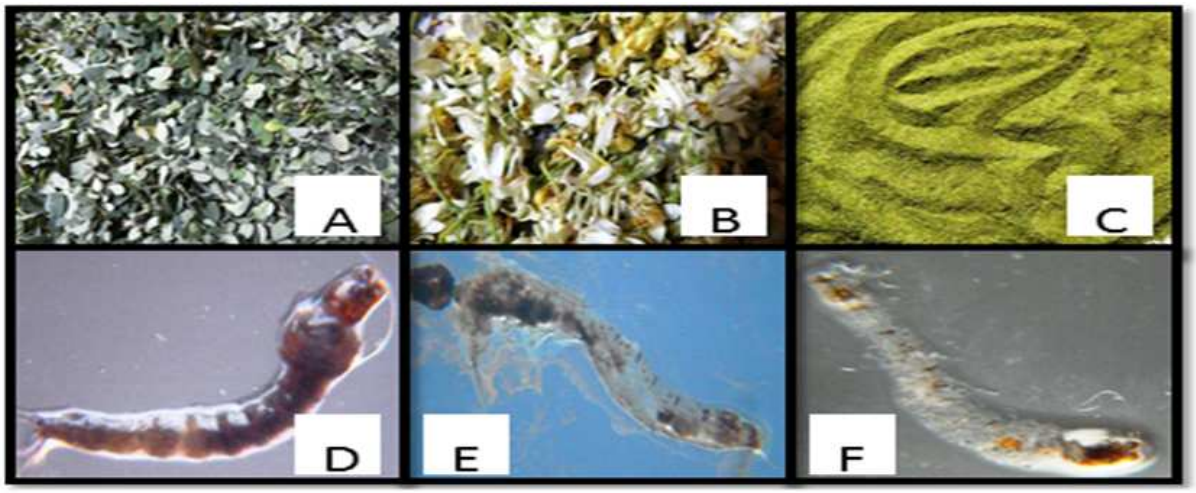


Figure 1: Toxicity Evaluation (A), Moringa oleifera Lam. leaves, (B) Moringa oleifera Lam. flowers , (C) Moringa oleifera Lam. leaves powder, (D) Normal Anopheles stephensi (L) larvae, (E) Anpheles stephensi (L) larvae treated with Moringa oleifera Lam. flower extract, (F) Anpheles stephensi (L) larvae treated with Moringa oleifera Lam. seeds extract.

Table 1: Efficacy of leaf extract of Moringa oleifera Lam. against Anopheles stephensi (L) at 24 and 48 hrs

Concentration (ppm)	Time (hrs)	% of mortality ± SD
20	24h	36.67±1.26
	48h	53.33±0.96
40	24h	50.00±0.96
	48h	63.33±0.58
60	24h	70.00±0.96
	48h	76.67±0.50
80	24h	83.33±0.58
	48h	86.67±0.50
100	24h	86.67±0.58
	48h	93.33±0.58

Table 2: Efficacy of flower extract of Moringa oleifera Lam. against Anopheles stephensi (L) at 24 and 48 hrs

Concentration(ppm)	Time (hrs)	% of mortality ± SD
20	24h	53.33±0.58
	48h	63.33±0.58
40	24h	66.67±1.26
	48h	73.33±0.58
60	24h	86.07±0.50
	48h	87.67±0.80
80	24h	93.33±0.58
	48h	95.33±0.58
100	24h	96.07±0.58
	48h	96.67±0.58

Table 3: Efficacy of seed extract of Moringa oleifera Lam. against Anopheles stephensi (L) at 24 and 48 hrs

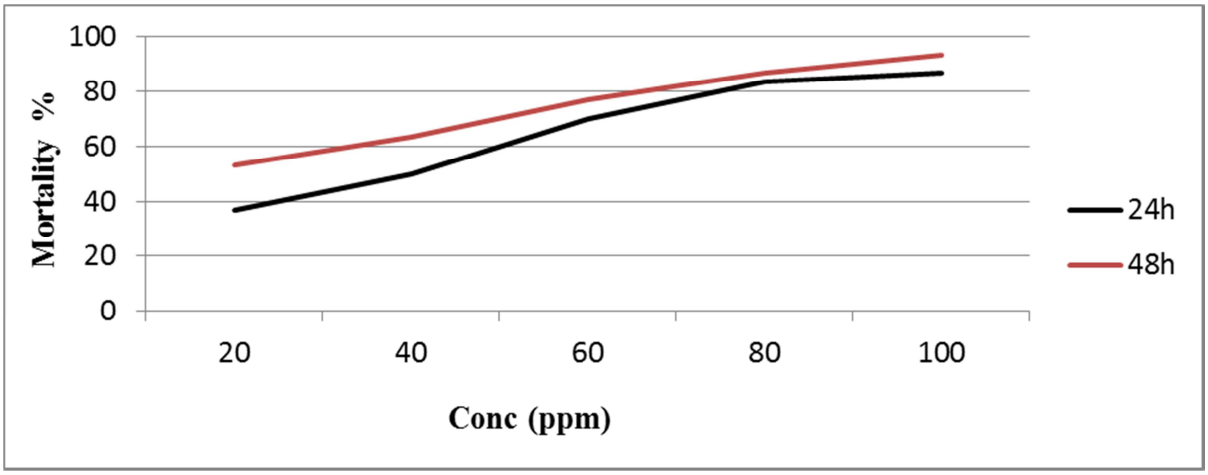
Concentration (ppm)	Time (hrs)	% of mortality ± SD
20	24h	66.67±1.26
	48h	70.00±0.50
40	24h	83.33±1.00
	48h	85.33±0.50
60	24h	93.33±0.50
	48h	95.33±0.58
80	24h	96.67±0.50
	48h	100.00±0.00
100	24h	100.00±0.00
	48h	100.00±0.00

Table 4: Toxicity of leaves, flowers and seeds extract of Moringa oleifera Lam. against Anopheles stephensi (L) under 24h and 48 h exposure time

Plant material	Exposure time (h)	LC_{50} (ppm)	Regression equation
Moringa oleifera leaves	*Moringa oleifera* after 24 h	38.99	Y=1.45+2.24X
	Moringa oleifera after 48 h	25.11	Y=2.17+2.02X
Moringa oleifera flowers	*Moringa oleifera* after 24 h	23.99	Y=1.36+2.64X
	Moringa oleifera after 48 h	20.46	Y=2.36+2.02X
Moringa oleifera seeds	*Moringa oleifera* after 24 h	15.17	Y=2.20+2.36X
	Moringa oleifera after 48 h	12.62	Y=2.83+1.97X

If the efficacy is compared between the plant parts, seed extract exerted maximum effects followed by flowers and leaves. The result (Table 1, 2 and 3) clearly depicts that at lower concentration of 20ppm, 70±0.50percent mortality was observed with seeds whereas same dose exerted 63.30± 0.58 and 53.33±0.96 percent mortality after 48 hrs of treatment time. Trends of mortality were time as well as dose dependant with all the three plant parts and a linera co-relation could be established between time and concentration (Fig 2).

Table 1 revealed the efficacy of leaf extracts of *Moringa oleifera* Lam. against *Anopheles stephensi* (L) at 24 and 48 hrs and generally found that highest larval mortality took place at highest (100 ppm) concentration i.e. 93.33±0.58% at 48 hrs of exposure time. Larval mortality was maximum in 48 h (86.67±0.50%) exposure time as compared to the 24 h (83.33±0.58%) in 80 ppm concentration and this trend was generally observed with all the concentrations. Toxicity of flower extract at the different concentrations revealed highest mortality at the 100 ppm concentration (96.67±0.58%). As compared to the leaf extract high larval mortality was observed at less time exposure (Table 2). *Moringa oleifera* Lam. seed extract is 100% mortality at 80ppm concentration and at the lowest concentration i.e. at 20 ppm it reaches the value at 66.67±1.26%. (Table 3 and Figure 2)



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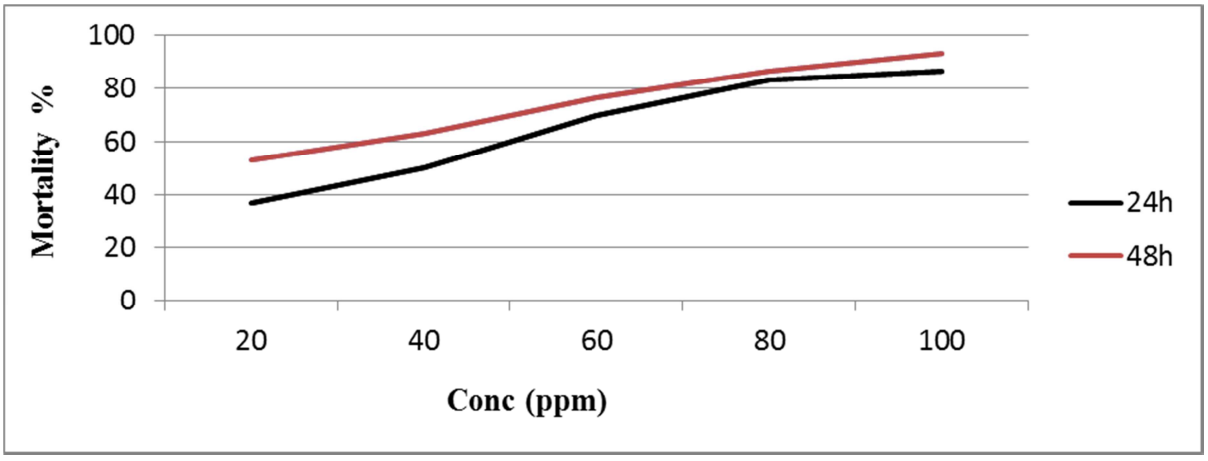

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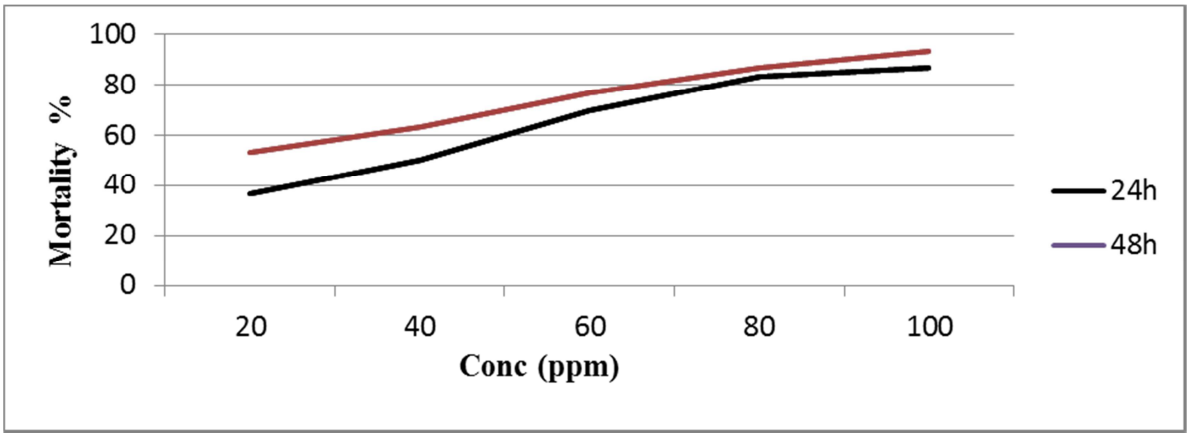

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Figure 2: Dose response relationship for extracts of Moringa oleifera Lam.for 24h and 48h against Anopheles stephensi (L) . A) Leaf extract B) Flower extract C) Seed extract

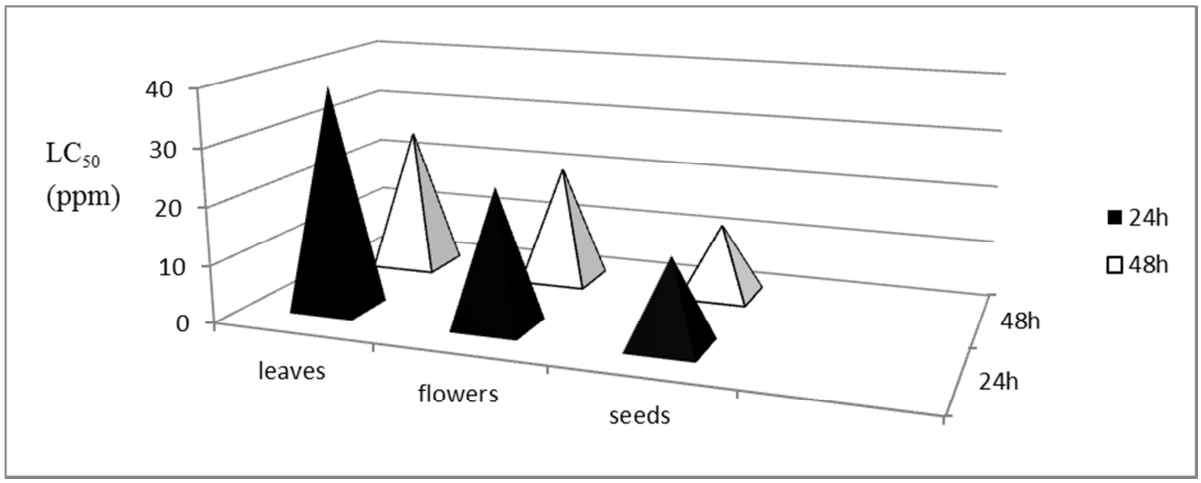

Figure 3: LC_{50} value of leaves, flowers and seeds extract of Moringa oleifera Lam. against Anopheles stephensi (L).

Table 4 shows the LC_{50} value and the regression equation which was calculated using the Probit analysis. LC_{50} value was calculated against different concentrations. Lowest LC_{50} value was observed with *M. oleifera* Lam. seeds followed by flowers and leaves i.e. 15.17 ppm, 23.99 ppm and 38.99 ppm respectively after 24 h of exposure and 12.62 ppm, 20.46 ppm and 25.11 ppm. respectively at 48 h of exposure (Figure 3)

4 DISCUSSION

Many researchers have been done on plants and natural derived chemicals which are non toxic to man and domestic animals and serve as useful basis for development of safer and more effective mosquito control agents. In the present research, encouraging results have been obtained after the treatment of *M. oleifera* Lam. against *An. Stephensi* (L). The obtained larval mortality may be due to active chemical compounds present in *M. oleifera*. *M. oleifera* seeds extract also act as larvicidal agent and studies have been reported on water extracted *M. oleifera* seeds (WEMOS) against *Aedes aegypti* larvae and methanol- extracted *M. oleifera* roots against *Culex quinfasciatus* and *Aedes albopictus*. The present findings have important implication in the control of mosquito larvae in the polluted aquatic ecosystems. The plants studied are explored in large quantities and these extracts are easy to handle, inexpensive and safe natural product for mosquito control. The extract of Murungai (Local tamil name) seed can also be used for water purification (Patterson et al. 1975). In view of residue problems in the environment and development of insect resistance to synthetic insecticides like DDT, the recent trend is to explore our natural resources to obtain extracts that are safe for non target and do not pose any residue problems but are still able to suppress pest populations. These advantages encourage the dispersion of *M. oleifera* tree around the world as well as the exploitation of its seed as a way to reduce the exorbitant costs of water treatment, mainly in developing countries and rural areas (Jahn et al. 1988). An additional benefit is that *M. oleifera* seed is available throughout the year, preferrably when the mosquito population is higher. The plants grow in nature without any extra care or cost and simple technology would be necessary to separate the most suitable fractions to be exploited as a possible chemical to be employed in mosquito control programs.

Quercetin and Kaempferol are flavnoiods, compounds of phenolic hydroxyl groups of *M. oleifera* with antioxidant action of potential therapeutic and pharmaceutical uses. (Asciak et al. 1995). Since many of the plant extracts have been subjected to risk factor in mosquito control and *An. Stephensi* breeds in drinking water tank. The plant extracts which are highly toxic against this malarial mosquito are also toxic to human beings. In the present study *M. oleifera* seed extract shows good effect on *An. stephensi* and it is also non toxic to human beings. Much previous study proved that extract of this plant is a water purifying agent. *M. oleifera* seed can also be used as a natural coagulant in household water treatment as well as in the community water treatment. Hence it is considered that the seeds extract of *M. oleifera* is not only a mosquitocidal agent but also a water treatment agent for the community. The present observation also revealed that seed extract of *M. oleifera* has a promising larvicidal efficacy. Plants are rich sources of bioactive organic chemicals and offer a promising advantage over synthetic pesticide as naturals are non toxic, less prone to development of resistance and easily biodegradable. The seed extract of *M. oleifera* therefore plays an important role in the mosquito control. Many compounds of plant origin are reported as larvicidal (Jeyabalan and Murugan 1996; Curtis et al. 1989; Curtis et al. 1990; Foidl et al. 1990; Murugan et al. 1996; Pushpanathan and Muthukrishnan 1995; Rajkumar et al. 2004; Saxena et al. 1993; Sharma and Saxena 1994; Vatandoost et al. 2010), there is wide scope for the discovery of more effective plant products (Saxena and Yadav 1986).

These results indicated a metamorphosis inhibiting effect of the plant extracts, which possibly based on the disturbance of hormonal control (Sharook et al. 1991), as the noticed morphogenetic aberrations suggesting a type of insect growth regulating activity. The most important deformities, larval-pupal intermediates and ecdysal failure, seemed to be the major cause of the mortalities. Likewise, such abnormalities were noted following treatment of immature mosquitoes with juvenile hormone (JH) analogues and chitin synthesis inhibitors (El-Barky et al. 1993; Khater 2003; Sivagnaname and Kalyanasundaram 2004)

5 CONCLUSION

An attempt has been made to evaluate the role of *Moringa oleifera* L. leaves, flowers and seeds extracts for their larvicidal bioassay against *Aopheles. Stephensi* L. The results reported in this study open the possibility for further investigations of the efficacy of larvicidal properties of natural product extracts as a potential agent for combating mosquitoes. Further research work undoubtedly will lead to improved formulations with enhanced activities which may eventually become environmentally acceptable and replace objectionable conventional insecticide for control of mosquitoes. It may be concluded that nature posses numerous natural herbs which may be useful for vector borne diseases.

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