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Toxic and biological effects of *Moringa oleifera* Lam. crude seed extract against *Culex pipiens* L. (Diptera; Culicidae) larvae

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ABSTRACT

Mosquitos' resistance to chemical insecticides motivated the need for alternative control strategy. Therefore, botanical extracts have become one of the controls of revolutionary methods in the sustainable vector strategy. The current study was conducted under laboratory conditions to evaluate the toxicological efficacy of the crude seed extract of *Moringa oleifera* against the 3rd larval instar of *Culex pipiens* mosquito. A series of aqueous concentrations had been prepared (100, 80, 60, 40 and 20 ppm) to carry out the laboratory bio-assay experiment. The highest larval mortality took place at 100 ppm concentration (81.39%). LC_{50} and LC_{90} values of *M. oleifera* seed extract recorded 67.91 ppm and 144.63 ppm after 120 hr exposure time, respectively. The obtained data revealed that larvicidal effect, malformed feature incidence and the cumulative larval death percentages were of a time and concentration-dependent manner. A similar trend had been documented for the total death percentages of both pupal and adult stages. On the contrary, the percentages of viable pupation and the emerged mosquito showed reverse responses. Accordingly, *Moringa* extract could be considered as a promising non-chemical candidate in the IPM programs of *Culex* mosquito.

INTRODUCTION

Dipterous insects are implicated in the transmission of serious public health problems for both humans and animals (Linthicum, 2012). This is attributed to their worldwide distribution, efficient capacity and potentiality as vectors of pathogenic organisms either biologically or mechanically (Hossain *et al*. 2000 and Njabo *et al*. 2013). Among the most widely distributed dipterous insects, mosquitoes constitute major vectors of several diseases affecting humans and domestic animals around the world such as malaria, lymphatic filariasis, dengue fever, rift valley fever, yellow fever,…*etc*. (James, 1992; Pavela, 2009 and Benelli *et al*. 2016). In Egypt, *Culex pipiens* has been stated as a vector of several diseases (Abd El-Samie and Abd El-Baset, 2012 and El-Zayyat *et al*., 2017). *Cx. pipiens* is implicated as the vector of the West Nile Virus that distributed throughout Africa, the Middle East, and Southern temperate and tropical Eurasia, and was recently introduced into North America as well (Campbell *et al*., 2002).

Since the start of the twentieth century, traditional insecticides were used to control mosquitoes **(**El-Wakeil, 2013 and Killeen *et al*., 2017**)**, but because of the repeated use of insecticides and rapid generation time, flies had arisen different levels

of resistance besides the cross resistant phenomenon to most available insecticides **(**Acevedo *et al*., 2009**)**.

Accordingly, the search for natural insecticides which do not have any unkind effects on the non-target population and are easily degradable remains the top priority (Redwane *et al*., 2002). In this concern, plant extracts and phyto-products revealed good promising effects in terms of easily extractable, ecofriendly, biodegradable, low persistence in soil as well as water and possess low or no toxicity against vertebrates, fishes, birds and mammals (Khater *et al*., 2011).

Moringa oleifera Lam. (drum stick tree) (family: Moringaceae) is one of the widely cultivated plant species worldwide, which originated in Western and Sub-Himalayan region, Pakistan, India, Africa and Asia (Mughal *et al.*, 1999). Rapidly growing and easily naturalizing pattern of *M. oleifera* tree besides fitting for human consumption, medicinal purposes and lastly its promising insecticidal activity were the reasons for its global expansions (Santos *et al*., 2009). Leaf, fruit and seed extract of *M. oleifera* possess promising insecticidal potentialities against larval and pupal stages of many insect species including bruchid beetles (Adenekan *et al*. 2013), grasshoppers, termites, mealy bugs (Ndubuaku *et al*. 2015) and mosquitoes; *Anopheles stephensi* Liston (Prabhu and Murugan, 2011 and Kumar *et al*. 2016) and *Aedes aegypti* (Coelho *et al*. 2009 and Ferreira *et al*. 2009). The current work aims to assess the potentialities of the aqueous crude seed extract of *M. oleifera* on *Culex pipiens* larvae and their accumulative effect on pupation and adult emergence.

MATERIALS AND METHODS

Collection of *M. oleifera* seeds

Moringa oleifera groves are cultivated at different districts in South Sinai governorate. The compatibility of the weather conditions with its growth schedule besides the benefits such as its multiple uses that returned on Sinai local communities were the main motivator for its propagation. Fully matured *M. oleifera* legumes were provided from Habiba Organic Farm, Nuweiba City, South Sinai governorate, Egypt, (29° 1' 20.74" E and 34° 40' 21.20" N). *Moringa* seeds were removed from the legumes and preserved in a dry clean paper bag to be ready for the extraction purpose.

Extraction of *M. oleifera* seeds

Collected seeds were washed with current tap water then left in a shaded place till complete dryness. A fixed amount of the dried seeds (40 gm.) were crushed by an electrical grinder. The grinded seeds were soaked in an adequate volume of ethanol 70% (100 gm. / 400 ml.) with shaking for two days to facilitate the extraction of the active ingredients. The ethanolic *Moringa* seed solution was filtrated using filter paper (Whatman® qualitative filter paper, Grade 1). The supernatant was evaporated using a rotary vacuum evaporator (Labo-Rota C311) to collect the crude extract (Hafiz *et al*., 2012). Aqueous stock solution was prepared by dissolving 1 ml of the crude extract in 100 ml of distilled water presenting 1% concentration. Thereafter a series of aqueous concentrations were prepared from this stock solution (80, 60, 40 and 20 ppm) to carry out the bio-assay experiment.

Laboratory production of the northern house mosquito, *Culex pipiens pipiens*

Egg rafts of *Cx. pipiens pipiens* generation five were obtained from the Research and Training Center on Vector of Diseases (RTC), Faculty of Science, Ain Shams University, Egypt. Egg rafts were placed in white enamel dishes 35-40 cm in diameter and 10 cm in depth filled with 1500 ml of distilled water. Newly hatched

larvae were fed on fish food (Tetra-/Min, Germany) as a diet sprinkled twice daily over the water surface of the breeding pans (Kasap and Demirhan, 1992). Distilled water in each dish was stirred daily and changed every two days to avoid scum formation on the water surface or on the walls and bottoms of pans. Small air pump was used to aerate the breeding water gently every day for about 5 minutes. Pupae were collected routinely and separated in plastic containers filled with distilled water then introduced into screened wooden cages until emergence. Newly emerged adults were supplied by 10% of sucrose solution as an energy source and to get new egg batches they got the chance to suck a blood meal from an offered pigeon host. Mosquito rearing schedule was held at 27±2°C and 70±10% R.H. with a photoperiod of 14:10 (light: dark) hours.

Bio-assay test

The larvicidal activity of the aqueous *Moringa* seed extract was estimated against the 3rd instar larvae of *Cx. pipiens* using immersion method in accordance with WHO (2005). Four groups of twenty five early third instar larvae of *Cx. pipiens* were transferred by a plastic dropper into 4 small test cups, each containing 100 ml of water for each corresponding concentration and incubated under laboratory conditions of 27±2ºC, RH 70±10%, and 14-10 light-dark regime. Each of the five concentrations was represented by 4 replicates. Distilled water was used in the check treatment. Daily larval mortality, the morphogenetic features and the total larval death were determined every 24 hrs. Observed mortality was corrected using Abbott's formula (Abbott, 1925) and the LC_{50} value was calculated (Finney, 1979). The latent effect of the examined concentrations was also detected on both pupal and adult stages.

$$\% \text{ corrected mortality} = \frac{\%\ mortility\ in\ treatment - \%\ mortality\ in\ untreated}{100 - \%\ mortality\ in\ untreated} \text{ X } 100$$

RESULTS

Aqueous extract of *M. oleifera* seeds was subjected to a laboratory bioassay test to examine its effectiveness on the 3rd larval instar of *Cx. Pipien*s as an eco-alternative of the synthetic insecticide. The mortality percentages of the five applied concentrations in terms of the daily larval death are shown in Table (1). Data in Table (2) shows that as the concentrations of the crude seed extract of *M. oleifera* increase, the mortality percentages increased in a dose and time dependent manner. On the 1st day post-treatment, larval death recorded 5 and 8 individuals in 80 and 100 ppm, respectively and 1, 2 and 3 larval death in 20, 40 and 60 ppm, respectively. The continuous daily monitoring matched with the proportional increase in the larval death, which recorded its highest pattern at the 5th day post-treatment.

Table 1: Efficacy of crude seed extract of *M. oleifera* on the number of *Cx. pipiens* dead larvae after 24, 48, 72, 96 and120 hrs.

Concentrations (ppm)	No. of larval death (days) after				
	24hrs.	48hrs.	72hrs.	96hrs.	120hrs.
Control	0	2	4	0	2
20	1	4	8	10	15
40	2	6	10	15	27
60	3	6	10	16	28
80	5	8	12	16	30
100	8	13	16	20	35

Table 2: Susceptibility of 3rd instars larvae *Cx. pipiens* to the crude seed extract of *M. oleifera*.

Concentrations (ppm)	% of larval mortality (days) after				
	24hrs.	**48hrs.**	**72hrs.**	**96hrs.**	**120hrs.**
Control	0	2	4.08	0	2.12
20	1	4.04	8.42	11.49	19.48
40	2	6.12	10.86	19.51	40.29
60	3	6.18	10.98	19.75	43.07
80	5	8.42	13.79	21.33	50.84
100	8	14.13	20.25	31.74	81.39
LC_{25}(ppm)	372.30	389.72	247.95	79.004	26.91
LC_{50}(ppm)	1132.09	2230.87	2281.75	462.31	57.88
LC_{90}(ppm)	9366.90	61404.90	154789.11	13268.72	248.11
Slope± SE	1.3965±0.5593	0.8902±0.3753	0.6998±0.3086	0.8791±0.2743	2.0276±0.2523

On the 5th day post-treatment, detected larval death had been fluctuated between its maximum value (81.39%) at the highest applied concentration (100 ppm) and the minimum one (19.48%) at the lowest (20 ppm) (Table 2) (Fig.1).

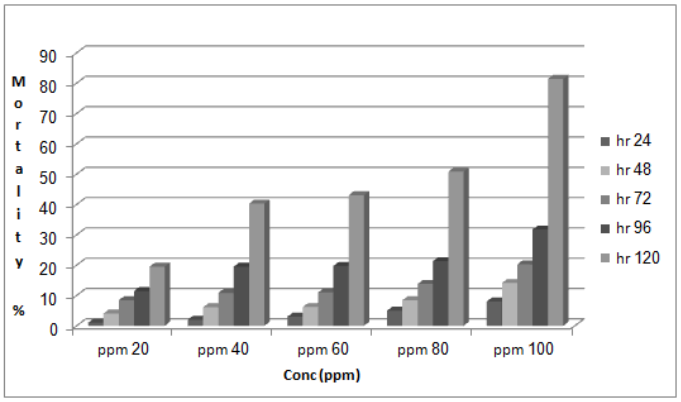

Fig. 1: Dose response of *Cx. pipiens* to *M. oleifera* seed extract after 24, 48, 72, 96 and 120hrs.

Culex pipiens pipiens mosquito larvae treated with *M. oleifera* seed extract revealed characteristic features in terms of their development and metamorphosis patterns (plate 1). The dead larvae showed darken signs on its body, losing its siphon (the respiratory organ) and/or the head got detached from its body.

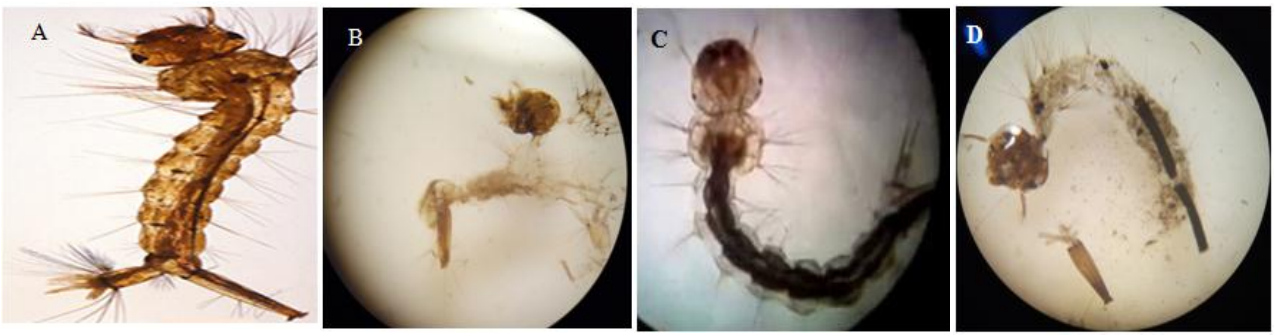

Plate 1: Normal and treated larvae of *Cx. pipiens*: a, Normal larva; b, c &d larvae treated with *Moringa* crude seed extract.

On the 6th day of the experiment, larval stage ends with the formation of the pupal one at the untreated larvae while showed 2 days delaying pattern at the lower applied concentrations (20 and 40 ppm), 3 days at 60 ppm and 4 days at the highest ones (80 and 100 ppm) (Table 3).

Concentrations	Number of Pupae (days) after	Total pupal	% of viable

	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th		
Control	14	17	20	19	22	-	-	-	-	-	8	84
20	0	0	0	14	10	15	8	13	1	1	10	52
40	0	0	0	8	8	9	7	3	2	3	15	25
60	0	0	0	8	8	7	5	4	3	2	15	22
80	0	0	0	0	7	9	8	2	2	1	17	12
100	0	0	0	0	5	3	-	-	-	-	3	5

Table 3: Impact of the crude seed extract of *M. oleifera* on the percentage of *Cx. pipiens* viable pupae and pupal duration.

The pupae produced from the treated larvae showed certain abnormality patterns in their life scenarios upon compared with that produced from the untreated larvae. Pupal calculations were in the favor of the control trial. As the first two days showed zero pupal formation at the tested concentrations, 25 pupae were recorded at that period in the control treatment. The 9th and 10th days showed higher pupal formation in the control trail than that in the treated ones. Viable pupae that managed to emerge into adult mosquitoes were also considered over here (Table 4). At the control trial, 84% of the pupae were viable and from which new adult mosquitoes got emerged with zero adult death percentage. On the contrary, the viable pupal percentages at the proposed concentration schedule were suffered great drop till reached 5% with only 3% adult emergence at the highest applied concentration (100 ppm) and mild decline at the lowest one (20 ppm) (52% of viable pupation categorized as 37% emerged mosquitoes and 15% dead ones) (Table 4).

Table 4: Impact of crude seed extract of *M. oleifera* on the percentage of *Cx. pipiens* adult emergence.

Concentration (ppm)	Number of emerged adults after (days)												Total adult death	% of adult emergence
	9th	10th	11th	12th	13th	14th	15th	16th	17th	18th	19th	20th		
Control	15	10	12	8	9	7	9	8	6	-	-	-	0	84
20	-	-	9	7	9	9	8	5	3	2	-	-	15	37
40	-	-	5	5	4	5	2	2	1	1	-	-	11	14
60	-	-	-	5	4	4	2	3	2	1	1	-	13	9
80	-	-	-	-	-	5	4	3	-	-	-	-	5	7
100	-	-	-	-	-	2	2	1	-	-	-	-	2	3

The LC_{50} and LC_{90} values of *M. oleifera* seed extract were estimated to be 67.91 ppm and 144.63 ppm after 120 hr exposure time, respectively as shown in Fig. 2.

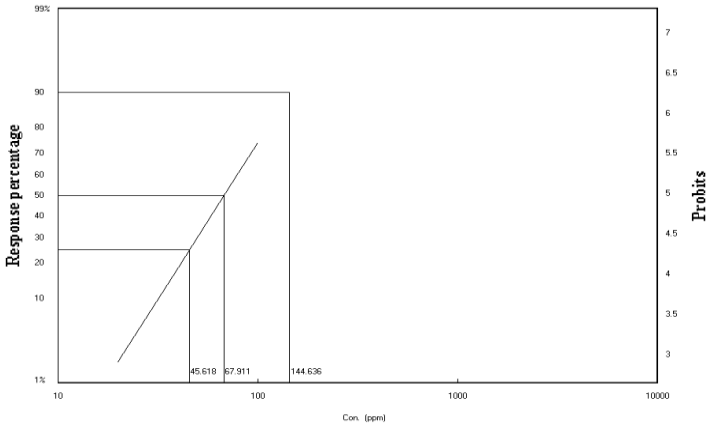

Fig. 2: Toxicity regression line of 3rd instar larvae of *Cx. pipiens* treated with crude seed extract of *M. oleifera*.

DISCUSSION

The development of insect resistance to synthetic insecticides was considered the main driven influence to adopt new environmental trend (Jahn *et al*. 1988). Plant extracts fulfilled such concern through their mild impacts on the non-target organisms besides their abilities in hindering insect pest populations (Choochote *et al*. 1999 and Murray, 2006).

Comparing with other plant extracts, *M. oleifera* seed extract proved its toxicological activity as an advantageous beginning for the development of an alternative method employing in mosquito control programs besides being non-toxic for both man and domestic animals (Paulo *et al*. 2009). Many phytochemicals of the plant are reported as larvicides on many insect orders (Anwar *et al*. 2007; Amaglo *et al*. 2010; Coppin *et al*. 2013 and Saini *et al*. 2014). The obtained data during the context of the current study revealed the promising effect of the aqueous *Moringa* seed extract as a larvicide. Similarly, Ohia *et al*. (2013) estimated the aqueous extract of the *M. oleifera* seed as a control agent against third instar larvae of *An. gambiae*, under the laboratory conditions. The increase of larval death percentages of *Cx. pipiens* with the applied concentrations as presented in table (2) was also observed by Prasad and Sharma (2015) against larvae of *An. Stephensi* Liston. The prolongation of larval developmental period and the appearance of deformed larval features in this study may be due to the presence of low juvenile hormone levels in the larvae or may be due to chemical compounds in the plant extract suppressing the presence of ecdysone; preventing normal pupation and preventing movement to the next developmental stage thus preventing adult emergence from occurring with the resultant effect of reducing the mosquito population. These results had been matched with the findings of Ohia and Ana (2017) on *An. gambiae*. Another effect of *Moringa* seed extract had been noticed on the pupal and adult stages.

The observed pupal mortality resulted from using *Moringa* extract (table 3) could be attributed to a hormonal disturbance, *i.e*., *Moringa* active ingredient may interfere with the ecdysteriod titre and/or function that consequently may induce an inhibitory effect during the development from larvae to pupae. Similar explanation had been reported on ecdysis of *Rhodnius prolixus* by Garcia and Rembold (1984) and on tobacco hornworm, *Manduca sexta* by Schluter *et al*. (1985). The cumulative or latent effect of *Moringa* active compounds in the pupae may cause a state of poisoning that could be another reasonable key for the pupal mortality. Similarly, Abdel-Rahman *et al*. (2002) assessed the latent effect of NeemAzal application on pink bollworm, *Pectinophora gossypiella*. The remarkable reduction in the pupal mortality that was observed at the highest applied concentrations could be due to the strongest mortality effect of these concentrations on the larval stage. Accordingly, only 8 pupal percentage at 100 ppm had been formed and more than half of which got died (3% at the pupal stage + 2% at the adult one). The observed drop off in the emerged adults' fitness under their long-lasting exposure to the *Moringa* active ingredients during the juvenile stages may be one of the causes for adult mortality. As a final conclusion, *M. oleifera* crude seed extract could be directly used in the dwelling habitats of mosquitoes for effective control. This is an environmentally safe and ecofriendly approach for the vector control programs.

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