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Laboratory evaluation of Aster 20SP against Culex pipiens (Diptera: Culicidae) immature stages

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Abstract

The purpose of this research work was to investigate the larvicidal activities of Aster 20SP against Culex pipiens and biological effect of this compound. The larvicidal activities of Aster 20SP were tested against the third and fourth instar larvae of Culex pipiens, the mortality was observed after 24h post the treatment. The data were subjected to probit analysis to determine the lethal concentration (LC_{50} and LC_{90}). The obtained results show a high mortality with the increase in the concentration, at third stage, larval mortality increased from 18.23 % at 0.7 mg /L to 45.16 % at 3mg /L in direct effect. The LC_{50} and LC_{90} were measured as 3.61 and 5.16 mg/L respectively. Cumulate mortality increased from 35.30 to 54.34% at 0.7mg/L and 3mg/L respectively. The LC_{50} and LC_{90} were 3.38 and 4.68mg/L respectively. At fourth stage, larval mortality increased from 5.50 % at 0.7 mg /L to 19.29 % at 3 mg /L in direct effect, the LC_{50} and LC_{90} was 4.34 and 6.52 mg/L respectively. In indirect effect, the mortality increased from 9.16% to 30.80% at 0.7mg/L and 3mg/L respectively, the LC_{50} and LC_{90} was 4 and 5.87 mg/L respectively. The results indicated the larvae duration increased with the increased in the concentration. In contrast, in adult stage, the results indicated high reduction in fecundity.It was concluded from this study that Aster 20SP as a potential pesticide to control the larvae stage of mosquito Culex pipiens.

Keywords: Larvicidal; Biology; Mortality; Culex Pipiens; Aster 20SP; Insecticide; Pesticide.

1. Introduction

Mosquitoes are the most important vectors of certain human infections and diseases. Mosquito-borne diseases such as malaria, yellow fever, filariasis and dengue contribute significantly to mortality in most tropical countries. Culex pipiens L. (Diptera: Culicidae) is one of the most widely distributed mosquitoes in the world. The species, commonly referred to as "house mosquito", can be found in urban and suburban areas and lives near people, but feeds primarily on birds (Bernard et al. 2001). This mosquito can transmit many arbovirus encephalitides and lymphatic filariases. More than 120 million people worldwide are infected with the Wuchereria bancrofti form of lymphatic filariasis (Cao et al. 1997; Turell et al. 2000). Several commercially available insecticides (e.g. temephos, chlorpyrifos-methyl, diflubenzuron) can be effective to control the species at immature stages (Cetin et al. 2006a, 2006b). However, many of these chemical insecticides are expensive and harmful to the environment as well as to humans.

Insect growth regulators are comparatively safer to nontarget organisms (Mulla, 1995) and have been recommended for mosquito control (WHO, 2006). Growth regulators include chemicals with unique mode of actions such as juvenile hormone analog, chitin synthesis inhibitor, ecdysone agonist (Mulla and Su, 1999; Mordue et al. 2005; Soin et al.2010) Insect growth regulators have shown significant larvicidal efficacy against Aedes albopictus(Skuse) mosquito at low lethal doses as compared to microbial, organophosphates and synthetic pyrethroids insecticides (Ali et al.1995). Few studies have also shown disrupted hormonal balance inside developing embryo (Berger and Dubrovsky, 2005). Partial exposure of Culex quinquefasciatus (Say) and Aedes aegypti (L.) eggs were observed to various doses of insect growth regulators (Miura et al. 1976; Vasuki, 1990; Su and Mulla, 1998; Umar et al. 2007). Several other groups (Zahiri and Mulla, 2006; Albernaz et al. 2009; Govindarajan et al. 2008) tested low doses of microbial, fungal and plant products to inhibit egg hatch but none of these products were effective as ovicide. In the present study, we assessed the toxicity of Aster 20SP against third and fourth larval stages of Cx. pipiens, and the duration of both stages were determined. In addition, its effects on the fecundity of females and the body weight of the fourth larval stage of Culex pipiens under laboratory condition.

2. Material and Methods

2.1. Rearing of Mosquito

The larvae of Culex pipiens (Diptera: Culicidae) were obtained from a stock colony of the laboratory. Each 25 larvae were kept in Pyrex storage jar containing 250 ml of stored tap water and maintained at temperature between 25-27°C and a photoperiod of 14L:10D. Larvae were daily fed with fresh food consisting of a mixture of Biscuit Petit Regal-dried yeast (75:25 by weight), and water was replaced every four days.

2.2. Bioassays and Larval Mortality

Newly ecdysed third and fourth-instars larvae of Culex pipiens were exposed to the different concentrations (0.7, 2 and 3 mg/L). For 24 hours when the control larvae were exposed to water only. After the exposure time of 24 hours, according to the World Health Organization [WHO, 2005], the larvae were removed, and placed in clean breading water. The test was carried out with three repetitions containing 25 larvae each. The growth and development was examined and mortality was registered daily until adult emergence.

2.3. Fecundity and Sterility

The fecundity experiments were conducted by taking equal number of male and female Cx. pipiens, which had emerged from the control and treated sets of each concentration. They were mated in the cages of (20 x 20 x 20) cm dimension individually to each concentration. Three days after the blood meal, eggs were collected daily from the small plastic bowls containing water kept in ovitraps in the cages. The fecundity was calculated by the number of the eggs laid in the ovitraps divided by the number of female let to mate (The death of the adult in the experiment was also considered) The Sterility Indices experiments were conducted by the method of Sexina et al (1993).

2.4. Total Larval Duration

To assay the growth factors of Cx. pipiens, test solution of concentration of Aster 20SP (0.7; 2; and 3mg/L) were used. A known number of eggs were made to hatch and the total larval duration (days) was calculated from hatching to pupation period, the pupa was placed in a small container closed with a transparent mesh, so that the adults were kept trapped.

2.5. Statistical Analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90}, and other statistics at 95% confidence limits of upper confidence limit and lower confidence limit. Data from duration and fecundity deterrence and effective concentration were subjected to analysis of variance (ANOVA), results with $P < 0.05$ were considered statistically significant.

3. Results

3.1. Insecticidal activity

Dose-response relationship was determined for Aster 20SP applied for 24 h to newly ecdysed third and fourth-instar larvae of Culex pipiens, and the mortality was recorded up to adult emergence. For third stage, the highest concentration tested 3mg/L in direct effect caused 45.16% mortality (**Figure1**). With probit, the regression equation as $Y = 1.19X + 0.67$, the LC_{50} was calculated as 3.61 mg/L (95% CI=3.3-3.9mg/L; Slope=1.29) and the LC_{90} was 5.16 mg/L, (**Table1**). For indirect effects, the highest concentration caused 54.34% mortality (**Figure2**), the LC_{50} was 3.38 mg/L (95% CI=2.9-3.5 mg/L; Slope=1.60) and LC_{90} were 4.68 mg/L, respectively, (**Table1**). For fourth stage, the highest concentration tested 3mg/L in direct effect caused 25.91% mortality (**Figure3**). With probit, the regression equation as $Y = 1.08X + 0.29$, the LC_{50} was 4.34 mg/L (95% CI=4.05-4.64mg/l; Slope=1.27) and the LC_{90} was6.52mg/L (**Table2**). For indirect effects, the highest concentration caused 33.53% mortality (Figure4), the LC_{50} was 4 mg/L (95% CI=3.73-4.28 mg/l; Slope=1.25) and LC_{90} was 5.87 mg/L, respectively, (**Table2**). After a comparison between the two stages showed that, the third stage is the most sensitive than the fourth (**Figure 5**).
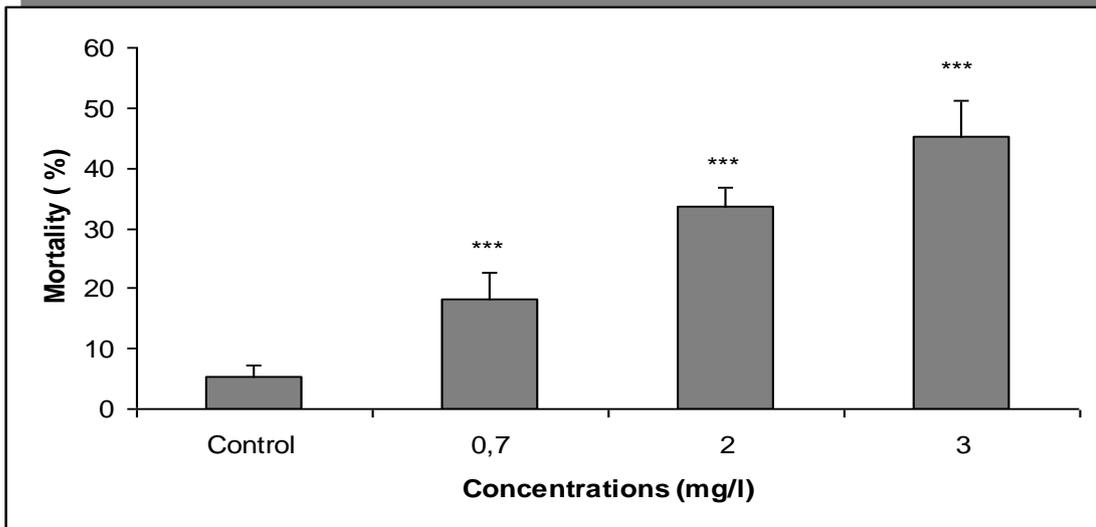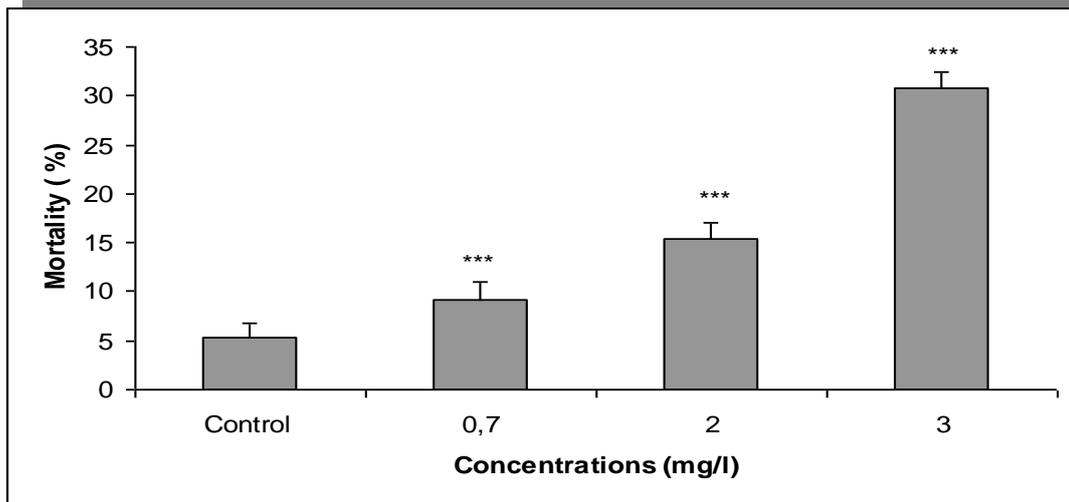
Figure1. Larvicidal activity of Aster 20SP against the third instars larvae of Culex pipiens (effect direct) (Data following by *** are significantly different from control, p<0.001)
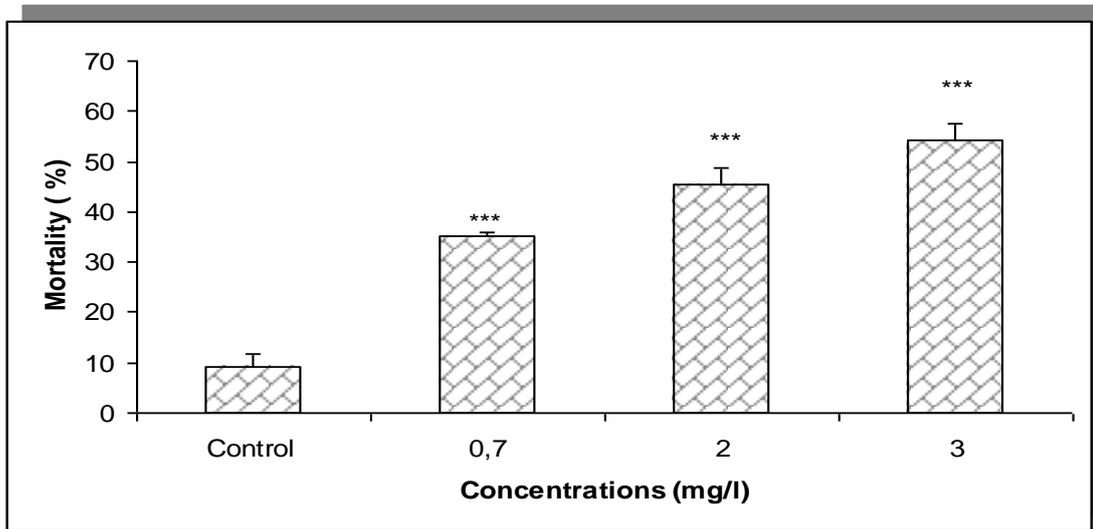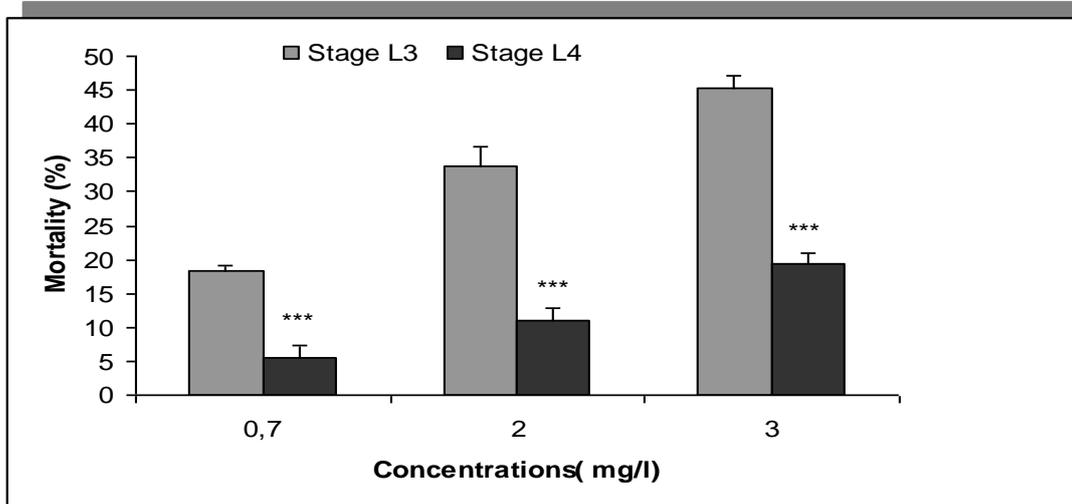

Figure2. Larvicidal activity of Aster 20SP against the third instars larvae of Culex pipiens (effect indirect) (Data following by *** are significantly different from control, p<0.001)
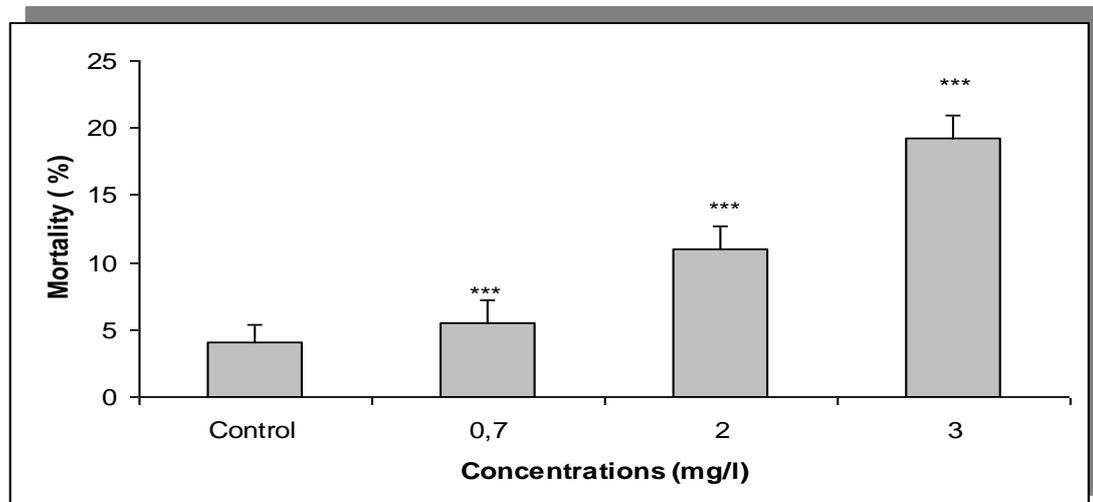
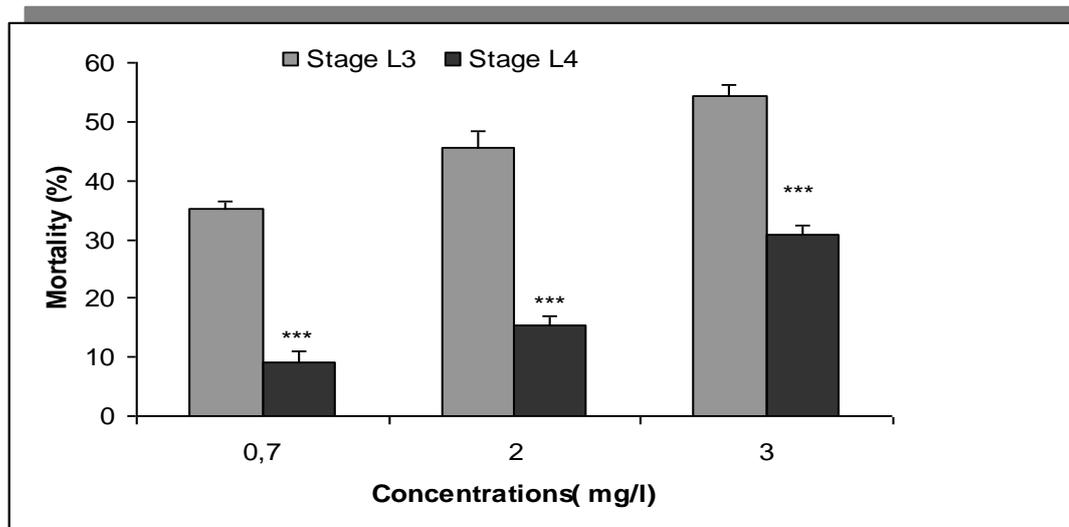**Figure3.** Larvicidal activity of Aster 20SP against the fourth instars larvae of Culex pipiens (effect direct) (Data following by *** are significantly different from control, p<0.001)

Figure4. Larvicidal activity of Aster 20SP against the fourth instars larvae of Culex pipiens (effect indirect) (Data following by *** are significantly different from control, $p < 0.001$)

Figure5. Effect of the Aster 20SP on the two stages: Comparison of mortality between the third and fourth instars larvae of Culex pipiens (effect direct) (Data following by *** are significantly different between the two stages larval, $p \leq 0.001$).

Figure6. Effect of the Aster 20SP on the two stages: Comparison of mortality between the third and fourth instars larvae of Culex pipiens (effect indirect) (Data following by *** are significantly different between the two stages larval, $p \leq 0.001$).

Table 1. Larvicidal activity of Aster 20SP at various concentrations applied on third instar larvae of Culex pipiens at 24 hrs exposure period.

Effects	LC_{50} (mg/l)	95% Confidence limits(mg/l)		LC_{90} (mg/l)	95% Confidence limits(mg/l)		Régression equation	Slope
		Lower	Upper		Lower	Upper		
Direct	4.34	4.05	4.64	6.52	6.15	7.55	Y=1.08X+0.29	1.27
Indirect	4	3.73	4.28	5.87	4.90	6.32	Y=1.19X+0.21	1.25

Effects	LC_{50} (mg/l)	95% Confidence limits(mg/l)		LC_{90} (mg/l)	95% Confidence limits(mg/l)		Régression equation	Slope
		Lower	Upper		Lower	Upper		
Direct	3.61	3.3	3.9	5.16	4.33	6.05	Y=1.19X+0.67	1.29
Indirect	3.38	2.9	3.5	4.68	4.22	5.30	Y=0.72X+2.55	1.16

Table 2. Larvicidal activity of Aster 20SP at various concentrations applied on fourth instar larvae of *Culex pipiens* at 24 hrs exposure period.
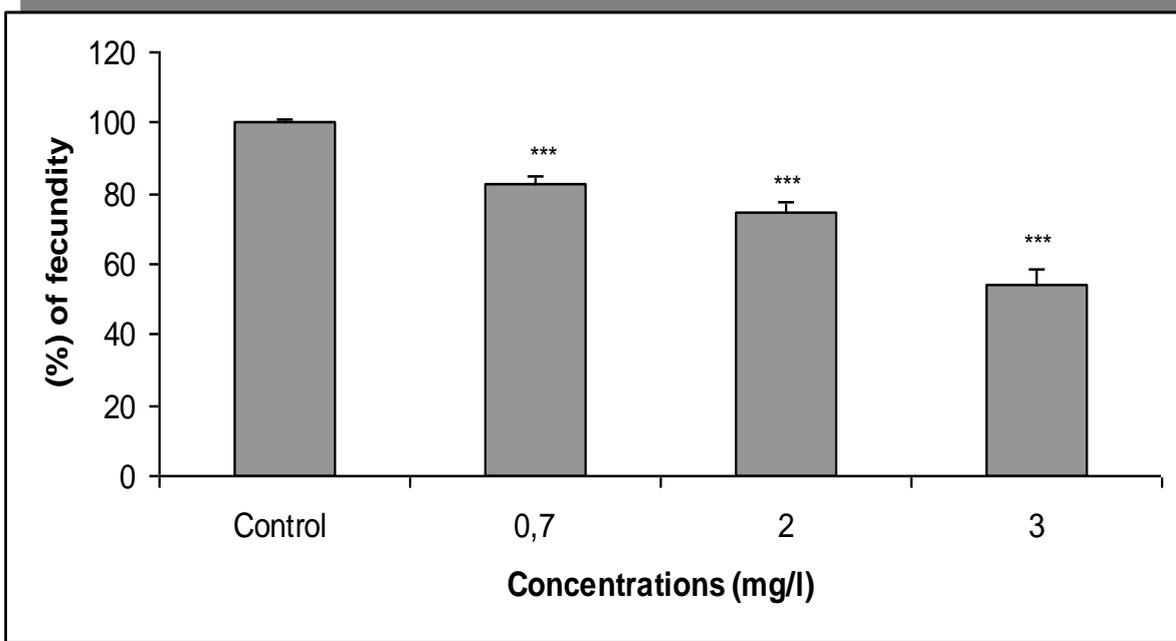
3.2. Effect on Fecundity and Sterility

The results showed that the fecundity of Cx. pipiens females decreased after the treatment of the third and the fourth instars larvae (**Figure 6, 7**), and the sterility indices with the females was increased (**Figure 8, 9**).

Figure7. Fecundity (%) of Cx. pipiens females after treated the third instars with Aster 20SP (Data following by *** are significantly different from control, p<0.001)
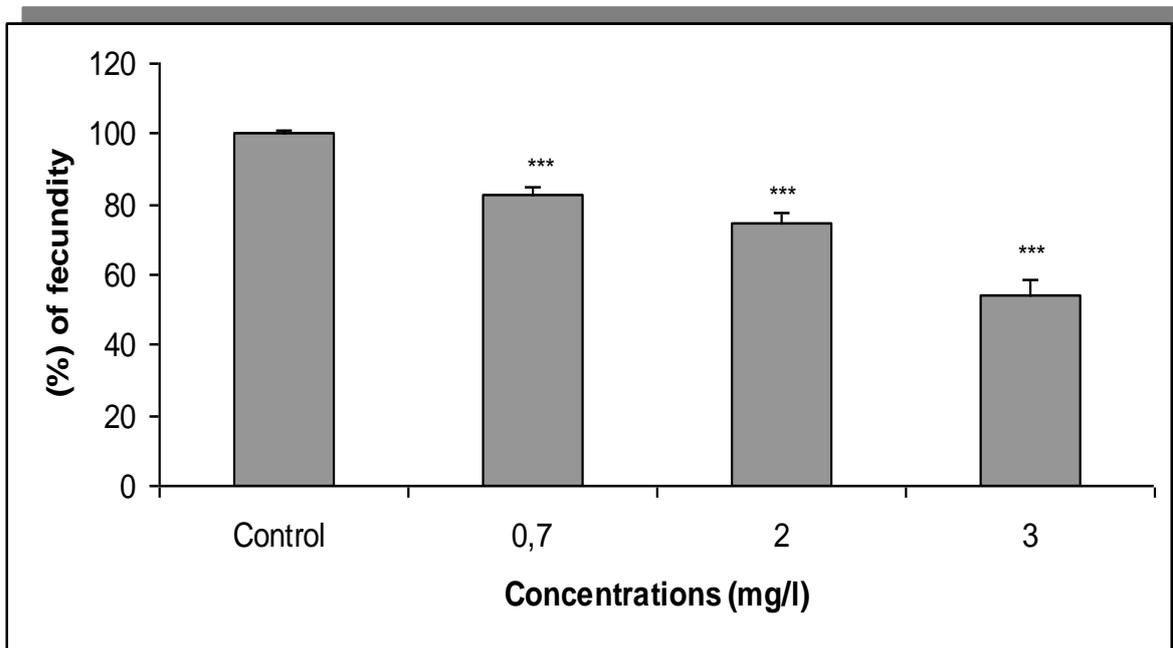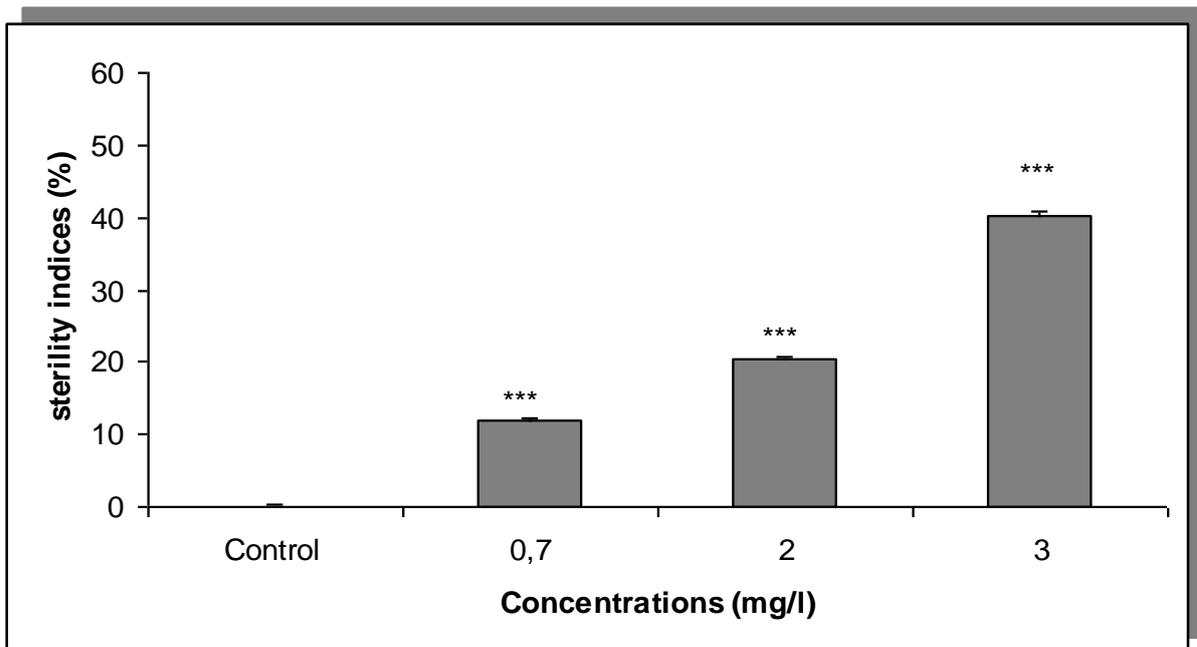

Figure8. Fecundity (%) of Cx. pipiens females after treated the fourth instars with Aster 20SP (Data following by *** are significantly different from control, p<0.001)
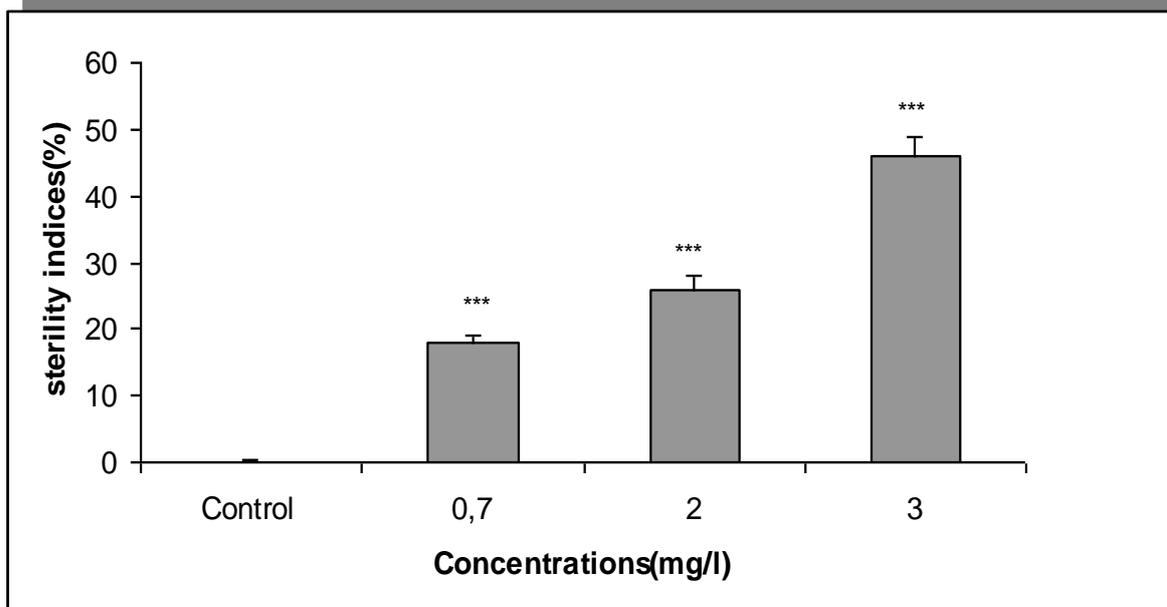
Figure9. Sterility Indices (%) of Cx. pipiens females after treated the third instars with Aster 20SP (Data following by *** are significantly different from control, p<0.001)

Figure10. Sterility Indices (%) of Cx. pipiens females after treated the fourth instars with Aster 20SP (Data following by *** are significantly different from control, p<0.001)

3.3. Effects on larval development duration

The effect on development duration of the third and the fourth instars larvae treated with the different concentrations is presented in (**Table3**). The results show that Aster 20SP interferes with growth by increasing the larval development duration. There was a significant difference between control and treated series. With these concentrations, the development duration of the third larval stage was recorded at 4.60 and 8.70 days at 0.7 and 3 mg/L respectively, compared with control that was 3.66. However, the development duration of the fourth instar was recorded at 6.24 and 10.55 days at 0.7 and 3 mg/L respectively, whereas for the control the age was 5.75 days.

Table 3. Effect of Aster 20SP applied to newly ecdysed third and the fourth instar larvae of Cx. pipiens on the duration of development. (Data are expressed as means ± SD)For each duration and each instar, means values followed by different letters are significantly different (P<0.05)

Concentration (mg/l)	Duration (days) Mean ±SE	
	3rd larval instar	4th larval instar
0	3.66 ± 0.28 a	5.75±2.99 a
0.7	4.60 ± 1.04 b	6.24±1.28b
2	7.67 ± 1.53 c	8.24±1.28d
3	8.70 ± 0.60 d	10.55 ±2.82d

4. Discussions

In the present study, Aster 20SP has displayed varied toxicity on third and fourth instar larvae of Culex pipiens under laboratory conditions. The results showed that an increase in mortality with the increase in concentration and the early instar larvae are much susceptible than the later ones. The effect of this compound on the biology, reproduction, and adult emergence of the mosquitoe are remarkably greater than those reported for other compounds in the literature. For example, 50% inhibition of the emergence of the adult mosquitoes was observed by the use of C. inophyllum, S. suratense, S. indica and Rhinocanthus nasutus leaf extracts (Muthukrishnan and Puspalatha, 2001). Similarly, 88% of the adult mortality was observed by the use of P. citrosa leaf extracts at 2% concentration (Jeyabalan et al. 2003). The Meliaceae plant family is used as growth regulator against many insect pests (Saxena et al. 1984; Jacobson, 1987; Schmutterer, 1990). In the other study, the hexane extract of hairy root callus culture of Tagetes patula L. which contains a high quantity of thiophenes showed larvicidal effect against Culex quinquefasciatus mosquitoes. The extract showed 50% mortality at 0.06 mg/L as compared to standard thiophenes, which showed 55% mortality at the same concentration (Rajasekaran et al.2004). Similarly, application of pyrethrins extracted from the callus tissue of Tagetes erecta plant (5 mg/mL) against Tribolium spp. showed immediate 'knock down' effect on the tested insects (Sarin, 2004). Using 50 mg/L of the root-derived callus of the Balanites aegyptiaca, which contained a high quantity of saponin that induced 18% adult emergence inhibition of Ae. aegypti population relative to the control. The extracted saponin also showed dose-dependent larvicidal effect on Aedes mosquito larvae (Chapagain et al.2008). Results of this study indicated that a sublethal dosage of the crude extracts of C. aromaticus cultured cells can exert effect on the biological and morphological aspects of the treated mosquitoes and even on their F1 generation. The finding of the current study showed that phytochemicals with insect growth regulator activity can significantly inhibit normal ovarian maturation and egg production in mosquitoes and therefore decrease the vector mosquito population. Possibly, variation in egg production of mosquitoes in response to chemical exposure is due to factors which are involved in the regulation of egg production in mosquitoes such as genetical factors as well as hormonal and nervous system stimulations (Attardo et al .2005; Gulia-Nuss et al . 2011). Variability among the anti fertility effect of active compounds of various plant extracts could cause different effects on the reproduction potential (Muthukrishnan and Pushpalatha , 2001) . The extract of C. aromaticus cultured cells might produce their effects on fecundity and hatchability in mosquito species through its influence on the endocrine system due to the JH III content. Both embryogenesis and embryonic ecdysis are inhibited in eggs of many insects species exposed to juvenile hormone active IGRs in the female body or after the egg deposition (Staal , 1975). Prolongation of larval and pupal periods of insects following exposure to phytoextracts indicates the interference by the bio-active compounds on the normal hormonal activity coordination of the metabolic processes of the developing stages probably due to interference in the endocrine mechanism (Sakthivadivel and Thilagavathy, 2003).

Large alterations in the fecundity and sterility of insects exposed to neem have been extensively reported, such as those in the fly, Ceratatis capitata (Steffens and Schmutterer 1982); banana root borer, Cosmopolites sordidus (Germar) (Musabyimana et al. 2001); and mosquitoes, A. stephensi and A. culicifacies (Dhar et al. 1996). The work published by Khan et al. (2007) microscopically demonstrated that the decrease in fecundity of Bactocera cucurbitae and Bactocera

dorsalis exposed to neem compound was due to the block of ovarian development. Likewise, mixing of a commercial formulation of neem in the adult diet caused reduction in the fecundity of C. capitata by interfering with oogenesis (Di Ilio et al. 1999). The block in the ovarian activity of C. capitata, resulting from neem compound, was verified by histological observation (Di Ilio et al. 1999). Results from the study of Lucantoni et al. (2006) clearly indicated that the neem treated female mosquito, A. stephensi, displayed a delay in oocyte development in the vitellogenesis. As discussed by Weathersbee III and Tang (2002), the disruption of reproductive capability could lead to substantial population decline over time. Furthermore, Dhar et al. (1996) revealed that exposure to neem extract suppressed rather than inhibited oviposition in mosquitoes.

Conclusions

Thus, it is concluded from the above study that is a potent larvicide against vector mosquito specie, and its toxic potentiality depends on concentration of insecticide.

Acknowledgements

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