Delayed Effects of Some Plant Extracts on Some Biological Aspects of *Culex quinquefasciatus* (Diptera: Culicidae)

Al-khalaf ,A.A[1] and Al-mehmadi, R .M[2.]

ABSTRACT

The present study was conducted to evaluate the effects of LC_{50} concentrations of ethanolic plant extracts, *Artemisia herba alba* (Compositae), *Matricharia chamomilla*(Compositae), and *Melia azedarach*(Meliacae), which were 1.807, 0.505, and 1.035 mg/L, respectively (after 24 h exposure), on the 3rd larval instar of *Culex quinquefasciatus* (Say). Delaying effect of the LC_{50} were followed up on the life cycle of the insect. The results showed that the concentration used led to a prolongation of the 3rd larval stage as: 23.20± 1.009 and 23.10±1.006 days for each treated group of *A. herba alba* and *M. chamomilla*, respectively, while those treated with *M. azedarach* complate larval stage after 26.3 ± 1.004 days, the untreated larvae 10.78±0.25 days ,and affected proportions entering the pupal stage and developing to pupal stage 20% and 26.6% compared to the control (86.49%). It had an impact on female longevity, where females lived longer than males, as well as on sex ratios, with more males than females. These results clearly demonstrate the effectiveness of these extracts to influence the life cycle of this insect. Plant extracts apparrentally acted as a growth regulation and suppressed the develping of the *Cx.quinquefasciatus* thereby impeding the natural growth of the insect and reducing its damage.

Keywords: delayed effect, plant extract, *culex quinquefasciatus*, larval control.

INTRODUCTION

Saudi Arabia is characterized by plant diversity.Each plant consists of different biochemical components of which many have some potential effects in controlling pests and diseases. The use of plant-derived compounds to control pests has gained increasing importance over conventional chemical pesticides, because of their excellent biodegradation in addition to their safety to humans, hot blooded animals and natural enemies of insects, in addition to their unstable nature. In recent years there were reports about many plants with active properties as pesticides. As the use of pyrethrum powder *Chrysanthemum cineraiaefolium*, *Nicotiana tobaccum*, and *Azedaracta indica* (Subramaniam, 1993) in some pesticides, can seriously affect the life cycles of insects by breaking their cycle before they molt to the adult stage. Small concentrations should be used to prevent an imbalance in the environment where target organisms live. Mehta *et al.* (1999) found that an extract of *M.azedarach* leaves against *Henosepilachna vigintioctpunctata* caused an impact on the life cycle by extending it 6 days beyond the normal cycle, along with malformation in the adult stage that resulted from the treated larvae. The natural components of the botanical extract may affect the stomach of the larvae, so it is expected to have morbid activity during the following stages of development (pupa and adult). Sujath *et al.* (1988) observed that a botanic extract of *Acorus calamus* led to severe malformation in the *Anopheles stephensi* mosquito and less deformation in the *Culex quinquefasciatus* mosquito. This experiment were conducted to evaluate the biological effects of LC_{50} of extracts of *M. chamomilla*, *M. azedarach*, and *A. herba alba*.and the delaying effect when treating the 3rd larval instar.

MATERIALS AND METHODS

1. Mosquito strain rearing

Culex quinquefasciatus (Say) egg-rafts were collected from a laboratory in the College of Science, King Saud University in Riyadh. Egg-rafts were obtained from females rearing laboratory, and transferred to medium-sized rearing containers (10 x 6 cm) filled with tap water. Each egg-raft was put in a separate container to avoid overcrowding on the food. The containers of eggs were provided with a small quantity of food. Water was stirred daily with a glass stem to prevent formation of a superficial fatty layer that could kill the larvae. During larval development to the 2nd instar, they were transferred to larger containers (25 x 8 cm). The water was changed every two or three days as needed by pouring the used water through a narrow-pore net to filter it, and then moving the larvae to another container with clean water. Any dead larvae were removed instantly, and an effort was made to maintain the level of water in rearing containers by restoring any evaporated water. Large basins were used to raise growing numbers of larvae and were provided with oxygen pumps.

2. Larval feeding

Different feeding media to mosquitoes larvae were reported in many studies, Goldberg and Ford (1982),

[1]Email:aj_khalaf@yahoo.com
[2]King Abdul-Aziz University, P.O.Box 19516, Jeddah, 21445 Saudi Arabia.
Email: ralmehmadi@kau.edu.sa , d_almehmadi@yahoo.com
Received July 10, 2010, Accepted August 2, 2010

who used tetramine powder to feed *An. multicolor* and *T. longiareolata* mosquito larvae, adding dog biscuits to feed *An. caspius* and *Cx. pipiens* mosquito larvae. Saleh,(1985) used a mixture of equal parts of yeast, biscuits, and skim milk, and Al-mehmadi(1990), Fallatah(1997) and Al-Ghamdi(2005) used tetramine for *Cx .univittatus* and *Cx.pipiens* mosquito larvae. In this study, fish food (Tropical Flakes) was used to feed *Cx.Quinquefasciatus* mosquito larvae. An appropriate amount was scattered over the rearing container, and this food proved highly efficient for larval rearing.

3. Plant extracts used and bioassay:

Three plant extracts namely *Artemisia herba alba*, *Matricaria chamomilla* and *Melia azedarach* were tested against 3rd instar larvae of *Cx. quinquefasciatus* mosquitoes. The plant extracts were obtained from Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), College of Pharmacy, King Saud University in Riyadh. They were reserved in dark glass bottles at a low temperature (4°C) until use. Experiments were conducted to find the delayed effect of plant extract, by using a concentration that caused 50% larval mortality 24 hours after the start of treatment (on 3rd instar larva) which was 1.807, 0.505 and 1.035 mg/l, a lethal concentration (LC_{50})of the botanical extracts of *Artemisia herba alba*, *Matricaria chamomilla* and *Melia azedarach*, respectively. Tests were carried out on 3rd instar larvae immediately after molting. The larvae were divided in 80 ml glass vials and four replicates were used each of 20 larvae with 200 ml of each concentration of extract for every replicate. In addition, a control of 80 larvae was placed into four replicates each of 20 larvae in tap water. The treated larvae of this concentration were used to determine the following biological aspects: duration of larval stage, duration of pupal stage and percentage of pupal stage. Determination of reproductive efficiency, longevity of females and males, Sex ratio were record. The number of eggs, incubation period and percent hatchability were also considered. All biological measurements were carried out at 75 ± 5% RH and 26° ± 1 ºC.

RESULTS

Table (1) shows the durations of 3rd instar larvae treated with LC_{50} of each botanical extract before developing into pupae, as well as in the pupal durations before developing into adults. The control larvae took an average of 10.78±0.25 days to develop into the pupal stage, while for larvae treated with LC_{50} of *A. herba alba*, *M. chamomilla*, and *M.azedarach* the results were as follows:

23.20± 1.009 and 23.10±1.006 days for each treated group of *A. herba alba* and *M. chamomilla*, respectively, while those treated with *M. azedarach* reached the larval stage after 26.3 ± 1.004 days and failed in the process of transition to pupae. For pupal duration, no clear difference was found in efficiency between treated and control groups. For the controls, the duration was 2.44 ± 0.17 d, but the groups treated with *A. herba alba* and *M.chamomilla* extracts were pupae for an average 2.81 ± 1.02 and 2.32 ± 1.01 day, the proportion of transition to pupae was 20% and 26.6% compared to the control group(86.49%).

As shown in Table (1) , the percentage of pupae developing to adults was affected by treatment with LC_{50}. The mean percentage developing into adults in the various treatments were as follows: 16% when treated with *A. herba alba*, 24% with *M. chamomilla*, 94% in the control group.

The results in Table 1 shows that the female mosquitoe lived longer than the males.. Tthe average lifespan of the females in the control group being 12.83±0.47 and 11.11±0.15 days for males. In the treated groups, the plant extracts had an effect on the longevity of both males and females. For both, life span was shorter than in the control, with an average longevity of both female and ma le larvae treated with *A.herba alba* of 7.33 ± 1.10 and 3.95 ± 1.03day, and with *M. chamomilla* = 6.77 ± 1.04 and 4.16 ± 1.02 day respectively. The results also indicated that the response of 3rd-instar larvae depended on the plant extract. In Table 1 it appears that the sex ratio of the adult resulting from the treated 3rd- instar larvae was affected being 1:1.4 and 1:1.5 resulting from treatment with *A. herba alba* and *M. chamomilla* , respectively, while the control group had a sex ratio of 1:1.07 females to males. The larvae treated with LC_{50} of *M. azedarach* extracts failed to develop. As for an effect on reproductive capacity of females, the results showed that the females and males failed to reproduce.

DISCUSSIONS

Many varieties of plants have an effect on many species of pests. A large number of those plant species probably have toxic properties, but their effect on the development of insects has not been studied. The results of the plant extracts examined in the present study clearly showed prolongation of the larval period after exposure to LC_{50} of each of the plant extracts. The longevity of the larvae was prolonged maximally in the case of treatment with *M. azedarach*. Almost the same maximum prolonged duration was achieved when the larvae were treated with *M. chamomilla* and *A. herb alba*. The number of pupae resulting from the treated

larvae was less than among those untreated, although the duration of the pupal stage was not much different for both groups (treated and untreated).

The prolongation of the tested 3rd larval period might be due, in part to the effects of these plant extracts on the tissues of insects. Khalaf (1998) Shoukry and Hussein (1998) and Abou El-Ela *et al*. (1995) reported that the total carbohydrates, proteins and lipids decreased in the last instar larvae of *Galleria mellonella* caused by treating third larval instar with some plant volatile oils. Moreover, two of the investigated plant extracts produced effects on the percentage of adult emergence.. No adults emerged when the larvae were treated with *M. azedarach*, because all the larvae and pupae died before developing into the adult stage. Therefore these botanical extracts are able to create an imbalance in the biology of the insect in terms of prolonging the larval instar, as in the case of all the botanical extracts, or preventing development into the pupal stage, as in the case of *M. azedarach*. The current study found that the total period of development of the mosquito *Cx. quinquefasciatus* may have been affected because the larval duration was prolonged compared with the control group. It was also noted that the LC_{50} of the group treated with *M. azaderach* extracts was more intense, which prevented molting to the pupal stage, besides the malformation that occurred in every individual treated during larval instars. Using *M. chamomilla* and *A. herb alba* extracts in the pupal and adult stages produced wing malformation or inability to molt properly from pupae, or impeded the growth of the adult insect. These results correspond with those reached by Khalaf (1999), who found that when the larvae of mosquito *Cx. pipiens* were treated with LC_{75} concentration of essential oils of *Lantana camara* and *Conyza dioscoridis*, none succeeded to emerge in the adult stage.

Previous studies have shown that certain plants exhibit toxic or growth regulator activities against mosquito larvae at concentrations higher than 10 mg/L (Deschmukh and Renapurkar, 1987; Thangam and Kathiresin,1988), but the values of LC_{50} of the ethanolic extract of plants used were less than 2 mg/L. Another study has indicated that the meliacin and meliacarpin found in *M. azedarach* are considered growth inhibitors (Lee *et al*., 1991). The low percentage of pupation "that preceded larvae's failure to develop to this stage" may be explained either by the effective impact of the components of the botanical extracts, which are here considered pathological agents spreading in the digestive system of the larvae, causing the death of numbers of larvae, possibly because of physiological impediment processes interfering with the process of transformation to the pupal stage, although the pupation percentage of formed pupae was not affected by the treatment. This low percentage of emergence from pupae to adult, or the malformation that appeared in the adult insects, led to a decrease in the total number of adult individuals resulting from the larvae treated with botanicl extracts Al-Rubaie, *et al*., (2004)and Schmuttere (1990) mentions that the effects of *Alazadirachtin* substance may continue for 8–14 days, depending on environmental conditions and the variety of treated plant. Dilawari *et al*. (1993) recorded the occurrence of genetic abnormalities and deformations in the adult stages of *Coccinella septempunctata* (L.) and *Plutella xylostella* insects when treated with extracts of *M.azedarach* kernels. All these results correspond with the results of the current study. The results of this study also indicate that female mosquitoes live longer than males. As for an impact on the adult stage, in the case of 3rd-instar larvae treated with LC_{50} of plant extracts (*A. herba albe* and *M. chamomilla*), the sex ratio was affected in the resulting adult individuals, with the number of males greater than the females.In addition, the plant extract treatment led to diminished longevity of males resulting from the treated larvae, compared with control group. The effect on sex ratio by the extract means that there is a difference in the sensitivities of pupae that resulted from the treated larvae, which would produce males or females, compared with the pupae from the control group. This means that treatment with the plant extracts described here had an effect on determining the sex, meaning that the toxicological effect on the larval stage may lead to a difference in the proportion of males to females. As for the impact of the plant extracts on the longevity of adult mosquito *Cx. quinquefasciatus* from 3rd instars treated with LC_{50} from plant extracts, the results showed a reduction in the duration of survival of these insects, whether female or male. This means that the larvae surviving after treatment with botanical extracts result in an adult insect with shortened longevity, compared with adults emerging from the control one, and this is considered one of the positive effects of the use of all types of botanical extracts, decrease longevity of females reducing the extent of damage, as it is known that the females have the ability to transmit serious diseases by feeding on blood, and they are the ones which lay eggs. The reason for shortened longevity of adult insects from treated larvae could be that the cells of the larvae's midgut were slightly affected by these extracts and remained affected until the process of adulthood began, accordingly affecting the insect's survival. It may also be a result of the low stocks of food inside the fat body of the insect because of its low rate of metabolism and increase in catabolism over anabolism because of the toxic components, and thus a lack of completion of the

functions performed by various organs of the insect due to incomplete growth (Al-Mehmadi, 1990). Theresearch regarding the impact of botanical extracts on the longevity of adult mosquitoes is almost nonexistent, but some authors have studied the effects of chemical pesticides on the longevity of mosquitoes. Blaz-quez and Maier (1950) noted that the longevity of adults of *Cx. fatigans* (Wiedmann) and *Ae. aegypti* exposed to nonlethal doses of the pesticide DDT was not affected, and that generations resulting from these treated mosquitoes were normal, and their longevity was not affected. Nnakumusana (1986) found that females of *Ae. aegypti*, *Ae. gambiae*, and *Cx. quinquefasciatus* died quickly after exposure to *Coelomomyces indicus* fungus infection in larval stages, meaning that they had a short longevity. The researcher also found that, the sooner the larvae were infected in the first instars, the shorter the longevity of the females. In the present study, an increase in the longevity of females over males was noted, whether from the larvae treated with the plant extracts, or from the control. This may be due to the fact that females are responsible for laying the eggs and require a longer period for laying a maximum of eggs to increase reproductive efficiency.

REFERENCES

Al-Rubaie, H. F.; Tamimi N. K. and Darraji ,S. F. (2004).The effectiveness of crude extracts of Sobhbh *Melia azadarach* L. And neem *Azadirachta indica* A. Juss in the killing of nymph and adults whitefly *Bemisia tabaci* (Gennadius). Journal of Plant Protection Arab 47:52-22.

Al-Ghamdi, Mariam Mohamed (2005). The impact of Propolis Extract (bee glue) on the mosquito *Culex pipiens* (Diptera: Culicidae) Master Thesis - College of Education faculty departments in Makkah.

Abou El-Ela, R.G.; Helmy, N.M.; El-Monairy, O.M. and Salah, H. (1995). Effect of certain plant extracts on some biochemical aspects of the house fly larvae *Musca domestica* (Diptera: Muscidae). *Bull. Ent. Soc. Egypt. Econ.* 22: 17-25.

Al-Mehmadi, R.M.(1990). Evaluation of the efficiency of the Bacteria *Bacillus thuringiensis* and *Bacillus sphaericus* as A biological control agent on *Culex univittatus* in Saudia Arabia. Jeddah, Saudi Arabia. ph.D.Thesis, Girls College of Edu. Zool.Dept.

Blaz-Quez J. and Maier J. (1950). Effects of doses of DDT on the reproduction and susceptibility of *Culex pipiens* L. *Bull. Wld. Health Org., 38: 459-467.* (Cited after zaghloul and Brown, (1968)

Deschmukh, P.B. and Renapurkar, D.M. (1987). Insect growth regulatory activity of some indigenous plant extracts, *Insect Sci. Appli*., 8: 81- 83.

Dilawari, V.K.; Zebitz, C.P.W. and Kraus, W. (1993). Effect of *Melia azedarach* L. on the fitness of *Coccinella septempunctata* L. Amultitropic interaction approach. *Indo-German Conference on Impact of Modem Agriculture on Environment*, CCS Haryana Agricultural University, Hisar, 1-3, :.202-203.

Khalaf, A.A. (1999).Evaluation of toxicity of two plant volatile oils against laboratory and field strains of *Culex pipiens* larvae.*J.Egypt.Ger.Soc.Zool.,Vol.28(E):* 61-71.

Khalaf, A.A. (1998). Biochemical and physiological impacts of two volatile plant oils on *Muscina stabulans* (Diptera-Muscidae). *J. Egypt. Ger. Soc. Zool*., 27 (E): 315-329.

Fallatah, S.A.(1997).Efficacy of *Bacillus thuringiensis* and *Bacillus sphaericus* as biological control agents against larvae of *Culex pipiens* complex prevailing in the Eastern area of Saudia Arabia.*M.Sc.Thesis*,Fac.Sci.,Girls College.Dammam,Saudi Arabia.

Lee, S.M.; Klocke, J.A.; Barnby, M.A.; Yamazaki, R.B. and Balandrin, M.F. (1991). insecticidal constituents of *Azadirachta indica* and *Melia azedarach* (Meliacae). :.293-304. *In*: P.A. Hedin(ed.). Naturally Occurring Pest Bioregulators, *American Chemical Society ACS Symposium Series*.

Mehta, P.K.; Thakur, M. and Chandel, R.S. (1999). Effect of some plant extracts on growth and development of *Henosepilachna vigintiopunctata* (Fabr.), *Pest Management and Economic Zoology*, 7(2): 119-123.

Nnakumusana E. S. (1986). The effect of *Coelomoyces indicus* on the fecundity and longevity of *Anopheles gambiae, Culex fatigans* and *Aedes aegypti* exposed to infection at each larval instar, *Insect Science and its application 7 (2): 139-142.*

Saleh, M.S. (1985): Larvicidal activity of *Bacillus thuringiensis* serotype H-14 against mosquito larvae. *Insect Sci.Applic.* 6(5): 617-620.

Shoukry F.I.I. and Hussein, K.T. (1998). Toxicity and biochemical effects of two plant volatile oils on the larvae of the greater wax moth *Galleria mellonella* L. (Pyralidae: Lepidoptera). *J. Egypt, Ger. Soc. Zool., Vol*. 27(E): 161-169.[a]

Subramonia, T.T. and Kathiresan, K. (1988). Toxic effect of seaweed extracts on mosquito larvae. *Indian J. Med. Res*., 88: 35-37.

Sujatha, C.H.; Vasuki, V.; Mariappan, T.; Kalyanasundaram, M. and Das, P.K. (1988). Evaluation of plant extracts for biological activity against mosquitoes, *Int. Pest Control*, 30: 122-124.

Schmutterer, H. (1990).Properties and potential of natural pesticides from the neem tree, *Azadirachta Indica. Ann. Rev. Entomol*.:35-271.

Spielman, A. and Skaff, V. (1967). Inhibition of metamorphosis and ecdysis in mosquitoes. *J. Insec physiol., 13, 1087-1095.*

Thangam, T.S. and Kathiresan, K. (1988). Toxic effects of seaweed extracts on mosquitoe larvae, *Ind. J. Med. Res*., 88: 35-37.

الملخص العربي

التأثير المتأخر لبعض المستخلصات النباتية على بعض المظاهر البيولوجية لبعوضة *Culex quinquefasciatus* (Diptera: Culicidae)

أريج عبد الكريم الخلف و رقية محمد المحمادي

أجريت هذه الدراسة لتتبع تأثير المستخلص النباتي على بعض المظاهر البيولوجية نتيجة معاملة يرقات العمر الثالث لبعوضة *Cx quinquefasciatus* بتركيز LC_{50} الذي تم تحديده بعد التعرض لمدة 24 ساعة من المستخلص الايثانولي لكل من نبات الشيح *Artemisia herba alba* ونبات البابونج *Matricharia chamomilla* ونبات الزنزلخت *Melia azedarach*، والتي كانت 1.807، 0.505، 1.035 ملجم/ لتر، على التوالي، وتمت متابعتها طوال فترة دورة الحياة. أظهرت النتائج أن استخدام تركيز الـLC_{50}أدى إلى إطالة فترة طور اليرقة حيث كانت 23.20±1.009 و23.10±1.006 يوماً عند معاملة اليرقات بالبابونج والشيح في حين اكملت الطور اليرقي عند المعاملة بالزنزلخت بعد 26.3 ± 1.004 يوماً أما اليرقات غير المعاملة فكانت فترة حياتها 10.78±0.25 أيام. كما أثرت المعاملة على نسبة الخروج من طور العذراء حيث وصلت إلى ,20% و 26.6% عند المعاملة بكل من الشيح والبابونج على التوالي أما المعاملة بالزنزلخت ففشلت في التحول إلى طور العذراء في حين كانت نسبة الخروج في غير المعاملة 86.49%. كما أثرت على طول عمر الإناث، حيث عاشت لفترة أطول من الذكور، وزادت النسبة الجنسية للذكور عن الاناث. ولم تنجح الاناث الناتجة من المعاملة في عملية وضع البيض. هذه النتائج تدل بوضوح على فعالية هذه المستخلصات في التأثير على دورة حياة هذه الحشرة، حيث قامت بدور منظمات النمو وبالتالي أدت إلى عدم نمو الحشرة مما يعني عدم كفاءة الحشرة المعاملة وتقليل أضرارها.