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The effect of jujube oil on some biological activities of Culex pipiens mosquito

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ABSTRACT

Synthetic insecticides are quick, effective and most popular methods of pest control. But because of their unfavorable effects on environment and non-target organisms, there is a growing need to find new, safe effective alternatives. Plant extracts represent the most promising alternative. The present study aims to evaluate jujube plant as a promising candidate. LC40 of Jujube oil and Jujube extract using petroleum ether as a solvent were investigated against Culex pipiens larvae. The results showed the reduction of egg hatchability, interruption of molting stages and the inhibition of adult mosquitoes emergence cause by application of LC40 jujube oil and jujube extract at 3rd larval stage. Sex ratio was reduced by jujube extract but pure jujube oil has no effect on sex ratio. In the light of these results, jujube plant is recommended as a promising herbal candidate for integrated pest management instead of chemical insecticides.

KEYWORDS

jujube plant; mosquitocidal effect; culex pipiens; egg hatchability; adult emergence; sex ratio; morphological abnormalities.

Academic Discipline And Sub-Disciplines

Entomology, Biological control

SUBJECT CLASSIFICATION

Mosquito control

TYPE (METHOD/APPROACH)

Plant extract application in biological control

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INTRODUCTION

Synthetic traditionally-used insecticides are quick, effective and most popular methods of pest control. But because of their unfavorable effects on environment and non-target organisms, there is a growing need to find new, safe effective alternatives. Plant extracts represent the most promising alternative because they are non-pollutant, easy degradable and less-toxic to the environment. Plant oils consist of mixture of volatile and odorous terpenoids which affect voltage gated and/or ligand gated ion channels in the CNS such as tyramine, octopamine, GABAA, TRP type ion channels and acetylcholinesterase (Blenau *et al*., 2012). Certain plants have been reported to possess insecticidal action against stored grain pests (Gundu Rao & Majumdar, 1962; Ahmed and Eapen, 1986; Nawrot et. al., 1982; Behal 1998), a neem formulation, NeemAzal T/S against *Anopheles stephensi*, *Cx. quinquefasciatus* and *Aedes aegypti* (Gunasekaran *et al*., 2009) and sunflower *Heliunthus annus*, mustard *Brassica juncea*, groundnut *Arachis hypogaea*, sesame *Sesamum indicum*, soybean, olive *Olea europea* and oil palm *Elaeis guineensis* against the pulse beetles *Callosobruchus chinensis* L. (Khalequzzaman *et al*., 2007). Crude plant extracts such as *Agava sisalana* (Pizarro *et al*., 1999), *Allium sativa* (Thomas and Callaghan, 1999), flowers of *Anthemis nobilis* (Soliman and El-Sherif, 1995), *Alnus glutinosa* (David *et al*., 2000), *Thymus capitatus* (Mansour *et al*., 2000), leaves of *Origanum majoranal* and *Jasminum fructicans* (Soliman and El-Sherif, 1995), seedlings of *Sorghum bicolour* (Jackson *et al*., 1990), rhizome of *Vetiveria zizanioides* (Soliman and El-Sherif, 1995) and peel of *Citrus limon* (Thomas and Callaghan, 1999) have been reported for their mosquitocidal activity against *Cx.pipiens* mosquitoes. Repellency effect of jujube plant against *Trogoderma granarium* has been reported by Dwivedi and Shekhawat (2004). No reference is available regarding the use jujube plant against *Cx.pipiens* larvae except El-Husseiny et al. (2014) but the biological effects of jujube plant on *Cx.pipiens* remain uncharacterized.

The present study aims to estimate the effectiveness of jujube plant oil and plant extract against *Cx. pipiens* mosquito to evaluate whether it could be a promising herbal alternative for chemical insecticides.

MATERIALS AND METHODS

Mosquito larvae

Culex pipiens egg rafts were collected from a fresh water pool near the Faculty of Science, Tanta City, Egypt. They were placed in white enamel pans containing dechlorinated tap water. After hatching, larvae were reared at insectary conditions of 27±2 °C temperature, 70–80 % relative humidity and under a 16:8 h (light:dark) photoperiod. The larvae were fed TetraMin (Tropical Fish Food, Tetra WerkeW. Germany).

Zizyphus jujube extracts

Jujube oil was obtained from the Egyptian Company of Natural Oils, Cairo. Jujube leaves were obtained from planted trees in the Faculty of Agriculture, Cairo University, Giza. Jujube leaves were washed, dried in the air and then ground into fine powder in a spice grinder. Jujube extract using petroleum ether as a solvent was prepared according to El-Husseiny et al (2014). The jujube extract was applied to *Cx.pipiens* larvae in sublethal dose (LC_{40}) which were calculated according to its LC_{50} value (El-Husseiny et al, 2014).

Estimation of female fecundity, egg hatchability, emergence inhibition and emerged adult's sex-ratio

One hundred early fourth instars' larvae of *Cx. pipiens* were exposed to LC_{40} of jujuba oil and jujube plant extracted in petroleum ether. Twenty four hours post-treatment, the survivor larvae were gently washed and transferred to labeled breeding pans. For each treatment three replicates were made. Treated larvae and control were kept under laboratory conditions of 27±2^{o}C and 75± 5% RH and a photoperiod of L-D/16-8hrs/day. Emerged pupae were collected daily using small droppers then transferred to small plastic cups filled with dechlorinated water and placed in adult wooden framed cages (30x30x30cm3) screened with a fine narrow mesh till adult emergence. Emerged adults were counted, sexed and the sex ratio was determined. Adult mosquitoes were daily provided with a piece of cotton soaked in 10% sucrose solution for adult feeding.

Four to five days post- emergence, adult females were allowed to feed on pigeon. Each single engorged female was transferred into a propylene tube (4 cm in diameter and 7 cm deep) 1/3 filled with water, and covered with muslin. The tubes were observed daily for egg laying. The number of eggs in each obtained egg raft was calculated. The number of hatched and unhatched eggs obtained from all treatments and control was calculated. The data obtained were subjected to statistical analysis to evaluate the differences in action between tested agents.

Light microscopy

To detect signs of abnormalities occurred in some stages of life cycle of *Cx. pipiens* mosquitoes due to jujube oil application, all dead pupae and adult mosquitoes were transferred to glass slides using a fine brush and wet-mounted for microscopic examination using Olympus BX61 light microscope.

RESULTS

The purpose of this study was to determine the effects of sublethal treatments of jujube oil and its dried plant extracted in petroleum ether on Culex larvae on adult emergence, sex ratio and reproductive potentials of Cx.pipiens.

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Fecundity reduction:

The estimation of fecundity is based on the number of eggs per egg raft per female mosquito. LC40 treatment of Jujube oil and the plant extracted in petroleum ether resulted in insignificant reduction (students t-test) in the fecundity of mosquitoes (p=0.67) and (p=0.45) as compared to the control group (161±20 and 167±26) respectively compared to (170±12) table (1).

Hatchability inhibition:

Egg hatching was significantly reduced (students t-test) by larval exposure to LC40 treatment of Jujube oil and the plant extracted in petroleum ether (p=0.0003and p=0.0137) respectively as compared to the control group the percentage of egg hatching in treatments of Jujube oil and the plant extracted in petroleum ether were (40.6% and 51.5%) respectively. the percentage of egg hatching in control specimens was (100%) table (1).

Emergence inhibition and sex ratio:

The estimation of adult emergence is based on the relation between the total numbers of emerged adults to the total number of treated larvae (mortality in all stages is included). The effect of larvicidal agents on adult emergence and sex ratio is shown in table (2).The LC40 treatment of jujube oil and the plant extracted in petroleum ether were reduced adult emergence by 52% and 67% respectively compared with 30% in control. The proportion of emerged adult males was slightly increased with jujube extracted in petroleum ether being 1:0.9(♂:♀). The proportions of emerged adult males and females were equals with jujube oil being 1:1(♂:♀) compared with proportion of female biased in the check group 1:1.3(♂:♀).

Pathological effects of jujube oil

Light microscopic examination results revealed morphological abnormalities on the stages of life cycle of Cx. pipiens after exposure to LC40 of both pure jujube oil and jujube plant extract compared to the unexposed control. Generally, jujube oil treatment causes darkness of dead pupal and adult stages. Pathological effects of jujube oil were obvious in dead late pupal stages which appear to be uniformly black, extended more than they should be but failed to reach adult stage (Fig.1A). Some of fully-formed, partially-emerged adults died immediately after several trails and failures of emergence because their legs and wings still attached to the pupal exuvae and subsequently these adults were unable to pull themselves out of pupal exuvae and dead cadavers remain float on the water surface (Fig.1B). Application of LC40 of jujube extracts in petroleum ether as an organic solvent, cause morphological abnormalities in pupal and adult stage. Some pupae extended but failed to develop to adult stage (Fig.2A). Some pupae in late pupal stage developed partially into adult stage and tried but failed to emerge out of their pupal case (Fig.2B and C). Some fully-formed adults remain hardly attached with their legs and mouthparts to pupal exuvae (Fig.2D).

Table (1): Mean number of eggs per-female and egg hatchability of Cx.pipiens resulting from the treatment of 4th instar larvae with sublethal concentrations of jujube larvicides. S.E= standard Error

Tested agents	No. of egg rafts	Total No. of eggs	Mean±SE of eggs	No. of hatched eggs (%)
Jujube oil	8	1285	161±20	522 (40.6%)
Jujube extract	8	1241	167±26	640(51.5%)
Control	10	1695	170±12	1695 (100%)

Table (2): The percentage of adult emergence and sex ratio of Cx. pipiens resulting from the treatment of the 4th instar larvae with sublethal doses of larvicides.

Tested agents	Total No. of larvae tested	No.of dead larvae	No. of pupae(%)	No. of adult emerged(%)	No. of males	No. of females	Sex ratio	
							♂	♀
Jujube oil	81	31	50	42	21	21	1	1
Jujube extract	95	45	50	33	17	16	1	0.9
Control	79	9	61	70	30	40	1	1.3

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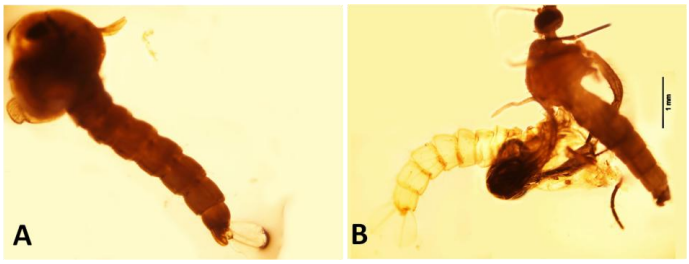

Fig.1: pathological effect of LC40 jujube oil treatment on pupal and adult stage of Culex pipiens. A, dead fully-formed pupa extended but it is unable to develop to adult stage. B, dead partially-emerged adult stage. Note that adult mosquito is unable to pull itself out of pupal exuvae because legs and wings still glued to the pupal exuvae.

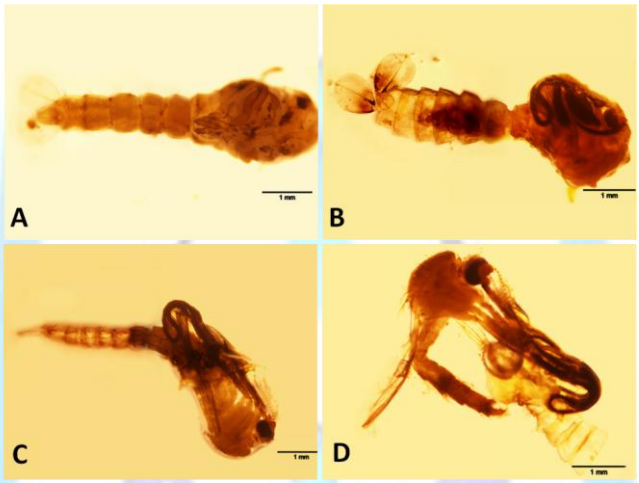

Fig.2: pathological effect of LC40 jujube extract in petroleum ether treatment on pupal and adult stage of Culex pipiens. A, fully-formed pupa, extended but failed to develop to adult stage. B and C, late pupal stage that failed to transform completely into adults and failed to emerge out of their pupal case. D, dead fully-formed adult stage. Note that they are unable to pull themselves out of pupal exuvae because they pulled out its wings but legs, mouthparts and the proximal tip of its abdomen are still glued

DISCUSSION

Being unsafe to the non-target organisms and environment, there is a growing need to find new natural alternatives for chemical, sysnthetic insecticides. The main goal of this work was to evaluate insecticidal potency of jujube plant, as promising candidate, against *Cx.pipiens* mosquitos. The present investigation indicated that adult emergence was reduced when larvae were treated with LC_{40} of jujube oil and jujube plant extracted in petroleum ether. The reduction of adult mosquitoes emergence as a result of application of plant extract was reported before by Shaurub and El-Bassiony (2014) who found that the LC_{50} of diethyl ether extract of *Nerium oleander* leaves significantly decreased percentage of adult emergence of *Culex pipiens* mosquito and Kamiabi *et al*. (2013) who revealed that sublethal dose (EI_{50}) of the crude extracts of *Cyperus aromaticus* cultured cells inhibited the adult emergence of *Aedes* mosquito species.

Our results reveal that the egg production of female *Cx.pipiens* was insignificantly decreased due to application of jujube oil and jujube plant extracted in petroleum ether compared to control. These results are in disagreement with Shaurub and El-Bassiony (2014) who showed that the reduction in fecundity of *Culex pipiens* emerged from treated larvae with LC_{50} of diethyl ether extract of *Nerium oleander* leaves was about 36.57 % of the control and Kamiabi et al. (2013) who reported that the crude extract of *C. aromaticus* cultured cells reduced the mean number of eggs oviposited by treated female of *Ae.aegypti* mosquitoes. Also, Priya et al. (2013) tested the acute toxicity of the acetone extracts of *Anamirta cocculus* (L.), Wight and Arn fruits and *Sphagneticola trilobata* (L.) Pruski (leaf) against *Culex pipiens* (L.). Their data shows that the effect of the selected plant extracts is dose dependent and reduced the fecundity level very significantly when compared with the control. These findings may be run counter to our results because the ingredients and mode of action of these plants are different from jujube plant.

The present study reveals that treatment of *Culex* larvae with LC_{40} of Jujube oil and jujube plant extract in petroleum ether caused reduction in egg hatchability. This finding is in agreement with Shaurub and El-Bassiony (2014) who found that application of LC_{50} of diethyl ether extract of *Nerium oleander* leaves significantly decreased egg hatchability of *Culex pipiens* mosquito. Kamiabi *et al.* (2013) found that application of the crude extract of *C. aromaticus* cultured cells cause significant reduction in the hatching percentage of eggs produced by the parental generation of *Ae. aegypti* over the first gonotrophic cycle (24.03%) and *Ae. albopictus* over the first and second gonotrophic cycles (28.82% and 19.22%

respectively) compared to the controls. Priya et al. (2013) reported that *Anamirta cocculus* fruit extract in acetone showed a significant reduction in fecundity and hatchability of *C. pipiens.*

Our results reveal that the proportions of emerged adult males and females were equal when the larvae were subjected to the action of LC_{40} of jujube oil. Same effect was obtained in *Aedes* mosquitoes after exposure of the 3rd instar larvae of each mosquito to the EI_{50} dose of the crude extract of *C. aromaticus* cultured cells (Kamiabi et al., 2013). In our study, sex ratio was changed by larval exposure to LC_{40} of the plant extracted in petroleum ether. The proportion of emerged adult males tended to be increased more than that of adult females.

Application of LC_{40} dose of jujube oil and jujube extract in petroleum ether interrupt the life cycle which is very clear due to obtaining a molting stage, pupal-adult intermediates, and the failure of some adults to emerge out of their pupal exuvae. The same interruption of the life cycle was recorded in *Anopheles stephensi*, *Cx. quinquefasciatus* and *Aedes aegypti* induced by application of a neem formulation, NeemAzal T/S (Gunasekaran et al., 2009). During the emergence test, most mortality occurred in larval and pupal stage and few occurred in adult stage (data not shown).

Our results reveal that pathological effects of jujube oil were observed in late pupal and adult stages which appear to be uniformly black after death. Darkness of stages of life cycle due to application of plant extract was observed before in *Cx.Pipiens* larvae after application of jujube plant extracts (El-Husseiny et al, 2014).

Taken together, the reduction of egg hatchability, interruption of molting stages and the inhibition of adult mosquitos' emergence cause by application of LC_{40} jujube oil and jujube extract suggested that jujube plant could be a promising candidate for integrated pest management instead of chemical insecticides.

CONCLUSION:

The present study explores the potential role of the different leaf extracts of *jujube* as control agents to *Cx. pipiens* . Petroleum ether extract of *jujube* leaves should reduce the population dynamics of *Cx. pipiens*, either directly through larval kill, or indirectly through its latent effects expressed in reduction of egg hatchability, inhibition of adult emergence, interruption of life stages and effect on sex ratio. The insecticidal property of this extract can be considered for use in future integrated management strategies of *Cx. pipiens*.

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