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EFFECT OF *WITHANIA SOMNIFERA* PLANT EXTRACTS IN IMMATURE STAGES OF *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE)

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Abstract

The study was planned to prepare the ethyl alcohol and hexane to extract of the plant *Withania somnifera* and studying their effect in Immature Stages of *C. quinquefasciatus*. The study results showed that the ethyl alcoholic extract of *Withania somnifera* plant scored mortality rates in immature stages of *Culex quinquefasciatus* higher than hexane extracts. The mortality rate of eggs, larvae and pupal was 100% after 72 hours of treatment at 0.8% concentration. While the highest mortality rate for eggs, larvae, virgins and pupae recorded 72 hours of hexanal extract treatment was 75.22, 87.08, 85% respectively, at the same concentration. Furthermore, results showed various abnormalities on the larvae, including black spots on the larval bodies and the larvae dead during Ecdysis into the next instar or to the pupal instar. In addition, some larvae were elongated, while the others become larger than the normal size. Head malformation, abdominal rings and the disappearance of some hair tufts at the end of the abdomen, as well as the brush at the front of the head were noticed. Low concentrations of (0.2%) ethyl alcohol extracts of *W. somnifera* plant leaves increased, the growth period of immature stages resulted from treated eggs, especially, the first larval instar when reached 41-43 days compared to 13-16 days for the control treatment with. In addition to the external malformation represented in atrophy the thick hair tufts in the front of the head (the brush) used by the larva to collect food, as well as the hair tufts and bronchial gills at the end of the abdomen was observed.

Key words: Extract, *Withania somnifera*, *Culex Quinquefasciatus*.

Introduction

Mosquito is one of the most important medical and veterinary vectors. They transmit many parasites and pathogens, having a devastating effect on humans. The diseases transmitted by Pipiens are one of the major health problems in worldwide, including malaria, dengue fever, yellow fever, filariasis and chikungunya (Mittal *et al*., 1995). The *Culex quinquefasciatus* is the main carrier of nemathelminthes that causes filariasis and impacts various parts of the tropical and subtropical regions (Sabra, 1981). About 120 million people were infected by lymphatic filariasis and more than 1.3 billion were in danger causing an approximated loss up to 1.3 billion dollars per year (Conteh *et al*., 2010). This disease has been reported to spread in more than 83 countries according to World Health Organization (WHO, 2012). Plant extracts have been used as an alternative to chemical pesticidesin pest control, including as they are environmentally safe. *Withania somnifera* (Ashwagandha, *Withania somnifera*, winter *Prunus cerasus*) is one of the plant extracts that have effectively been used to control Pipiens immature stages. Furthermore, the extract of this plant was used in the oldest Indian medicine for 3000 years. It consists of many compounds that cure many diseases such as arthritis, anxiety, neurological diseases, cardiovascular disease, etc. (Vijay *et al*., 2016). Bansal *et al*., in 2011 noted the importance of *W. somnifera* extracted by different solvents against *Anopheles stephensi* larvae, *Aedes aegypti* and *C. quinquefasciatus*, which are important disease vectors in semi-arid areas (Sumathi and Gasper, 2016).

Materials and Methods

Cx quinquefasciatus Pipiens culture

The Pipiens were reared and maintained inside a 50cm × 50cm cubic wooden cage covered with clamp wire in front and topside and the other sides and the base were covered with wood. A cylindrical rag of 50cm length was placed on one side in order to insert and remove the breeding containers. The larvae were placed in 300 mL plastic containers containing 250 ml of tap dechlorinated water. The larvae were fed using fish diet and bread

crumbs to obtain egg rafts, while the adult were fed on the pigeon's blood after three days from emerged as follows: had feathers was removed from the chest and abdomen, the wings and legs were tiered and the bird was placed on the breeding cage overnight so that the females could get the blood meal easily. A water vase was placed inside the cage to lay eggs. A piece of cotton was saturated with a 10% sugar solution and placed in a glass dish for adult males feeding (Alissa, 1999).

Collection and diagnosis of *W. somnifera* plant samples

W. somnifera seeds were collected from experimental fields in Horticulture and Landscape Design Department, College of Agriculture engineering science/ University of Baghdad. These seeds were sown in pots containing peat moss and after growing into the appropriate size (25-30 cm), they were transferred to the permanent field (Northeast of Baghdad). The plant species was identified by the National Herbarium of Iraq-Department of Seed Certification and Testing, Ministry of Agriculture. For the extraction process, older leaves were collected from plants at flowering stage. Leaves were separated from the branches, rinsed and cleaned well. Leaves were air dried by spreading them in the shade with good ventilation at room temperature and flipped regularly. An electric mill was used to crush the dried leaves and the leaf powder was placed in a clean plastic bag. Each bag was marked with a paper indicating the name of the plant sample, the place and time of collection and the dried plant parts and stored during the extraction process later.

Preparation of ethyl alcohol and hexane extracts

About 100 g of *W. somnifera* leaf powder was weight and placed into a 1000 ml flask. Four hundred ml of both ethyl alcohol and hexane was added into the flask and left for 72 hours at room temperature 25±2°C and then filtered through Whitman No1 filter papers. The filtrate was concentrated using a rotary evaporator with a vacuum pump at 40-50°C for (Ethyl alcohol) and 35-40°C for (Hexane), then the thick liquid placed in the Petri dishes and left on the room temperature in order to remove the remaining solvent. The raw material collected was placed in glass bottles and kept at 4°C until use (Harborne, 1973).

Effect of Ethyl Alcohol and Hexane extractions of the W. Somnifera plant leaves on mortality rates of Cx. Quinquefasciatus pipiens eggs

Egg rafts were collected from breeding cages at 24 hrs, age by a soft brush and transferred in 200 ml capacity plastic containers. Each contains a 100 ml of water at an average of one raft per container, treated with one of the following concentrations (0.2, 0.4, 0.8)% with four replicates per each concentration. The same treatment was applied to the control treatment except for using water and solvents only (Karim, 2016). After the egg hatched, the mortality rate was calculated then corrected using the Abbott equation, 1925.

Effect of Ethyl Alcohol and Hexane extracts to the W. Somnifera plant leaves in mortality rates of Cx. Quinquefasciatus pipiens larvae

The experiment was carried out by placing the larvae at final ages (3rd and 4th) in three plastic containers. Each container contained 20 larvae treated with one of the three concentrations previously mentioned, with four replicates. The same procedure was followed in control treatment, which was treated with water and organic solvent only. All experiments were carried out at 25±2°C and the mortality rates were calculated after 24, 48 and 72 hours, then corrected according to the Abbott equation 1925. The effect of Ethyl Alcohol and Hexane extracts of *W. Somnifera* leaves on mortality rates of *Cx. Quinquefasciatus Pipiens* pupal was tested based on the same procedure carried out as mentioned above.

Results and Discussion

Effect of Ethyl Alcohol and Hexane extract of W. Somnifera leaves on mortality percentage of Cx. Quinquefasciatus pipiens eggs

Results confirmed the eggs mortality scored higher rate in the ethyl alcoholic leaf extract of *W. somnifera* treatment at all concentrations compared to hexane extract (Table 1). The highest eggs mortality rate was 90.19% in the at 0.8%, concentration. Whereas, of Hexanes extract increased the mortality rates up to 79.99% at the same concentration. The increase in the mortality rates of the alcoholic extract indicated most effective compounds in *W. somnifera* could be extracted by this solvent. The reason for the low hatching rate may be either due to that the

Table 1: Effect of Ethyl Alcohol and Hexane leaf extracts of *W. Somnifera* plant in the mortality percentage of *Cx. quinquefasciatus pipiens* egg.

Solvent type	Concentration %				Effect rate of solvent type	Effect rate of Concentration
	0.0	0.2	0.4	0.8		
Alcohol	22.15	32.42	60.80	90.19	51.39	23.77
						29.86
Hexane	25.38	27.30	53.81	75.22	45.45	57.31
						82.74
LSD (0.05)	Solvent type=3.928; Concentrations=5.554; Interaction=7.855					

extracts prevent the gases exchange or the rigidity of eggshell and thus the embryo died and could not hatch (Al- Adill and Abd, 1971). This plant may contain a hormonal analogue which disrupted the embryo growth and thus eggs did not hatch (Drexley, 1982). Similarly, Shaker in 2006 indicated that the Hexanes extract of tobacco plant was less efficient than ethyl alcohol extract and ethyl acetate in *Chrysomya albiceps* mortality. Furthermore, Al-Khafaji in (2010) found that the ethyl alcohol extract of the *Ricinus communis* plant scored the highest mortality rates up to 100% of immature stages in *Culex Pipiens* compared to the hexane and ethyl acetate. In contrast Karim in (2016) indicated that the hexane extract of *Chrysanthemum cinerariaefolium* leaves and flowers at all concentrations over the scored high mortality in *Cx. quinquefasciatus Pipiens* eggs compared to ethyl acetate and ethyl alcohol extracts.

When used at lowest concentration, mainly 0.2%, ethyl alcohol leaf extract, could increase the growth period of immature stages resulted from treated eggs. The first larval instar ranged 41-43 days compared to the control treatment amounted to 13-16 days for control treatment. Furthermore, the morphological malformation mainly, atrophy of the feeding brush the hair tufts and bronchial gills at the end of the abdomen. Similarly Arora *et al*., (2011) indicated a morphological malformation in the larvae treated with *W. somnifera* plant extract, in addition to the smaller size of treated larvae and the cuticle hardening. The long growth period of larva might be eggs were exposed to low doses, as the plant extract containing some substances that acted as anti-feeding, or the plant extract includes a juvenile hormone analogue which has an emergence inhibitory effect (Self *et al*., 1978). They noticed. When treating the *Cx. Quinquefasciatus Pipiens* with the juvenile hormone analogue. Emergence was inhibited by 52% thirty five days of treatment in the sewage canal". Similarly, (Al-Taee, 2003), found that there was an increase in the growth period in immature stages of *Cx. pipiens* when treated with leaves and flower plant terpenes Capparis spinosa, which scored 17 and 18 days, respectively, compared to 10 days for control treatment.

Effect of Ethyl Alcohol and Hexane extracts of W. Somnifera plant leaves in mortality percentage of Cx. Quinquefasciatus pipiens larvae

Results showed alcohol extract scored the highest mortality rates at all concentrations (Table 2). The highest mortality rate was 100% at 0.8% concentration 24 hours of treatment. Whereas, mortality caused by Hexane extract was 76.25% at the same concentration and time. Similarly, Al-Zaher, (2005) indicated that the alkyl alcohol extract of *Myrtus communis*, Eucalyptus and *Melia azedarach* caused mortality up to 100% in *Culex pipiens*. Moreover, (Makhlaf and Marwan, 2017) reported that the alcohol extracts of *Achillea fragrantissima* (Forssk), *Arnebia decumbens* and *Juglans regia* plants caused 100% dead rate of 2nd and 4th instar larvae at 250 ppm. In contrast (Shaker *et al*., 2010), mentioned that, the Hexanes extract of *Chara* spp. scored better results than the Ethyl alcohol extract when LC50 and LC90 of pipiens larvae were 1000 and 3000 ppm, respectively, 24 hours of treatment with 65.27% mortality rate of (Manimegalai *et al*., 2013) reported that the Hexanes extract of the *Abutilon indicum* plant was better than the ethyl alcohol extract, when the mortality rate of forth instar larvae of *Cx. Quinquefasciatus pipiens* scored 100% at 300 ppm concentration 24 hours after treatment.

The highest mortality rate indicated presence of a high poisoning cases and an accumulation of active substances occurred in *W. somnifera* plant in the digestive channels of larvae. This activity did not restricted to mortality, but also included the growth delays and malformation. These might be resulted from the effect of the extract active compounds on the enzymes in the digestive channel. The epithelial cells of the digestive channel for insects contained a group of enzymes that

Table 2: Effect of Ethyl Alcohol and Hexane leaf extracts of *W. Somnifera* in mortality percentage of *Cx. Quinquefasciatus pipiens* larvae.

Solvent type	Concent-ration %	Mortality Percentage after 24 hr. of treatment	After 48 hours	After 72 hours	Effect rate of solvent type	Effect rate of extract concentration	Time effect rate
Alcohol	0.0	0.00	0.00	0.00	49.38	0.83	37.19
	0.2	6.25	28.75	41.25			
	0.4	52.50	75.00	88.75		26.43	49.30
	0.8	100	100	100			
Hexane	0.0	0.00	2.50	2.50	44.63	69.42	
	0.2	10	32.91	39.44			
	0.4	52.5	70.69	77.08		91.32	54.51
	0.8	76.25	84.58	87.08			
LSD (0.05)	Solvent type= 3.663; Concentration= 5.180; Time=4.486; Interaction = 12.689						

Table 3: Effect of Ethyl Alcohol and Hexane extract to the *W. Somnifera* plant leaves in mortality percentage of *Cx. Quinquefasciatus pipiens* pupal.

Solvent type	Concent-ration %	Mortality Percentage after 24 hr. of treatment	After 48 hours	After 72 hours	Effect rate of solvent type	Effect rate of extract concentration	Time effect rate
Alcohol	0.0	0.00	0.00	0.00	41.46	00.00	26.09
	0.2	8.75	36.25	46.25			
	0.4	17.50	43.75	60.00		27.92	41.72
	0.8	85.00	100	100			
Hexane	0.0	0.00	0.00	0.00	35.42	37.50	
	0.2	6.25	31.25	38.75			
	0.4	15.00	38.75	50.00		88.33	47.50
	0.8	76.25	83.75	85.00			
LSD (0.05)	Solvent type= 2.822; Concentration= 3.991; Time=3.456; Interaction = 9.775						

have a major role in removing the toxic effect of natural compounds in the plants that feed on. Thus, any compound that affects these enzymes may cause toxicity to the digestive channel tissues and insect death (Wigglesworth, 1972). Morphological malformation varied in the dead larvae from black spots symptoms on the larval bodies to the larvae death during Ecdysis into the next instar. In addition, an elongation was shown in some larvae, while the others were larger than normal, or showed head malformed, abdominal rings abnormality and absence of some hair tufts at the end of the abdomen and the brush on the front of the head. Salih and Akron, (2010), reported, when treating *Cx. Quinquefasciatus Pipiens* larvae with the water leaf extract of the *Nerium* plant it caused a mortality rate of 100% at 3000 ppm concentration. Besides, it caused larva malformation, deadly blackness, inflation and malformation in the abdominal rings on the treated larvae.

Effect of Ethyl Alcohol and Hexane leaf extracts of W. Somnifera in the mortality percentage of Cx. Quinquefasciatus pipiens pupal

The alcohol leaf extract showed the strongest effect in the *Cx. quinquefasciatus* pupal stage at all concentrations when scored 100% mortality rate compared to 85% for the hexane extract at 0.8% concentration. While the lowest mortality rates were 45% and 35% in alcoholic and hexane extracts respectively, at 0.2% concentration. Moreover, there was an obvious correlation among mortality rate, extract concentrations and the time of exposure as the result showed mortality rate increased when the exposure time increased, as well. Mortality may be caused, either by the toxic substance effect in the cuticle hardening through inhibiting the tyrosinase enzyme, or through the effect of these toxic substance occurred in the extract on the body wall, the respiratory openings and prevent the gas exchange (Al Drexli, 1982). (Halify and Al-zubaidi, 1989) presented another explination which that the extract contains some compounds that act as an insect growth regulators and thier effect on the emergence hormone, leading to the emergence failure of adults. Al-Khafaji, (2003) pointed that the alcohol extract of *P. harmala* plant caused on the highest mortality rates among other organic solvents of *Culex Pipiens* pupal, when scored 81.8% at 20 mg/ml concentration. Finally, Mahdi, (2001) indicated the alcoholic fruit extract of the *Melia azedarach* plant caused mortality in *An. pulcharrhimus* pupae ranged 9.66-75.56% at 200-1000 ppm concentrations. Makhlaf and Marwan, (2017) noted that the death rate of *Culex pipiens molestus Forsakl* pupae was 100% when treated with the alcoholic extract of the *Achillea santolina* at 500-600 ppm, through two exposure time periods (24 and 48 hours). In contrast, Karim, (2016) indicated hexane flower extract of *Chrysanthemum cinerariaefolium* caused mortality rate higher than the alcoholic extract of the same plant which was 70.10% when used against *Cx. Quinquefasciatus Pipiens* pupae, at 40 mg/ml concentration.

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