The Effect of Alkaloids Extract of *Amaranthus gracilis* (L.) on Some Biological Aspects of House Fly *Musca domestica*(L.)

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Abstract

Present study is carried out to evaluate the effect of alkaloids extract of *Amaranthus gracilis* on some biological aspects of the house fly *Musca domestica* to find out the best way for their control. The highest mortality percentage on eggs of the house fly was 83.3% at concentration of 10 mg/ml followed by 44.4% and 31.1% at 7.5 mg/ml and 5 mg/ml respectively. Results also referred that the mortality rates of eggs, larvae, pupae were increased with increasing concentration. The pupal weights were decreased with increasing of concentration.

Keywords: *Musca domestica*, *Amaranthus gracilis*, Alkaloids extract.

تأثير المستخلصات القلوانية لنبات السرمك *Amaranthus gracilis* (L.) في بعض معايير الأداء الحياتي للذبابة المنزلية *Musca domestica* (L.)

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الخلاصة:

أجريت الدراسة الحالية لتقييم كفاءة المركبات القلوانية لنبات السرمك *Amaranths gracilis* (L.) في بعض جوانب الاداء الحياتي للذبابة المنزلية *Musca domestica* (L.) أظهر المستخلص القلواني نسب هلاك عالية لبيض الذبابة المنزلية, حيث وصلت أعلى نسبة 83.3 % عند التركيز 10 ملغم /مل بينما كانت أقل نسب هلاك لنفس المستخلص44.4% و 31.1% عند التركيز 7.5 ملغم/مل و 5 ملغم /مل على التوالي. أشارت النتائج إلى أن معدلات الهلاك للبيض واليرقات والعذارى تزداد بزيادة التركيزأما أوزان العذارى فبينت النتائج أنها تنخفض بزيادة التركيز.

Introduction:

House fly *Musca domestica* (L.), is a major domestic, medical and veterinary pest that causes irritation, spoils food and acts as a vector for many medical and veterinary pathogenic organisms [1 - 4] .In addition it may cause annoyance to humans and the agronomic livestock , resulting in considerable economic loss in livestock business [5]. House flies can also be controlled by making natural fly traps. A conventional method for fly control in the short term is the uses of insecticides. Never the less , the widespread and massive applications of chemical insecticides frequently produce the risk of developing insect resistance and accumulation of residual insecticidal in the environment [6]. These problems coupled with acute neuro–toxicity to man and his domesticated animals have stimulated the search for biologically based alternatives [7]. The majority of flies have been found to assess crude extracts from many botanical sources**.** Regarding this, assessment of the pure active compounds extracted from plants against flies is of interest, because synthetic

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insecticides can't be used organic dairy operation, hence, there is a need for alternate strategies to control these flies. Accordingly , bioinsecticides , a botanical type based on natural compounds from plants, are expected to be possible application as selective efficacious and toxicologically safe insecticides [8]. Several studies have also looked the possibility of using plant extracts in the control of eggs, larvae, pupae and adults of *M . domestica* [9 , 10]. Hence present study was undertaken to find eco-friendly, economical, readily, available and effective insecticides preparations, which are expected to be devoid of any residual or the accumulative toxicity to the end user.

Materials and methods

Collecting, rearing and identification of *M. domestica:*

Adult house flies were collected from dumpsters nearby the residential area in Al – Aziziya city at Wassit province during 2013 by using aerial net. The insects were transferred into cages and kept in the rearing room (in zoology lab in the collage of women \ Babylon university) with temperature of 30 ± 2 ˚c, and 65 ± 5% relative humidity, and 12hrs.Photoperiod. Adults of *M. domestica* were kept in rearing cage with dimensions of (60 x 60 x 60) cm for the stock culture, cages with dimensions of (30 x 30 x 25) cm for the treatment experiments. The bottom and the roof of the cages were covered with wood, the other four sides were made of muslin cloth, and one side had along sleeve of muslin cloth to allow cleaning and feeding process. Adults were supplied daily with artificial diet (cotton dipped with sugar solution) as described by [1]. Plastic containers were kept inside cages; these containers were contained rearing medium for eggs lying. Rearing media was consisting of Ruth calf and floured bread in ratio 2: 1 another container (125 ml capacity) filled with water and covered by cotton reached to the bottom of it as a source of water [3]. The ingredients were mixed and kept in 500 gm. capacity plastic containers. After materials decayed the female attracted to these materials to lay their eggs on the surface of these media . The containers then covered with muslin cloth and kept at temperature of 30± 1^0C , and relative humidity 65 ± 5 % , The colony was reared for three generations to obtain pure suitable colony level for studying purposes [12]. When the larvae were reached the last in star and pupated , the resulted pupae were collected and placed in rearing cages containing sawdust until the adult's emergence and mating occurred after 36 hrs [11].

The insects were identified at the Iraqi Natural History Museum and research center / Baghdad University.

Collection and identification of plant samples

Leaves of *A. gracilis* were collected from Al – Aziziya city at Wassit province, during flowering time. The plant was identified in Baghdad University Herbarium, College of science. Leaves of this plant were cleaned from dust, then left to dry at temperature at 35 – 40 ˚c in oven for three days. The samples were grounded into powder by electrical grinder, and then the powder was kept until use.

Extract of plant was prepared according to [6]. The stock solution of alkaloid compounds were prepared by dissolving 2 gm of plant extract (leaf) residue in 5 ml of ethanol then the volume completed to 100 ml with D.W. The concentration of the resulting stock solution was 2 % or equal to 20mg /ml , the reagents used to detection of alkaloids were mayer's , tannic acid & dragendorff reagents. In order to investigating the bioactivity of these plant extracts. Different concentrations (5, 7.5 and 10) mg/ml of plant extracts was prepared according to the following equation: C1V1= C2V2**.**

Determination bioactivity of alkaloids extracts of *Amaranthus gracilis* on *Musca domestica*:

1- Effect on eggs:

The concentrations of used extract was 5, 7.5 and 10 mg/ml to test its activity on eggs of .10 eggs were used (three replicates for each concentration), the eggs were treated with different concentrations by hand spray (5 ml in volume) , on the other hand control treatments were treated with D.W , and solvent (ethanol), then transferred to incubator, egg mortality recorded after 24hrs.

2- Effect on larval instars:

The same concentrations were used to test its activity on larvae. The larval development and mortality rate were tested by using 10 individuals of 1^{st} instar larvae approximately (24 hrs. old) in three replicates for each concentration. The larvae were transferred to containers supplied with larval diet (ruth culf & floured bread) treated with different concentrations of extracts. The dose was equal for all the treatments. They were kept in the rearing room at a temperature of 30 ± 2 ^{0}C and R.H. 65 ± 5 %. The resulting pupae were observed. The mortality rate was calculated.

3- Effect on pupae:

The development and mortality of pupae were tested by using 10 of newly formed pupae from the culture. The pupae were treated with the concentrations mentioned above (with three replicates for each concentration) by using hand spray. The control was treated with D. W. and solvent. The dose was equal for all the treatments and the control. The dead pupae and the pupal weight were calculated. Statistical analysis & correction of mortality was done using [13, 2].

Results and discussion:

The results show that the mortality percentage of eggs was increased with the increasing of concentration (Table 1). It was 83.3% at concentration of 10 mg/ml. followed by 44.4% and 31.1% at 7.5 and 5 mg/ ml respectively. The extract viscosity was formed layer on the external egg shell and preventing air exchange between embryo and environment. Significant differences were observed among the concentrations at ($P< 0.05$). [14] Reported that extracts may affect the embryo tissues and this lead to inability of hatching. [15] study reveals that the eggs mortality rate of *M. domestica* was increased from 17% at control treatment to 23.2 , 24.8 and 27.2% at concentration of 20 mg /ml of the alkaloid extracts of leaves , flowers and fruits of *Datura.innoxia* respectively. Present study results agree with those of [16] who mentioned that eggs mortality rates of *M. domestica* were 100% at the concentration of 10 mg/ml when treated with alkaloid and terpenoid extracts of *Euphorbia peplus*. [17] showed in her study that crude alkaloid extract of *E.heloiscopia* affect egg mortality rate of *M .domestica* significantly. The mortality rate was increased from 10.68% at control treatment to 81.74% at concentration of 20 mg/ml of the extract.

Also the results show that the crude alkaloids extracted from leaves of *A .gracilis* significantly affected the mortality of different larval instars of *M. domestica* , at concentrations of 5.0, 7.5, and 10 mg/ml, the highest mortality percentage of third larval instar was73.3% at 10 mg/ml concentration , while the lowest was 43.3% at concentration of 5 mg/ml of first instar larvae , the third instar larvae was the most sensitive instar than other larval instars. [18] found that the plant extracts appeared to have contact poison, stomach poison, respiratory poison and nervous poison properties. The alkaloid extract could cause larval mortality via growth inhibition, antifeedant, forming a complex with proteins and enzymes which cause toxicity and death [14]. In general, the mortality was increased significantly ($P < 0.05$) with increasing concentrations of the extracts. Significant differences were found between the concentrations. The alkaloid extract showed higher average mortality than other extracts, also the extract was more effective on first larval instar than third instars To the proximity of the process of dormancy. This is conflicted of [14] who found that the third larval instar was the most sensitive instar than other larval instars with variance of plants.

The pupal mortality was 30% at concentration of 10 mg/ml, table 1. The data showed inverse correlation between pupae weight and the extract concentrations. The death of pupae due to the treatment with different concentrations of plant extracts could be due to disorganization or interaction of these compounds with processes of hormonal balance. This interaction includes inhibition of hormonal release and delaying or prevention of insect's ecdysis, which delay or retard of their development processes and leads to morphological abnormalities or cause mortality [19,20]. In addition be possible because of low efficiency in the transformation of the stored food inside the body or due to the effects on the protein digestive enzyme or due to the interaction between the alkaloids and the proteins, or due to the effects of the extracts on the cells of the digestive tract responsible for absorption [21]. The cause in retardation or stopping process of the emergence may be attributed to continuous exposure to chemical compounds which lead to disorganization of ionic balance and change hydrogenion n umber values for digestive tract, or the chemical compounds were interacted with some physiological systems causing its inactivation . The pupal stage is considered to be a diapause stage before reaching the adult stage, thus insect parts and organs that form the different body parts were developed. Juvenile hormone is considered to be the key to metamorphosis regulation in insects. In the natural cases a decrease in levels of this hormone during last larval instar to the pupal stage and it is necessary for pupation process and adult emergence [22]. On the other hand, inability of the pupae to emerge from pupa case may be led to sclerosis of the ecdysis skin therefore, the pupae cannot get rid of it, or may be the alkaloids or terpenoids that found in the extract were affected the ecdysis hormone 20-hydroxyecdyson which is triterpenes [23]. [24] found that the aqoues extracts for many plants affect pupal weight. In the present study pupal weight was decrease to 1.5 mg when treated with concentration of 10 mg\ml of alkaloids while

it was 2.20 at control treatment , this agree with the study of [4] who found that the extracts of *Quercus brantti, Plantago laceolata, Eucalyptus camaldelulensis* and *Rumex dentatus* effect on weight of pupae of *M. domestica* in the presence of an inverse relation with concentrations. It was 1, .21, 1.75 and 2.8 respectively.

Table 1- The Effect of alkaloids extract of *Amaranthus gracilis* on mortality of different stages *M. domestica*

Concentrations Mg\ml	Egg mortality%	Larval mortality%			Pupal mortality%	Pupal weight Mg) (
0.0	7.7	1st	2nd	3rd	10	2.20
5	31.1	73.3	56.6	43.3	10	3.1
7.5	44.4	76.3	63.3	56.6	10	2.6
10.00	83.3	73.3	73.3	66.6	30	1.5
LSD	*4.82	Concentrations9.15* Stages 9.15*			Concentrations 3.37	Concentrations 0.63*

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