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Biological Effect of Crude Alkaloid Extract of Cordia Myxa L. Leaves on Some Stages of Musca Domestica (Diptera: Muscidae)

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Abstract--- Objective: *We found from present study effect of alkaloid extract of Cordia myxa on some stages of Musca domestica (third instar larvae and pupa with 24h.and 72h) the results showed that leaves affected performance in some stages of Musca domestica have increased distortions numbers for treatments(third instar larvae and pupa with 24h.and72h) compared to control treatment. Also results showed death of all naturally emergence insects for all treatment compared to control treatment.*

Methods: *The alkaloid extract tested against larvae and pupae of Musca domestica the efficacy test was achieved at different concentrations (2.5, 5, 7.5 and 10mg/ml)*

The Results: *results showed differences in treatments of third instar larvae and pupa with age 24h.and 72h. Also showed results increased numbers of distortions for larvae and pupa compared to control treatment.*

Conclusion: *The results described in this study the alkaloid extract showed distortions and differences in treatments (direct spray for larvae, spray pupa with age 24h.and72h.The best concentration in alkaloid extract was 10 mg/ml. The best treatments in alkaloid extract were the treatment of pupa with age 24h, in concentration 10mg/ml, Varied mortality rates of larvae and pupa to all treatment and to all concentrations.*

Keywords--- *Cordia Myxa, Musca Domestica, Crude Alkaloid.*

I. INTRODUCTION

The housefly, *Musca domestica* is one of the most common insects related with human settlements [1]. It is feed on breeds in decaying matter, human waste and food, is behold a mechanical vector for pathogens (bacteria, protozoa and viruses) to humans and livestock [2] and [3]. These vectors may also carry eggs from worm parasites [4] and [5]. The pathogens transferred by *M.domestica* may cause cholera, food poisoning, typhoid, diarrhea, anthrax and shigellosis, [6], [7] and [8]. Diverse studies Have so looked the probability of using plant extract in the control of eggs, larvae, pupae and adults of *M.domestica* [9] and [10]. Suggested use of *cordia myxa* to control on *M. domestica*, because for this plant medical importance due to contain its huge amounts of trace elements, within the installation of blood and enzymes body such as iron, zinc and copper. Also presence of flavonoids, styroids and alkaloids [11]. *C. myxa* plant is one of the species of genus Cordia, family Boraginaceae, comprise of trees and shrubs which are widely distributed in warmer regions [12].

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II. MATERIALS AND METHODS

Musca domestica rearing

Larvae and adult of *M. domestica* are tacked from Dr. Nawal S. mehdi /College of education for pure science/ Ibn Al-Haitham as the insect was identified in natural historic Museum and Research Center / Baghdad University, the insects were transferred to cages and kept in the rearing room in animal house / Biology department / College of science / Mustansiriyah University. With temperature of 30 ±2 c and 65 ±5 % relative humidity, and 12:12 dark : light. Adults of *M.domestica* were kept in rearing cage with dimensions of (30x30x30) cm. 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 a long sleeve of muslin cloth to allow cleaning and feeding process. Purification of strain for six generations to make sure there is no remaining effect of pesticide in environment and avoid presence natural distortions in the individuals. Plastic container were kept inside cages, these containers were contained rearing medium for eggs lying and another container (50 ml capacity)filled with water and covered by cotton reached to the bottom of it as a source of water. And cages are contained with plastic container (5 onz capacity contain plastic container, capacity (5 onz) with in ratio 1:1 milk : Suger to feed adults with take care of change it all three days. Grown larval in plastic container(20 onz capacity) contain modified media (fish diet) consist of crude protein (28.0%Min.), crude fat (4.0% Min.) and crude fiber (4.0% Max.) in addition to Amino acids, vitamins, minerals, where waiting 200 gm. of fish diet) with 10 gm. dry yeast and dissolved in 100 ml of distilled water [13] when larval reached the last instar larvae and pupated, the resulted pupal were collected and placed in rearing cages until the adults emergence and mating occurred after 48-72h.

Extraction with (ethyl alcohol 85%)

In order to extract alkaloids from the plant powder must be disposed from turbines located in which, therefore take the weight 100g of plant powder and add the amount of hexane to it to cover the powder in the volumetric flask 2 liter to leave the form for 72 hours with continued shaking between the period of the other filtered the form using the filter paper. After filtration taking the filtrate containing the turbines and leaving the lack of the need to him to take the remaining source of alkaloids and dry in the degree room temperature after the drought weight 100g of it and added to him ethal alcohol 85% and 400ml distilled water and left for a period 72 hours in the volumetric flask 2 liter with continued shaking between the period of the other and then filtered by using the filter paper NO.2 taking the filtrate and concentrates by using rotary evaporator with vacuum pump on 40-50 heat degree take concentrate it and put in the refrigerator until the use and this concentrate represent alkaloids. Repeat the process several times to reach the required quantity (Harborne,1973). Tacked 1 gm. Of dry matter of alkaloid extract and dissolved in 5ml of distilled water and complete volume to 100ml, become the concentration 1% and from it prepared another concentrations.

Impact of Alkaloid Extract to Cordia myxa Leaves in the Rates Mortality of Third Larvae Stage (treated with tow treatment)

Direct Spray Treatment

Tacked 10 larva / replicate within three replicates per concentration of concentrations previously prepared, treated larval with 2 ml of foregoing concentrations and spray larvae by sprinkler hand capacity of its 10 ml at a

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distance of 10 cm to ensure spray all the larvae and left to dry in the laboratory temperature and then transferred to plastic container with addition 10 gm. from earlier prepared media and covered with cloth to prevent larvae exit. While control treatment it has been larvae sprayed with 2 ml of distilled water,and examined samples to record the following:

Numbers distorted of larvae and pupa

Numbers dead of larvae and pupa

Numbers of natural emergence

Treatment of Media

Tacked 10 larvae/ replicate within three replicates from third larvae stage per concentration transferred larvae to pots plastic contain 10 gm. Of media earlier prepared to growth and breeding larvae after treated with 2 ml of concentrations previously mentioned and follow the same steep mentioned in previously paragraph

Impact Alkaloid Extract to Leaves in Ratios Pupa Mortality (age24 and 72 hours).

Direct Spray Treatment

Tacked 10 pupa / replicate within three replicates 24 h. and 10 pupa/ replicate within three replicates 72h. and sprayed with 2 ml per concentration of concentrations previously prepared by sprinkler hand is capacity 10 ml at a distance of 10 cm to ensure spray all the pupa and let dry in the laboratory temperature and then transferred to plastic pots and covered with cloth and stayed to dry in laboratory temperature to be transferred to petri dish to record following data :

Numbers mortality of pupa

Numbers distorted pupa

Numbers of natural emergence

III. STATISTICAL ANALYSIS

Differences between groups were calculated by one-way analysis of variance where appropriate using (Minitab VERSION 11) Values are expressed as mean ± SD. LSD (Least Significant Difference) a,b,c,d for rows,similar letters mean the absence of significant differences and different letters mean the presence of significant differences.A p value of less than 0.05 was considered statistically significant.

IV. RESULTS

Effect the alkaloid extract for Cordia myxa L. in third instar larvae of Musca domestica (direct spray)

Showed results of table (1) high rate destruction of third instar larvae in two concentrations 10,2.5mg/ml to 6.66% and control treatment where was 0%.Increased rate pupa destruction to 23.33% in 10 mg/ml concentration where differs from rates destruction pupa in 2.5,5 mg/ml where was 20% also no record rates destruction for pupa in control treatment. Total summation for distortions record variance between spray in concentrations 10, 7.5 mg/ml

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and other concentrations where summation of rates distortions was 16.66%,and 20% for concentrations 10, 7.5 mg/ml. Showed results in rates emergence of normal adults, highest ratio emergence was 66.66% for concentrations 2.5,5mg/ml which difference from normal emergence rates 53.33% in 10mg/ml compared to control treatment where the emergence rate was 100%. Naturally emergence insects cannot to complete life cycle where dead rate was 100% after 24h. From the emerging to all concentrations, all treatments with variance while control treatment dead rate was 0% after 24h. from emerging destruction rates which obtained in larvae was because the effect of alkaloid extract and reached to digestive system, and due to blocking it bio process led to presence distorted larval as in picture 1.

Picture 1: Dead larvae due to treatment direct spray of larvae with alkaloid extract (10X).

Table 1: Effect alkaloid extract for Cordia myxa L. with(2.5,5,7.5,10) mg/ml concentrations in third instar larvae of Musca domestica

Conc. (mg/ml)	Mortality rate of larvae (%)	Mortality rate of pupa (%)	The distortions %		Rate of natural emergence	Rate mortality naturally emergence adult(24 hr.)
			Distorted pupa	Distorted insect		
10	6.66	23.33	3.33	13.33	53.33	100
7.5	3.33	16.66	10.00	10.00	60.00	100
5	3.33	20.00	10.00	0.00	66.66	100
2.5	6.66	20.00	6.66	0.00	66.66	100
Control	0.00	0.00	0.00	0.00	100	0.00

Effect the alkaloid extract for Cordia myxa L. in media prepared to third instar larvae of Musca domestica

Results of table (2) showed that the highest destruction rate of third instar larvae was 36.66 in 2.5 mg/ml concentration and lowest destruction rate was 10.00% in 10 mg/ml concentration compared to control treatment where was 3.33%.When following rates of pupa destruction in table (2) developed from third instar larvae feeding on media treated with different concentrations for alkaloid extract,It is noticed that record 33.33 for concentration 2.5 mg/ml then 20.00% for two concentrations 10,7.5mg/ml which variance about rate pupa dead in control

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treatment where was 0.00%.The results in table (2) gave indicators to occurrence distorted insects increased by an increase of the concentration where reached 10% in 10 mg/ml concentrations and decreased to 0.00% in 2.5mg/ml concentration, and noticed from table (2) that distortions summation (distorted pupa+ distorted insects) was high in 5mg/ml concentration which variance from 2.5 mg/ml concentration and control treatment. Showed results of percentage rates variance in natural emerging rates for insects to concentrations 10,7.5,5 mg/ml where ranging between (46.66-53.33)% which different from rates of 2.5mg/ml concentration where was 30.00% comparison to control treatment where was 96.67%.Media treatment influenced on the insects death which naturally emerging where reached 100% in all concentrations which different about control treatment where was 0.00%.

Table 2: Effect alkaloid extract for *Cordia myxa* L. with (2.5,5,7.5,10) mg/ml concentrations in media prepared to third instar larvae.

Conc. (mg/ml)	**Mortality rate of larvae (%)**	**Mortality rate of pupa(%)**	**The distortions %**		**Rate of natural emergence**	**Rate mortality naturally emergence adult (24 hr.)**
			Distorted pupa	**Distorted insect**		
10	10.00	20.00	6.66	10.00	53.33	100
7.5	23.33	20.00	0.00	6.66	50.00	100
5	33.33	0.00	13.33	6.66	46.66	100
2.5	36.66	33.33	0.00	0.00	30.00	100
Control	3.33	0.00	0.00	0.00	96.67	0.00

Also results of table (2) showed treatment media of the third instar larvae with alkaloid extract caused the larvae dead or stay larvae for long duration in same stage without growth or development, also produce distorted adults with small size and without wings as in picture (2-A), partial emergence of adult as in picture (2-B) and adult with twisted wings as in picture (2-C). Where the feeding factor is a direct factor in induce the insect on growth and increase body size and that increase of cuticle area resulting from food absorption through larvae stage, is another factor stimulate on increase size and continue of growth through life cycle from egg to adults in normal state, the larvae swallow large amounts of food to storage large part of it in tissues to used it in pupa and adult stages to build body wall and complete systems growth and in reproduction, before stop feeding to transform later to pupa stage grow up its thin structure due to process of nutrition where grow up size and stop feeding. And because the plant extracts using in the study working in ingestion this mean it with stomaching impact more than working in contact, where the method depended on mixing of extracts with food,this led to failure larval feeding and stopping it or influenced on the mouth parts then caused distortions and prevent it feeding. Pointed [14] for role of some plant compounds in dead the cells lining mid gut for the insect feeding on plant containing this compounds, where this cells responsible for secretion the digestive enzymes and this led to dead of larvae.Also mention [15] that Lignan beta-peltatin-A methyl ether(II) compound extracted from *Libocedrus bidwillii* plant led to mortality 98% of *Musca domestica* larvae when treatment media of larvae with the concentration 100ppm of extract. Showed study of [16] that averages destruction for third instar larvae of *Musca domestica* increased from 17% in comparison treatments to 31.2,36.3,42.6% on respectively in alcohol extract for Leaves and fruits of *Datura innoxia* Mill plant [17] proved that treatment of the second instar larvae of *Musca domestica* with crude extract of *Brassica compestries* leads to prolong duration of larvae stage as well as impact it on pupation ratio and adults emergence. The plant extracts effected directly in growth and development duration of larval and pupa through its influences in the hormonal

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balance for insects as it led to imbalance between stimulating hormones growth and inhibiting hormones growth, may be found that the increase duration of larvae and pupa for insects due to influence of plant extracts in alienation process inhibition through increase juvenile hormone level in the insects body who works on alienation inhibition, where found in normal states reduction level this hormone in alienations times of last stage of larvae to pupa as well as was important to pupation also in alienation of pupa to adult [18] [19] Also pointed [20] when treatment third instar larvae of mosquito *Culex pipienus* molestus with Asetonic extract of *Melia azedarach* fruit led to dead of larvae and distortion its [21] mention that two types from physiological effects which her causes the toxic plant compounds for the tissue insect are, the not direct toxic effect who causes defect in the nervous system of insect, and the direct effect, where the toxic compounds works on the target tissues, or because found toxic compounds works on inhibition activity of digestion enzymes for protein in insect or because correlation this compounds with proteins and difficult to digested and led to dead the insect [22].

A B

C

Picture 2: A- Distorted adults her small size and without wings (10X)

B- Partial emergence of adult (10X)

C- Adult with twisted wings (10X)

(due to treatment media larvae with alkaloid extract)

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Effect of the alkaloid extract for Cordia myxa L. in pupa of Musca domestica with age 24h

Results of table (3) showed there is highest destruction rate for pupa 20.00% when sprayed with two concentrations 7.5,10 mg/ml and lowest destruction rate was 13.33% in concentration 5mg/ml, which different about control treatment where was 0.00%.Also not found variance in percentage rates of distortions (pupa distortions) where no record any rate distortions to all concentrations. Either in partial emergence highest distortion rate 26.66% in 10mg/ml concentration and lowest rate 3.33% in 2.5mg/ml concentration with differences between them compared to control treatment where distortions rate was 0.00%.Recorded results of table (3) that rates the natural emergence of insect treated with alkaloid extract for the different concentrations inversely proportional with concentrations, where showed highest natural emergence rate80.00% in 2.5mg/ml concentration and lowest natural emergence rate 53.33%in 10mg/ml concentration and which different from the control treatment the rate was100%..

Table 3: Effect of alkaloid extract for *Cordia myxa* L. with (10, 7.5, 5, 2.5 mg/ml) concentration in pupa stage with age 24 hr. of *Musca domestica*

Conc. (mg/ml)	**Rate of pupa dead (%)**	**The distortions %**		**Rates of natural emergence (%)**	**Rate mortality naturally emergence adult (24 hr.)**
		Partial emergence	**Distorted pupa**		
10	20.00	26.66	0.00	53.33	100
7.5	20.00	13.33	0.00	66.66	100
5	13.33	13.33	0.00	73.33	100
2.5	16.66	3.33	0.00	80.00	100
Control	0.00	0.00	0.00	100	0.00

Effect of the alkaloid extract for Cordia myxa L. in pupa stage of Musca domestica with age 72h

Results of table (4) showed no difference in rates of pupa destruction for two concentrations 10,7.5 mg/ml that reached 23.33, 20.00 on respectively, also not variance in rates of pupa destruction for two concentrations 5,2.5 mg/ml where was 13.33%. It is also noticed that increased summation of distortion pupa rates in table (4) and partial emergence increased to 13.33 in concentration 10mg/ml. and decreased to 0.00% in 2.5 mg/ml concentration. Influenced different concentrations for alkaloid extract in rates natural emergence of adults for decreased to 63.33% in 10mg/ml concentration compared with control treatment where was 96.67% which different about rates of natural emerging to different concentrations, and adults naturally emerging not can complete the natural work and rapid it death after emerging directly.

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Table 4: Effect of the alkaloid extract for *Cordia myxa* L. with (2.5,5,7.5,10) mg/ml concentrations in pupa stage with age 72 hr. of *Musca domestica*.

Conc. (mg/ml)	Rate of pupa dead (%)	The distortions %		Rates of natural emergence (%)	Rate mortality naturally emergence adult (72 hr.)
		Partial emergence	Distorted pupa		
10	23.33	3.33	10.00	63.33	100
7.5	20.00	0.00	10.00	70.00	100
5	13.33	0.00	6.66	80.00	100
2.5	13.33	0.00	0.00	86.66	100
Control	3.33	0.00	0.00	96.67	0.00

The present study results showed that pupa with age 24h. was more sensitive than pupa with age 72h. for different concentrations for alkaloid extract because of non-hardness of puprium in pupa with age 24h. As it be thinner than in pupa with age 72h. where led use the different concentrations for alkaloid extract to appearance distortions in treatment pupa with two ages 24 and 72h. as in picture (3) The results agreed with [23] that extract of organic solvents of *Callistemon citrinus* led to increase average rate the cumulative destruction and growth duration of non- adult stages as well as reduction weights pupa of *Musca domestica*. All of [24] pointed that the reduction weights of pupa of *Musca domestica* back to low efficiency of metabolism due to correlation of chemical compounds such as tannins and alkaloids with protein materials or effect in digestive protein enzymes or poisoning cells of gastrointestinal tract responsible for absorption.All of [25] also pointed that mortality rate pupa of mosquito *Culex pipienus* was 7.5, 10.3, 59.4% in extracts of hexan,athyl acetate and ethyl alcohol on respectively for *Callistemon citrinus* plant and showed most treated pupa was black color. As mention [26] not found significant effect is found for hot and cold extract,as well as organic solvents extract for leaves and fruits of *Datura innoxia* in destruction rate pupa of *Musca domestica*. Pointed [27] when treated pupa mosquito of *A. pulcherrimus* with different concentrations of alcohol extracts for *Melia azadarach* fruit raining between 200-700 ppm and led to destruction of 75.56% in the highest concentration, and this ratio may increase or decrease according to concentration. Also mention [28] that when treated pupa mosquito of *Culex pipienus* with oil extract of *T. foenum-graecum* and *Eruca sativa* plant led to destruction it with 50,55.5% on respectively in 62.5 ppm concentration.

Picture 3: Different distortions for pupa due to treatment of alkaloid extract(10X)

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V. CONCLUSION

Varied mortality rates of larvae and pupa to all treatment and to all concentrations.The alkaloid extract presence distortions for two treatments (direct spray larvae and spray pupa with age 24h.) and the best concentration was 10mg/ml.

The death of all insect which are naturally emerging for all treatment and for all using concentrations and for two extracts (hot aqueous and alkaloid) after 24h. From emerging.

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