



# BIOLOGICAL EFFECT OF CRUDE HOT AQUEOUS EXTRACT OF *CORDIA MYXA* L. LEAVES ON SOME STAGES OF *MUSCA DOMESTICA* (DIPTERA : MUSCIDAE)

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## Abstract

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

The hot aqueous 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)

Results showed significant differences in treatments of third instar larvae and pupa with two ages 24h. and 72h. Also showed results increased numbers of distortions for larvae and pupa compared to control treatment.

The results described in this study hot aqueous extract showed distortions and significant differences in treatments (direct spray for larvae, spray pupa with two ages 24h and 72h. The best concentration in hot aqueous extract was 10 mg/ml. The best treatments in hot aqueous 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.

**Key words :** *Cordia myxa*, flavonoids, *Musca domestica*, saponins and tanins.

## Introduction

The housefly, *Musca domestica* is one of the most common insects related with human settlements (Kumar *et al.*, 2011). 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 (Olsen *et al.*, 2001; Sangmaneedet *et al.*, 2005). These vectors may also carry eggs from worm parasites (Wattanachai *et al.*, 1996; Ugbugu *et al.*, 1996). The pathogens transferred by *M. domestica* may cause cholera, food poisoning, typhoid, diarrhea, anthrax and shigellosis (Banjo *et al.*, 2005; Fasanella *et al.*, 2005; Yap *et al.*, 2008). Diverse studies Have so looked the probability of using plant extract in the control of eggs, larvae, pupae and adults of *M. domestica* (Issakul *et al.*, 2004; Malik *et al.*, 2007). 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, steroids and alkaloids (I-Awadi *et al.*, 2001). *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 (Thirupathi *et al.*, 2008).

## 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  $30 \pm 2^\circ\text{C}$  and  $65 \pm 5\%$  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 (Cetin *et al.*, 2006) 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 hot aqueous**

Take 100g amount of plant powder and is added in glass flask liter capacity and add 400ml distilled water .put the beaker in the water bath on the degree of boiling for about 10 minutes and put in the electric rocking for about 24 hours and then filtered by using filter paper with the use of vacuum then concentrates the form using rotary evaporator with vacuum pump in 40-50 temperature. Repeated the process several times for the required quantity (Harborne, 1973). Where dried 50ml of filter leave in room temperature to reach 10 gm. of dry matter from which prepared the remaining concentrations.

#### **Effect the crude aqueous extract for *Cordia myxa* leaves in some stages of *Musca domestica***

Effect the crude aqueous extract in third instar larvae (direct spray) tacked 10 larva/replicate within three replicates per concentration of concentrations previously prepared, sprayed larval with 2 ml of foregoing concentrations and spray larvae by sprinkler hand capacity of its 10 ml at a 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

#### **Effect the crude aqueous extract in media prepared to third instar larvae (media treatment)**

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

#### **Effect the crude aqueous extract for *Cordia myxa* leaves in pupa of *Musca domestica* with two ages (24 h and 72 h)**

Can distinguish between pupa ages during the colors and size of it, where the pupa with age 24 h. Tend to wax white color and small size .while the pupa with age 72 h. ten to brown color and big size in the late stages of pupation as in picture 1. Pupa with tow ages treated by direct spray treatment. Tacked 10 pupa / replicate within three replicates with age 24 h. and 10 pupa / replicate within three replicates with age 72h. and sprayed with 2 ml per concentration of concentrations previously prepared and stayed to dry in laparotomy temperature to be transferred to petri dish to record following data :

Numbers mortality of pupa

Numbers distorted pupa

Numbers of natural emergence

#### **Statistical analysis**

Differences between groups were calculated by one-way analysis of variance where appropriate using (Minitab VERSION 11) Values are expressed as mean  $\pm$  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.

## **Results**

#### **Effect of hot aqueous extract for *Cordia myxa* L. with (2.5,5,7.5,10) mg/ml Concentrations on the third instar larvae (direct spray) of *Musca domestica* and it development**

Results of table 1 showed effect of different concentrations from extract in third instar larvae (direct

spray treatment), where recorded a concentration 7.5mg/ml highest average for larvae destruction ( $2.66 \pm 0.5$ ), then a concentration 5 mg/ml with average ( $2 \pm 1$ ) which significantly differed from control treatment. Results of table (1) also confirmed that crude extract of *C. myxa* leaves significantly effect in produce different distortions

for larvae as in picture (2), pupa and adults which developmental from direct spray treatment for larval in both concentrations (7.5, 10)mg/ml, while non- record any effect when treatment in a concentration 2.5mg/ml, and this refers to increase averages numbers of distortions by increase a concentration of leaves extract. Fig. 1

**Table 1 :** Effect of hot aqueous extract for *Cordia myxa* L. with (2.5,5,7.5,10) mg/ml concentrations on the third instar larvae (direct spray) of *Musca domestica* and it development.

Development stage	Control	Concentrations (mg/ml) Mean±SD					
		2.5mg/ml	5mg/ml	7.5mg/ml	10mg/ml	P value	LSD
N.Dead larvae	0.33±0.5D	1±1C	2±1B	2.66±0.5A	0.66±0.5D	0.02	0.1
N.Dead pupae	0±0C	1±1.7B	0±0C	0±0C	2.33±1.5A	0.07	0.9
The distortions	0±0D	0±0D	0.66±0.5C	2.33±2.1B	5±2.6A	0.01	0.7
Rates of natural emergence	9.6±0.5	8±1	7.3±1.5	5±2.6	2±3.5	0.009	—

**Table 2 :** Effect hot aqueous extract of *Cordia myxa* L. with (10,7.5,5,2.5)mg/ml concentrations in media to feed the third instar larvae of *Musca domestica*.

Development stage	Control	Concentrations (mg/ml) Mean±SD					
		2.5 mg/ml	5mg/ml	7.5 mg/ml	10 mg/ml	P value	LSD
N.Dead larvae	0±0	0±0	0±0	0.66±0.5	0.33±0.5	0.17	NS
N.Dead pupae	0±0	0.33±0.5	1±0	0.33±0.5	1±1	0.2	NS
The distortions	0±0	0.33±0.5	1±0	1±1.7	1.3±1.15	0.46	NS
Rates of natural emergence	10±0	9.3±1.5	8±0	8±1.7	7.3±2.3	0.19	NS

**Table 3 :** shows the active compounds for extract of *C. myxa* leaves, reagents and result.

Compound type	Reagent name	Result
Alkaloids for Alkaloid and aqueous extract	Dragendroff	+Orange brown
Flavonoids for aqueous extract	Alkaline reagent test byA-KOHB-NOH	+A-BrownB-Yellow
Glycosides for aqueous extract	Benedicts test	+Reddish brown
Polyphenols for aqueous extract	Benedicts test By ferric chloride 3%	+Brown precipitate
Sponins for aqueous extract	Saponins detection (formation foam)	+(formation foam)
Tanins for aqueous extract	Ferric chloride test	+Black color

**Table 4 :** Effect hot aqueous extract of *Cordia myxa* L with(2.5,5,7.5,10) mg/ml concentrations .on pupa with age 24h.

Development stage	Control	Concentrations (mg/ml) Mean±SD					
		2.5 mg/ml	5mg/ml	7.5 mg/ml	10 mg/ml	P value	LSD
N.Dead pupae	0.3±0.5	0.3±0.5	1.33±1.5	2±1.7	0.33±0.5	0.3	NS
The distortions	0.33±0.5C	1.6±2.08B	0±0C	0.33±0.5C	6.6±1.5A	0.001	1.2
Rates of natural emergence	9.66±0.5	4.66±4.1	8.66±1.5	7.66±2.08	3±1	0.2	NS

**Table 5 :** Effect hot aqueous extract of *Cordia myxa* L. with (2.5,5,7.5,10) mg/ml concentrations on pupa with age 72h.

Development stage	Control	Concentrations (mg/ml) Mean±SD					
		2.5 mg/ml	5mg/ml	7.5 mg/ml	10 mg/ml	P value	LSD
N.Dead pupae	0±0C	0.33±0.5C	2.33±1.15B	1.66±0.5B	3.66±1A	0.001	0.98
The distortions	0±0D	2±1.7C	3.66±2.08B	4.33±1.5A	4.33±2.5A	0.05	0.53
Rates of natural emergence	10±0A	7.66±2.08B	4±3C	4±2C	0.66±1.15D	0.001	1.8



Picture 1 : Pupa with ages 24h and 72h (10x).

*Trigonella foenum-graecum* show 100% fatal effect to third instar larvae of *Musca domestica* at 25-100% concentrations, while led to destruction 22.2 , 33.3, 44.4% from it at 1%, 2%, 5%, respectively. Ramos *et al.* (2006) also find treatment the third instar larvae of mosquito *Aedes aegypti* by crud extract of *Calotropis procera* led to 100% larvae destruction at 100 mg/ml concentration. (Shalan *et al.* (2005) also said that Lambdacy halothrin pesticide lead to destruction 69% of mosquito larvae *Aedes aegypti* at 8% of pesticide as its lead to increase destruction ratio of results pupa and low adult emergence ratio. Su and Mulla (1998) as well as proved the Azadirachtin compound, which extract from *Azadirachta indica* plant who working ant feeding material in insects



A-



B-

Picture 2 : A- normal larvae, B-dead larval (10X).

showed increase averages numbers of insects naturally emergency when a concentration decrease, but destruction all insects after 24h. from emergence.

**Effect hot aqueous extract of *Cordia myxa* L. with (10,7.5,5,2.5)mg/ml concentrations in media to feed the third instar larvae of *Muscsa domestica***

Results of statistical analysis in table 2 and fig. 2 non-found any significant differences in averages destruction for larval and pupa which development from larval feeding on treated media with a concentrations of crude aqueous of *C. myxa*, where observed on treated media with different concentrations may be due effect one effective compounds (flavonoids, saponins and tanins) which chemically diagnosed as in table 3.

From results noticed that treatment media, which prepared to feeding larvae with different concentrations led to larvae destruction because loss of neutrino. Abdel-Halim and Morsy (2006) find the crude extract of

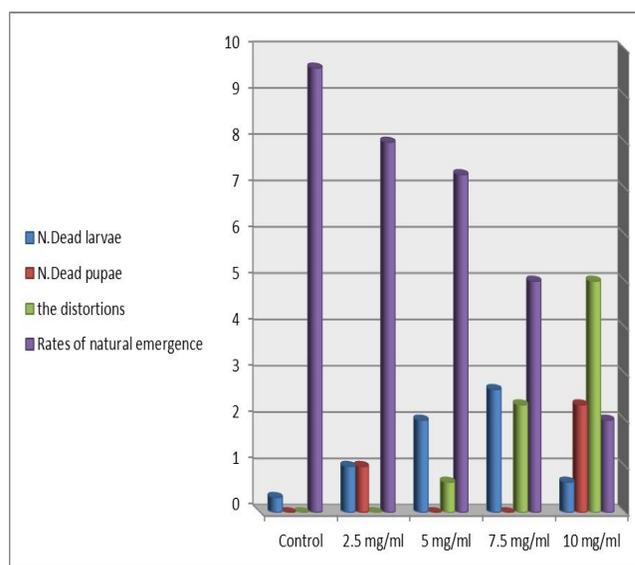


Fig. 1 : showed effect hot aqueous extract/third instar larvae (direct spray treatment).



**Picture 3 :** A- pupa a small size, B- elongated pupa, C-curved pupa and D- pupa as it empty (10x).



**Picture 4 :** Distorted adult without legs (10X).



**Picture 5 :** Adhesion of puparium in legs (10 X).



**Picture 6 :** Partial emergence of head and thorax (10X).

because effect it in chemoreceptors the found in insect mouth parts or its influenced in ingestion food process, through inhibition of gastrointestinal tract movement which send the food to inside midgut. As showed tissues studies of insects swelling consisting muscle of gastrointestinal tract and decomposition of epithelial cells lining of midgut lading to prevent insects feeding. Pointed (Naqvi *et al.*, 2005) when mixing media prepared to the second instar larvae with Neem extract lead to dead 62% of larval after 24h. of treatment at 12.5 mg/ml. Also

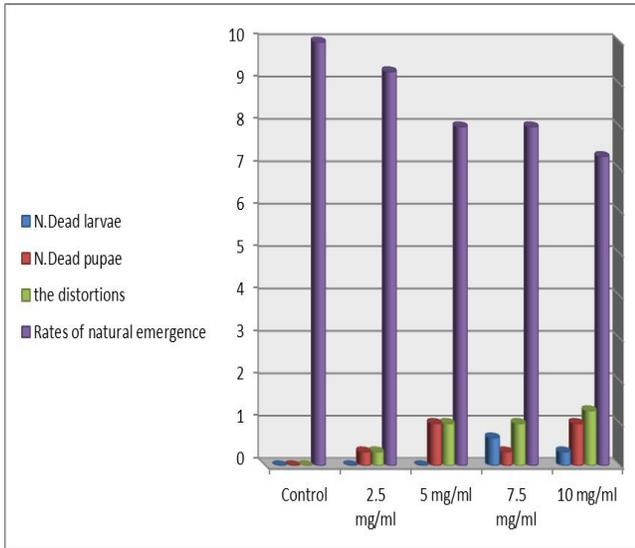


Fig. 2 : Effect hot aqueous extract / media treatment.

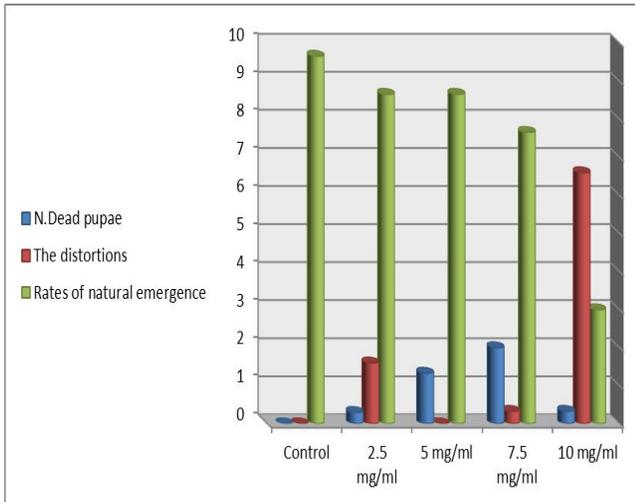


Fig. 3 : showed effect the hot aqueous extract of *C. myxa* on pupa of *Musca domestica* with age 24 h.

pointed by Talaat and Mulla (1984) when treatment the media third instar larvae of *Musca domestica* with growth regulator Cyromazine led to existing morphological distortions for larvae and pupa and adults not able to give up from puparium.

**Effect hot aqueous extract of *Cordia myxa* L with(2.5,5,7.5,10) mg/ml concentrations .on pupa with age 24 h and pupa with age 72 h**

Results of statistical analysis in table 4 and fig. 3 highest average of distortions numbers ( $6.6 \pm 1.5$ ) in a concentration 10 mg/ml and least averages for distortions in both concentrations (5 , 7.5) mg/ml as in pictures (3,4,5 and 6). As results showed in table 5 and fig. 4 significant differences destruction averages for pupa, where highest destruction average in 10 mg/ml concentration ( $3.66 \pm 1$ )

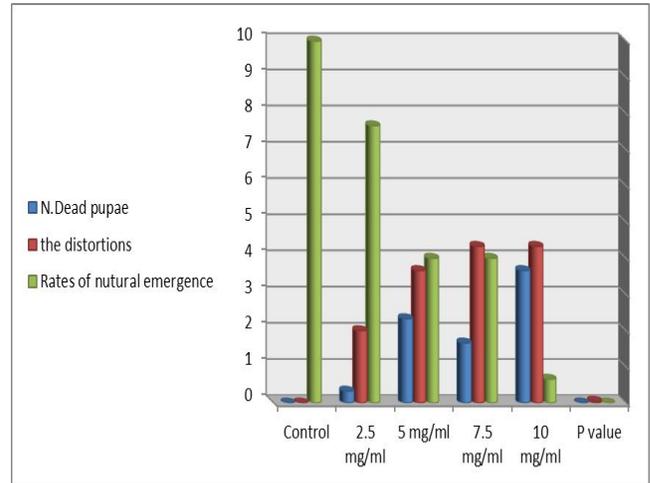


Fig. 4 : showed effect hot aqueous extract on pupa with age 72h.

and least average in a concentration (2.5) mg/ml, which significantly differ about control treatment.

The results also showed that pupa with age 24h. was more been affected with toxic compound which found in hot aqueous extract than pupa with age 72h. due to long duration exposure of pupa 24h. from pupa 72h. and this led to entry toxic compound which in aqueous extract to inside pupa body and according to this rate distortions in treatment pupa 24h. and this results similar to Alaa (2017). When used growth regulators Trigard and Match on *Musca domestica* and led to presence distortions in pupa. The cause in rates distortions which record in pupa with two ages 24 and 72h. to effective properties for chemical compounds found in aqueous extract of *Cordia myxa* L. including turbines where the extract is a mixture for different species from chemical compounds that within the group of turbines and the most important are (Saponin and 1,8-Cineol compound), where that 1,8-Cineol compound interferes with metamorphosis process for pupa, which led to the presence distortion states Rajendran and Sriranjini (2008). Rembold (1984) mentioned that destruction pupa rates in different concentrations due to interferes, which between chemical compounds and alienation hormone in pupa body.

**Conclusion**

The current study shows that hot aqueous extract of *C. myxa* caused distortions and significant differences in treatments (direct spray for larvae, spray pupa with two ages 24h and 72h.), also recorded the best concentration in hot aqueous extract was 10 mg/ml and the best treatments in hot aqueous extract were treatment of pupa with age 24h, in concentration 10mg/ml. Varied mortality rates of larvae and pupa to all treatment and to all concentrations.

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