

Some of the biological effects of ethanol extract of the green algae *Cladophora crespata* in the blow fly *Chrysomya megacephala* Fabricius, 1794 (Diptera:Calliphoridae)

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Abstract

This study was conducted to evaluate the effect of the ethanol extracts of green algae *Cladophora crespata* in some biological aspects of the blow fly *Chrysomya megacephala*. The results showed that the biological effects of ethanol extract of was high in the larval, pupal and adult stages. The 1st larval instar was most affected in the mortality after the treatment with ethanol extracts of two algae compared to 2nd and 3rd instars with many significant differences. The rates of mortality increases with the rise of extract concentrations; the most larval mortality of all experiments was in the concentration 2%, compared to 0.5% and 1%. The prolongation in the larval duration of the treated larvae was observed after the treatment with ethanol extract compared to control. A reduction of pupation percent was induced, a highly decrease in the pupation percent was observed in the treatments.

Larvae raised on tested extract diets recorded a highly lower pupal average weight, a highly prolongation in the pupal duration was observed in all tested concentration of ethanol extracts. Results revealed a reduction in the percent of total pupae developed to adults. The application of ethanol extracts induced different morphological abnormalities, including larval-pupal intermediates, compressed pupae, darkened pupae, spherical shaped pupa, and small sized pupae. Many adults could not emerge completely and remained concealed in the puparia. The adults that resulted from some abnormal pupa were with defective wings, and deformed head or abdomen. The algal ethanol extracts caused high mortality of adults and longevity or duration.

Key words: blow flies, *Chrysomya megacephala*, green algae, *Cladophora crespata*.

1. Introduction

Blow flies belonging to the family Calliphoridae are of considerable medical and veterinary insects, this family has non-metallic and metallic flies (Wall & Shearer 2001). Worldwide the blowfly family includes over 1000 species and 150 genera (Rognes 1991). Most species of blowflies have wide geographical distributions and they are habitually linked to the human activities. Some species are typically urban and others have medical and veterinary importance, cause medical problems and losses to the animal industry (Zumpt 1965). The blow flies are among the most widely recognized as being among the first wave of faunal succession to arrive on human cadavers (Smith 1986). Therefore, their life cycles, developmental rate and succession are utilized for estimating PMI, time between death to discovery of corpse and possible corpse movement (Sukontason 2000). The blow flies also cause a huge economic problem in the world; in the areas sun-drying is the major method of preserving fish, as ice is typically unaffordable (Wall & Shearer 2001). However, blowfly larvae tend to infect these sun-dried fish when the weather is warm and humid. The blow flies are among the most widely recognized as being among the first wave of faunal succession to arrive on human cadavers (Smith 1986). Therefore, their life cycles, developmental rate and succession are utilized for estimating PMI, time between death to discovery of corpse and possible corpse movement (Carvalho *et al.* 2000).

Among blowflies, the oriental latrine fly *Chrysomya megacephala* is one of the most common blowflies in many areas of the world, it is a warm-weather fly with a greenish-blue metallic box-like body (Sukontason 2000). The fly infests corpses soon after death, making it important to forensic science. This fly is implicated in some public health issues; it can cause accidental myiasis, and also infects fish and livestock. Greenberg (1973) reported that this species is among the most dangerous dipteran vectors of enteric pathogens. *C. megacephala* is the dominant vector of helminth parasite eggs (Carvalho *et al.* 2000). *C. megacephala* is attracted by carcasses, of mammals and birds, and human feces for oviposition (Soulsby 1982), and have also been reported as mechanical vector of several viruses, bacteria, protozoan cysts and other enteric pathogens (Greenberg 1973), occasionally causing myiasis in traumatic lesions of animals, including humans (Zumpt 1965). Under laboratory conditions, *C. megacephala* was able to develop on a variety of living animals including catfish, toads, frogs, lizards, and pigeons (Wall & Shearer 2001). Both sexes may cause economical loss or be vectors of disease. Studies are being done on *C. megacephala* to determine its role as a vector for diarrhea-causing bacteria such as *Escherichia coli* (Soulsby 2006).

C. megacephala are known to be the source of accidental (secondary) myiasis in humans, where the flies do not pierce the skin but invade an open wound (Hall 2001). This causes losses in cattle and fish industries all over the world. The first record of human miasis caused by *C. megacephala* and *C. rufifacies* was in Thailand, a man had a tumor lesion where the larvae accumulated (Greenberg 1973). Most recorded myiasis cases: cutaneous, intestinal, urethral and auricular, however, do not involve the fly (Soulsby 2006). *C. megacephala* is a carrier of pathogens, such as bacteria, protozoan and helminth eggs, to human food, because it lays its eggs on animal and human feces, and will land on human food soon after (Wall & Shearer 2001).

Insecticides are also used to control the flies, although these results in the development of resistance. Today, the environmental safety of an insecticide is considered to be of paramount importance. An insecticide does not have to cause high mortality on target organisms in order to be acceptable. But it should prevent the breeding. Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive, and are readily available throughout of the world (Schoonhoven 1982). The screening of some plants for pests control would generate good employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health (Singh & Saratchandra 2005). The biological activity of algae and plant extracts might be due to the various compounds, including phenolics, terpenoids, and alkaloids, existing in plants (Kracmar *et al.* 2000).

2. Materials and Methods

2-1. Algae collection and extraction

Samples of the algae *Cladophora crispata* were collected as biomass from many areas from Basrah city, South of Iraq. It's washed with a much amount of distilled water to remove all extraneous matters, and left to dry room, Preparation of the extracts was done according to Ladd *et al.* (1987) and Al-Mansour (1995).

2-2. Rearing technique:

The adult blow flies *Chrysomya megacephala* were collected from fields, markets and slaughterhouses in many areas of Basrah city. The flies were kept in glass cages measuring (60*40*40) cm in size. The flies were maintained in the laboratory at 27±2 °C temperature. Rearing technique was done according to Abdul-Fattah (1989). They were fed on a mixture of chopped meat and sugar solution, and provided in petridish as larviposition medium and was replaced daily to maintain hygiene and to avoid contamination. A layer of cotton was added in the cage as a place for oviposition and pupation; the eggs were moved to Petri dishes containing filter paper moisten by water. Control and treated eggs were incubated under the same laboratory conditions. The pupae when formed were removed from cotton and kept in meshed cages for adult emergence.

2-3. Biological studies

The first experiments were carried out on the larval instars of *Chrysomya megacephala*. The food media were treated with the concentrations of ethanol extract of *Cladophora crespata*. The study was done according to Al-Ezzi (1999) and Hatem (2011). The treated media was divided in 100 ml beakers each received 20g of media. Normal larvae were transferred from rearing media to each beaker (10 larvae). Control experiments were done as above but without any treatment. This procedure was repeated 3 times. All tests were carried out at laboratory conditions mentioned above. Larvae were examined daily to estimate the Mortality which was recorded after 48 hours, and calculated according to Abbot formula (1925). Larval duration was calculated as the intervals between the commencement of 1st instar larvae and that of pupation. It was calculated for each larva and then the mean value was taken. The resultant pupae were counted and weighed to determine the percent of pupation and the mean pupal weight.

Observations were carried out daily to record pupal duration. The reduction in pupal weight and adult emergence was calculated according to Khazanie (1979). Percentage of total pupae developed to adults was estimated according to Sripongpun (2008), and the emergence of successfully metamorphosed adults was estimated in percentage according to El-Kattan *et al.* (2011). The experiment of the ethanol extract against adult flies was done in same way and conditions; mortality was recorded after 48 hours. Any morphogenetic abnormalities that might occur in all developmental stages of *C. megacephala* were recorded and photographed.

2.3 Statically analysis

The results were analyzed with t test and chi-square and R.L.S.D. by using computerized SPSS program (Statistical Program for Social Sciences). P<0.05 was considered to be lest limit of significance (Al-Rawi 1984).

3. Results

In present study, the biological effects of ethanol extract of *Cladophora crespata* was high on *Chrysomya megacephala* in the larval, pupal and adult stages, while the *Cladophora crespata* were in the pupal stage.

3.1 Biological effects of the larval stage:

Data in Table 1 presented that treatment with ethanol extract of *C. crespata* produced: the 1st larval instar of *C. megacephala* was recorded highest mortality rate with 43.3, while the 2nd and 3rd instars with 34.4 and 28.8 respectively with significant differences. Also, the rates of mortality rose up with increasing the extract concentrations; the larval mortality rate was the highest in the concentration 2% with 63.3 compared to 0.5% and 1%, which recorded 13.3% and 30% respectively with significant differences.

Results in Table 2 revealed that the ethanol extract of *C. crespata* caused a clear prolongation in the duration of the *C. megacephala* larvae after the treatment, compared to control. There were clear effects on the larval period after treatment with tested algal extracts. The duration of larvae of *C. megacephala* may be up to 4-9 days of the 1st instar, 7-13 days of the 2nd instar, and 7-14 days of the 3rd instar. Also, the rates of prolongation rose up with increasing the extract concentrations; the highest was observed in the concentration 2% compared to 0.5% and 1%. Number of larvae showed obvious malformations after the treatment with ethanol extract, including darkened larvae, curved larvae, irregular shaped larvae and swelling larvae.

3.2 Biological effects of the pupal stage:

Results shown in Table 3 showed the ethanol extract of *C. crespata* was caused a high effect of the biological aspects of *C. megacephala* pupa, compared to control. A number of pupal mortality as treated larvae was observed, the mortality was recorded 26.5% in the 2% concentration. Also, a reduction in the percent of total pupae of developed to adults was noticed, as a result to the treatment of ethanol extract compared to the control. The percent pupation was decreased as the concentration of the extract increased with significant differences, the pupation percent 60.2% and was recorded in the 2% concentration. The duration of pupa of may be up to 5-10 days as result of the treatment with the three concentrations. Larvae raised on tested extract diets recorded a highly significant of lower pupal average weight, compared to control. The average pupal weights dropped with increased concentration. The larvae that treated with 2% concentration induced average pupal weight may be up to 0.129g/10 pupa with significant differences from 0.5 and 1%.

The application of ethanol extract of *C. crespata* against *C. megacephala* induced different morphological abnormalities. A number of pupae and adults showed obvious malformations after the treatment of larvae with the algal extracts, include larval-pupal intermediates (figure2), abnormal darkened pupae (figure3), spherical shaped pupa (figure4), and small pupae. Many abnormal adults could not emerge completely and remained concealed in the puparia. The adults that resulted from some abnormal pupa were with defective wings, and deformed head or abdomen.

3.3 The mortality of Adult stage:

Results in figure 1 presented that the ethanol extract of *C. crespata* was recorded a high rate of the mortality in the adult stage of *C. megacephala*. The 2% concentration caused most mortality of adults after 48 hours of the treatment with ethanol extracts compared to 1% and 0.5% concentrations with significant differences. As the mortality rates are as follows 83.3%, 50% and 23.3% respectively, while the control was 3.3%.

Table 1: Effect of ethanol extract of *Cladophora crespata* on the larval mortality of *Chrysomya megacephala*.

Larval instar	Control	0.5%	1%	2%	Mean
1 st instar	3.3	20	40	70	43.3
2 nd instar	0	13.3	26.6	63.3	34.4
3 rd instar	0	6.6	23.3	56.6	28.8
Mean		13.3	30	63.3	

Table 2: Effect of ethanol extract of *Cladophora crespata* on the larval duration of *Chrysomya megacephala*.

Larval instar	Larval duration (with days)			
	Control	0.5%	1%	2%
1 st instar	4-6	4-6	6-8	7-9
2 nd instar	6-8	7-8	8-10	10-13
3 rd instar	6-8	7-9	9-10	11-14

Table 3: Effect of ethanol extract of *Cladophora cespata* on some aspects of pupal stage of *Chrysomya megacephala* treated as larval instars.

Treatment	Pupal mortality %	Abnormal pupa %	Normal pupa %	Pupal weight (g/10pupa)	Pupal duration days
Control	3.3	0	96.6	0.784	5-6
0.5%	11	6.5	82.5	0.596	6-7
1%	15.3	13.6	71.1	0.238	7-9
2%	26.5	18.3	55.2	0.129	9-10

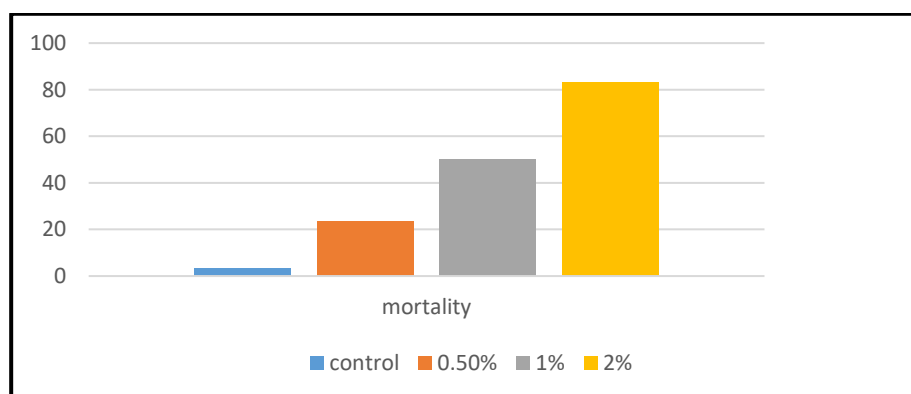


Figure 1: Effect of ethanol extract of *Cladophora cespata* in mortality of adult stage of *Chrysomya megacephala*.



Figure2: intermediate stage between larva and pupa results from treatment with extract.



Figure3: abnormal pupa results from larvae treatment with extract.



Figure4: spherical pupa results from treatment with the extract.

Discussion:

The present study revealed that the ethanol extract of green algae *Cladophora crespata* significantly affected the larval mortality, larval weight, larval duration, pupation, pupal weight, pupal duration, adult emergence and adult mortality of the blow fly *Chrysomya megacephala*. The World Health Organization (WHO) approximates that 80% of the world's inhabitants depend mainly on traditional medicine for their primary health care, Chlorophyta and Cyanophyta are rich source of structurally novel biologically active metabolite. Khalaf et al. (2011) reported that methanol extract of *C. crispata* were effected in *in vitro* activity against the protoscolices of hydatid cyst after 5 days post treatment, this means the time has played an important role in treatment since decreased concentration leads to increase the time of treatment. They stated that GC- Mass spectrum showed the activity of methanol extract returned to the presence of Phthalic acid, 3,5-difluorophenyl and undecyl ester compound. Athbi et al. (2011) isolated an alkaloidic compound similar to Calothrixin-A from the green algae *C. crispata*.

The high mortality rates in the larval stage may be due to the chemical compounds present in the ethanol extract of *C. crespata*. The biological activity of extracts might be due to various compounds, including phenolics, terpenoids and alkaloids present in plants (Rafeal et al., 2008). The presence of cytotoxic compound saponin was observed and the presence of saponin along with other phytoconstituents may be the reason for the high percent mortality observed with reference to the extracts of the plant extract. Pelah et al. (2002) have observed the presence of carbohydrates, saponins, phytosterols, phenols, flavonoids, and tannins in the plant extract having mosquito larvicidal activity. Similar observations were noticed in the present study and support the potential applications of some plant extracts in insects control measures. Al-Thameri (2006) reported the larvicidal activity of *Plantago lanceolata* against the larval stage of *Musca domestica*, and Al-Mansoor et al., (2010) by *Nicotiana tabacum* on *Chrysomya albiceps*. Prolongation of the larval duration with ethanol extract, that's probably due to the larvae observed to pupate faster as their environment increased in toxicity. This is clearly a self-preservation mechanism since the pupal form is less susceptible to the environment.

The decrease of pupal weight and prolongation in the pupal duration in the present study may be attributed to the decrease in total water content or decreased intensity of protein biosynthesis (Abdel-Aal, 1996), it may be due to the lack of proper sclerotization of the newly formed puparium, or evaporation of body fluids leading to decreased pupal weight. The effect of the tested algal extract on the mean pupal weight of pupae treated as larvae agrees with the results of using plant extracts obtained on *Chrysomya albiceps* by Al-Mansoor and Al-Thameri (2011). The morphological aberrations of some pupa induced by ethanol extract were concentration dependent, the higher concentration causes more morphogenetic aberrations. Adamski *et al.* (2005) observed that the degree of malformation was directly proportional to the pesticides. This results made also clear co- relation with the recent findings reported from Al-Mansoor and Al-Thameri (2010) where the phenols and alkaloids of *Citrullus colocynthis*, had been noticed to produced clear morphological abnormalities in *Sarcophaga haemorrhoidalis* .

Some deformed larvae were pigmented and larval-pupal intermediate, the resultant some individuals were abnormal shaped, and most of the pupae failed to reach adults, however, some emerged adult have various degrees of morphological abnormalities. This phenomenon was observed previously with Shoukry (1996) who studied the histopathological effects of Chamomile and Jasmine oils on the house fly larvae, Ultrastructure of muscles of the treated larvae showed that those compounds induced disorganization of light and dark bands of the muscles. This may be the possible explanation for the melanized patches of cuticle or may be due to the inhibition in melanin synthesis (Gelbič and Nĕmec 2001). El-Hadek (2002) reported that the malformation in pupal stage of *Musca domestica* as it appeared as larval-pupal intermediate, may be due to the treated larvae were unable or failed to free themselves from their old cuticle. Al-Thameri (2010) observed dark intersegment pigments on the 3rd larvae of *Calliphora vicina* and fully formed pupa but with a constricted puparium after treatment the 1st instar larvae with ethanolic extraction of leaves of *Conocarpus lancefolius*. In the present study, some pupae failed to reach adults. Ande (2001) stated that diet of house fly containing plant materials no doubt contains desirable primary or secondary principles which may have developed from the interactions of the components of the diet. These principles elicit biological activities in respect of larval-pupal transformation and pupal eclosion hindrances. Emerging of adults with malformed wings may be attributed to the failure of the wings to expand and flatten after adult emergence (Saxena *et al.* 1981). This may be due to the modification of the ecdysteroid titer, which in turn leads to changes in lysosomal enzyme activity causing overt morphological abnormalities (kumar *et al.* 1999).

The mortality rates in the adult stage may be due to the chemical compounds present in the ethanol extract of *C. crespata*. Klocke et al. (1986) reported the presence of cytotoxic compound saponin was observed and the presence of saponin along with other phytoconstituents may be the reason for the high percent mortality observed with reference to the extracts of the plant. Other studies were noticed the potential applications of some plant extracts in insects control. Al-Thameri (2006) showed the adulticidal activity of *Quercus brantii* against *Musca*

domestica, and Hatem (2012) reported the high mortality of the adults of *Calliphora vicina* by using ethanol extract of *Peganum harmala*.

Conclusion:

The results of the present study, suggest that the application of algal materials prevented normal development of the different stages of *Chrysomya megacephala*. Thus, the extract of *Cladophora crespata* were nearly comparable with the insect growth regulators and other insecticides in its effects. It's able to done more mortality of larva, pupa and adults. Also, the algal extract are harmful to *C. megacephala* not only mortality but also reduce pupal weights, adult emergence, resulted larval-pupal intermediates, and abnormal pupa. In general, show effective IGR-like activities and exhibit great promise in suppressing populations of *C. megacephala*. Furthermore, the results of the present study may contribute to a reduction in the application of synthetic insecticides, which in turn increases the opportunity for natural control of various important insects by algal extract. Further studies on the tested algae including mode of action, synergism with the biocides under field condition are needed.

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