COMPARATIVE TOXIC ACTIVITY OF FOUR ALGAE, AGAINST THE 2ND AND 4TH LARVAL INSTARS OF BLACK CUTWORM, *AGROTIS IPSILON* (HUFNAGEL)

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Abstract

The dry biomass of four cyanobacterial microalgal strains *Anabaena flos aquae, Anabanea laxa*, *Anabanea fertilissima* and *Nostoc muscorum* was evaluated in *invitro* assay as a biopesticides against 2nd and 4th instars of *Agrotis ipsilon*. Larvae were fed on leaf discs painted with the algal solution for 24h. The 2ndinstar larvae were more susceptible than the 4th ones to the four tested algal treatments. *N. muscorum* treatment was the most toxic one against the 2nd and 4th larval instars. Where, its LC_{50}values were 45 and 150 mg/ml, respectively while, *A. fertilissima* was the least one, its LC_{50} values were 250 and 660 mg/ ml, respectively. All the treated larvae were susceptible to four tested algal treatments and its resistance varied according to the larval instars and tested alga. Marked decrease in pupation for both 2nd and /or 4thinstar by the four algal treatments, whereas, the 2ndinstar treated with *N. muscorum* alga exhibited the most suppressive decrease to 44%, as compared to control (97%). Significant reduction for pupal weight, where, 4thinstar treated with *A. flos aquae* alga recorded the highest pupal weight decrease to average 290mg, as compared to 484mg of control. Besides prolonging pupal duration where, treatment for both instar with *A. flos aquae* and *A. laxa* algae induced the longest period and increased the pupal malformation percent where, 4thinstar treated with *N. muscorum* and *A. flos aquae* algae induced noticeable percent reached to21.3 and20% and increased adult malformation to30 and20% respectively, as respect to control. However, the larval treatment of 2nd instar with *N. muscorum* and *A. fertilissima* algae had the strongest effect in larval duration increase to average25 and 25.8day as compared to20.4day of control. In addition, the larval treatment of 4thinstar with the four algae decreased the adult fecundity and fertility. Moreover, *N. muscorum* and *A. flos aquae* algae inhibited the adult fecundity and eggs fertility to zero, as compared to505eggs/female of control. Marked declining of adult longevity with *N. muscorum*, *A. flos aquae* and *A.laxa* to average 7day, as compared to11day of control. Also, the treatment with the three algae disorder the sex ratio of males and females, as compared to that of control. While the larval treatment with *A. fertilissima* alga didn't affect the pupal malformation, eggs fertility, adult longevity and sex ratio.

INTRODUCTION

The need for natural pesticides is one of the scientific research goals. Biopesticides are an important group of naturally occurring, often slow-acting crop protectants that are usually safer to humans and the environment than conventional pesticides, and with minimal residual effects. Biopesticides can be biochemical or microbial. Biochemical pesticides may include plant-derived pesticides (botanicals) that can interfere with the growth, feeding, or reproduction of pests or insect (Copping and Menn 2000).The black cutworm, *A. ipsilon* attacks the seedlings of most crops. Crops attacked include beans, broccoli, cabbage, carrot, Chinese broccoli, Chinese cabbage, Chinese spinach, corn, eggplant, flowering white cabbage, green beans, head cabbage, lettuce, mustard cabbage, potato, spinach, sugarcane, sweet potato, tomato, turnip, as well as many other plants (Rings ,1975).They commonly feed on seedlings at ground level, cutting off the stem and sometimes dragging the plants into their burrows. Most of the plant is not consumed but merely eaten enough to cause it to topple. Since the larvae occur burrowed near the roots of the host, it sometimes feeds on roots and the below ground stem. Because of the nature of their feeding on young plants, this pest can do great damage in newly planted fields. The rising consumption of currently used insecticides in developing countries has led to a number of problems such as insect resistance, environmental pollution and the health hazards associated with pesticide residues .It is therefore necessary to complement our reliance on synthetic pesticides with less hazardous ,safe ,and biodegradable substitutes**.** These comprise inhibitory properties against microorganisms (bacteria, cyanobacteria, algae, viruses, fungi), and toxicity to invertebrates (crustaceans, bivalves) and vertebrates (fish, birds, mammals). Many of these activities are of allelochemical character. They strengthen cyanobacterial strains in their competition for nutrients, space and light, and protect them from viral, bacterial and fungal pathogens, as well as from grazing animals. Algae as primary producers in aquatic systems, are known to excrete some inhibitory effects upon certain components of aquatic fauna but few reports dealing with insecticidal activities of the algae the poisoning effects of blue green algae to fish and water fauna have been described by Allen (1956) who found that *chlamydomonas* and *chorella* spp. Liberate essential peptides and amino acids with different magnitude. The bioactivity of hapalindoles demonstrates that cyanobacterial biofilms can be considered as promising sources of insecticidal metabolites, which might be useful for the biocontrol of dipterans (Becher *et. al* 2007). Some of fractions derived from the algal extracts and after its purification and chemical characterization showed that they were either hydrocarbons or fatty acids(Sarkar *et al*., 1995) or some algae, such chlamydomonas and chorella spp liberate essential peptides and amino acids with different magnitude (Allen, 1956)

,sometimes some antimicrobial substances was found in green algae *Chlorella* and *Scenedesmus* (Saleh *et al*., 1984 and El-Baz *et al.* ,1985). Cyanobacteria produce numerous secondary metabolites exhibiting diverse bioactivities (Burja et al., 2001; Wiegand and Pflugmacher, 2005). The cyanobacterium, *A. flos aquae* that proved to be a good source for different bioactive compounds, may be an excellent candidate as a natural pesticide against (Lepidoptera). The cyanobacterium *Nostoc* strain ATCC 53789, a known cryptophycin producer, was tested for its potential as a source of natural pesticides (Biondi *et. al*., 2004). No doubt, Cyanobacteria are safe and promising agent for insect control.

The aim of the present study was performed to figure out the potent alga with bio-insecticidal activity of *Anabaena flos aquae, Anabanea laxa, Anabanea fertilissima* and *Nostoc muscorum* as natural pesticides to control one of the most serious pests (*A. ipsilon*) in Egypt.

MATERIALS AND METHODS

Insect rearing

The second and fourth instar larvae *A. ipsilon* used in this experiment were separated by plastic grids to form individual chambers (ice cubes) to avoid a cannibalism phoneme and fed on fresh castor leaves, *Ricinus communis*, they were incubated at 25^{o}C, 60-70 %R.H. in the laboratory followed according to Abdel-salam (1980) until the pupation. The newly emerged moths were mated within the large glass jars provided with a cotton piece soaked in 10%sugar solution as a feeding source and muslins as ovipostion substrate for the moths. The second and fourth instar larvae were used for the bioassay test.

Microalgae and growth medium

A. flos aquae, A. laxa, A. fertilissima and N. muscorum, is a cyanobacterial strain commonly prevailed in Egyptian rice fields at north delta. An axenic strain was obtained from the cyanobacterial culture collection of Agric. Microbial. Res. Dept., Soils, water and Environment Res. Inst. Agric. Res.center, Giza, Egypt. B.G11$_0$ (nitrogen free) medium was prepared according to Allen's and Stanier (1968).The algal strain was grown in 250-ml Erlenmeyer flasks at 28±2ºC with continuous illumination of 2500 Lux intensity.

Experimental set up

Growth conditions

The investigated cyanobacterial strain was sub-cultured, separately, in 5L Erlenmeyer bottles, containing 3L B.G11$_0$ (nitrogen free) medium inoculated with 30 ml of pre-cultured isolates during exponential phase. The isolate cultured under mixotrophic growth conditions, was created by adding glucose (1%, w/v) , and was aerated with bubbling air at regular pressure (200 ml min^{-1} with 50 Hz frequency) and

sealed with rubber plug having a narrow glass tube as an air outlet. This condition was depending on CO_2 (0.03%), which exists normally in air.

Preparation of algal solution

The obtained microalgal biomass at stationary phase was separated by filtration and air dried, then it crushed by mixer blind, the obtained powder of algal material. And a series of six different concentrations of the algal powder of four tested microalgae (0, 62.5, 125, 250, 500, 1000 and 1500 mg/ml) were prepared by dilution with absolute ethanol (99% purity). Absolute ethanol free from algae materials was used as control treatment

Bioactivity tests

The toxic activity of the tested algal material was assayed by using leaf disc method (Rani and Rajasekharreddy, 2009). Where, fresh leaf *Ricinus communis* were cut into small discs of (9 cm^2) by using a sharp cutter. Some of the leaf discs were treated uniformly on both sides with ethanol and used as control, while the other leaf discs painted equally on both sides with algal solution of 62.5,125,250,500,1000 and 1500 mg/ml/cm^2 of *A. flos aquae, A. laxa, A. fertilissima and N. muscorum*. The treated and control discs were completely air dried. All discs were placed for feeding in the plastic chambers (ice cubes) lined with moistened paper towel to avoid a cannibalism phoneme. There were eight replicates of eighty larvae of both 2nd and 4th instars of *A.ipsilon* were tested at each concentration for the four treatments beside control. Effect of LC_{50} on some biological activities such as larval and pupal duration, percent of pupation and adult emergence, the pupal weight, pupal and adult malformations, fecundity, eggs hatchability and adult longevity and sex ratios of *A. ipsilon* were carried out by feeding on treated discs and control as well. Also, the observed malformations were recorded and photographed.

Statistical analysis:

Percentage of larval mortality of 2nd and 4th instars resulted from the four algal treatments at the tested concentrations was recorded after 24h.and corrected according to Abbott formula **(**Abbott, 1925). The data were then analyzed using the probit analysis (Finney, 1971) and the LC_{50} values were estimated for both instars at each algal treatment. Effect of LC_{50} on biological activities of the surviving larvae was estimated. The obtained data were statically calculated through Excel for windows computer program to determine the P-value and L.S.D (least significant difference) at 0.05 freedom degrees.

RESULTS AND DISCUSSION

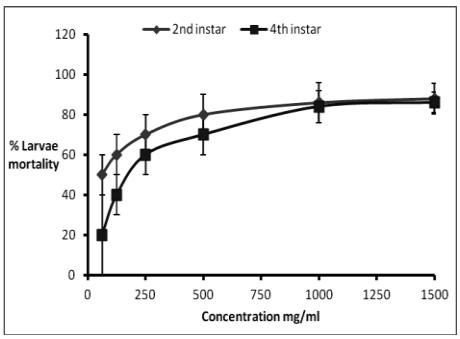
1-Insecticidal activity

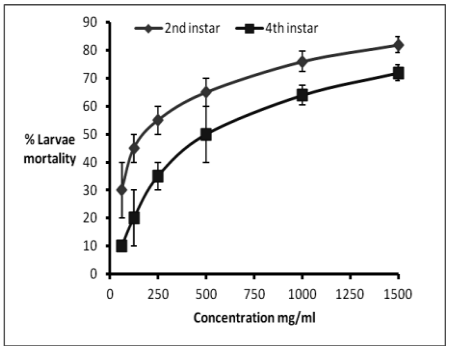
Data presented in Table (1) showed the toxic effects of the four microalgae, *A. flos aquae, A. laxa*, *A. fertilissima* and *N. muscorum* against the 2nd and 4th larval instars of *A.ipsilon* that fed on leaf discs treated with these algal materials. The 2ndinstar larvae were more susceptible than the 4th ones at the four tested algal treatments. The *N. muscorum* treatment was the most toxic one against the 2nd and 4th larval instars. The LC_{50}values were 45 and 150 mg/ml, respectively .While, *A. flos aquae* was the second one, the LC_{50}values were 62.5 and 200 mg/ml, respectively. Whereas, *A. laxa* was the third one, its LC_{50} values were 180 and 500 mg/ml, respectively. While, *A. fertilissima* was the least one, its LC_{50} values were 250 and 660 mg/ ml, respectively as showed in Figs 1,2,3 and 4.

Table 1. Insecticidal activity of *Anabaena flos aquae, Anabanea laxa*, *Anabanea fertilissima* and *Nostoc muscorum* expressed as LC_{50} values against the 2nd and 4th instar larvae of *A. ipsilon*

Treatment	Larvae							
	2ndinstar				4thinstar			
	LC_{50} values P.p.m	Slope function	95% confidence limit		LC_{50} values P.p.m.	Slope function	95% confidence limit	
			Upper	Lower			Upper	Lower
Anabaena flos aquae	62.5	10.1	200	19.5	200	4.5	520	76.9
Anabanea laxa	180	10	594	55	500	5.6	1500	167
Anabanea fertilissima	250	10.7	850	73.5	660	6.1	2112	206
Nostoc muscorum	45	16.8	153	13	150	5.4	420	54

(a) **(b)**





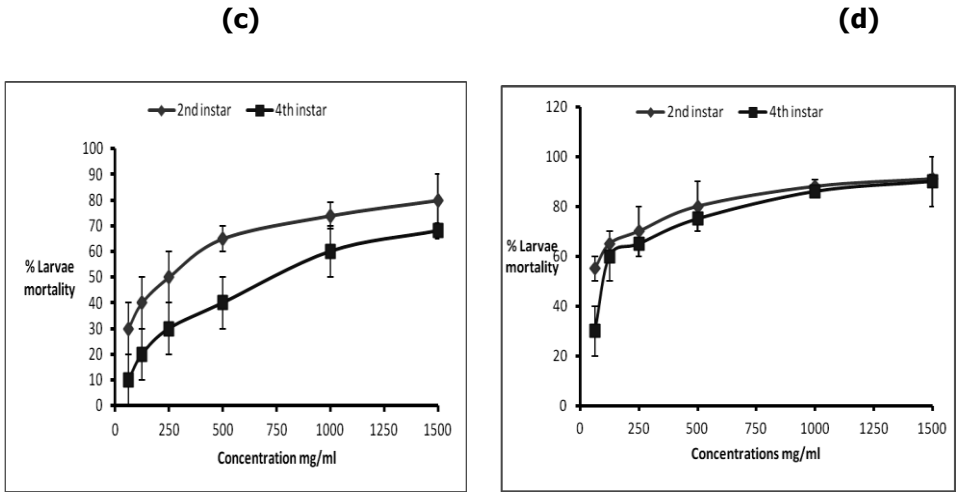


Fig. 1.% of larval mortality of 2nd and 4th instars of *A.ipsilon* treated with (a)*Anabaena flos aquae*,(b)*Anabanea laxa*, (c)*Anabanea fertilissima* and (d)*Nostoc muscorum*

These results agree with those obtained by Aly and Abdou (2010) who found that the cynobacteria Spirulina platensis cell content gave 100% mortality of *Spodoptera littoralis* larvae at 5% concentration. Saleh *et al.* (1984) and Sharaby *et al.* (1993) found that the green Scenedesmus acutus have insecticidal activity against S. littoralis.Also, Zaki and Gesraha (2001) reported that algae have insecticidal effect on several insect groups like cotton leafworm. Similarly, Nassar *et al.* (1999) established acute lethal toxicity of cyanobacteria (blue-green algae) against 4th larval stages of *Spodoptera littoralis* and *Agrotis ipsilon*. They recorded the LD50s were 7.59 and 9.10µg for *S. littoralis* and *A. ipsilon* respectively. Also, Sarkar *et al*. (1995) established antimicrobial, ovicidal and insecticidal properties of a petroleum ether extract of aquatic alga Chara zeylanica Klein ex Wild and its fractions (P_1 to P_{19}) against cotton pests.

Latent effect:

Larval and pupal period:

Data presented in Tables (2and 3) indicated that the 2nd larval instars of *A. ipsilon* fed on leaf discs treated with *N. muscorum* and *A. fertilissima* algae at the LC_{50} level inducing highly significant ($p<0.01$) increase of the larval duration to average 25.8±2.3 and25.1±3days, respectively, as compared with 20.4±2.2days control. While the 4th instar larvae fed on *A. flos aquae* and *A. laxa* alga gave significant ($p<0.01$) increase in the larval duration to average21.2+1.8 and 21.4+2.2 days, respectively, as compared to18.1+3.9days of control. Whereas, the treatment of both 2nd instar with *A. flos aqua, A. laxa* and 4th instar with *A. fertilissima* and *N. muscorum*

algae gave none significant increase in the larval duration, as compared to that of control. .

Treatment of the 2nd instar larvae of *A. ipsilon* with the *A. flos aquae, A. laxa* and *Nostoc muscorum* algae at LC_{50} values increased significantly ($p<0.01$), the pupal duration to average 13.7±1.6, 16.4±1.7 and 9.6±2.1 days,respectively,as compared to 6.9±1.1 days of control. Whereas, the 4th instar treated with *A. flos aquae, A. laxa* and *A. fertilissima* gave significant ($p<0.01$) increase in the pupal duration to average 14.8±1.6, 15±1.6 and 12.8±1.5 days, respectively,as compared to 10.2±1.7 days of control. While, the larval treatment of 2nd instar with *A. fertilissima* and 4th instar with *N. muscorum* alga didn't affect the pupal duration, as respect of control.

Table 2. Biological activities of Anabaena flos aquae, Anabanea laxa, Anabanea *fertilissima* and *Nostoc muscorum* at their LC_{50} values against the 2ndinstar larvae of *A. ipsilon*

Treatments	Larval periods (days) ± SD	% Pupation ± SD		Pupal duration days	Pupal weight mg	% Moth emergence		
		Normal	Malfo.			Normal	Malfo.	Total
Anabaena flos aquae	21±2n.s	50±15.4**	10n.s	13.7±1.6**	345±60**	68.8**	31.3**	100
Anabanea laxa	21.4±1.3n.s	53±15**	10n.s	16.4±1.7**	343±46**	88.9n.s	11.1n.s	100
Anabanea fertilissima	25.1±3**	45±18**	12.5n.s	6.4±0.9n.s	359±49**	77.8**	22.5**	100
Nostoc muscorum	25.8±2.3**	44±19**	10n.s	9.6±2.1**	364±18**	88.9n.s	11n.s	100
Control	20.4±2.2	97±3.9	0	6.9±1.1	462±6.2	100	0	100
P value	0.00009	0.000615	0.107	0.00408	0.0201	0.0156	0.0195	
L.S.D. at 0.05	1.4	19.4	23.9	1.3	54.5	12.3	12.1	

** = Highly Significant ($p<0.01$) * Significant ($p<0.05$)
S.D.=Standard deviation Malfo.= Malformation%
L.S.D. = Least significant difference Lab. =Laboratory strain
n.s=none Significant ($p>0.05$)

Table 3. Biological activities of *Anabaena flos aquae, Anabanea laxa, Anabanea fertilissima* and *Nostoc muscorum* at their LC_{50} values against the 4th instar larvae of *A. ipsilon*

treatments	Larval periods (days) ± SD	% Pupation Mean± SD		Pupal duration Mean+S.D days	Pupal weight Mean+S.D mg	% Moth emergence		
		Normal	Malfo.			Normal	Malfo.	Total
Anabaena flos aquae	21.2±1.8*	60±7.1**	20**	14.8±1.6**	290±86**	80**	20**	100
Anabanea laxa	21.4±2.2*	61±7.4**	0	15±1.6**	396±61**	80**	20**	100
Anabanea fertilissima	20.4±2.2n.s	70±14**	0	12.8±1.5**	366±76**	87.5n.s	12.5n.s	100
Nostoc muscorum	19.3±4.2n.s	50.2±7**	21.3**	11±1.1n.s	408±32**	71**	30**	100
Control	18.1±3.9	99.2	0	10.2±1.7	484±33	99.7	0	100
P value	0.0358	0.00347	0.0115	0.0083	0.0204	0.0503	0.039	
L.S.D. at 0.05	2.75	37.8	11.5	1.9	101.6	11.95	9.1	

** = Highly Significant (p<0.01) * Significant (p<0.05)
S.D.=Standard deviation Malfo.= Malformation%
L.S.D. = Least significant difference Lab**.** =Laboratory strain
n.s=none Significant (p>0.05)

This result is in agreement with that obtained by Nassar *et al.* (1999) who established acute lethal toxicity of cyanobacteria (blue-green algae) against 4th larval stages of *Spodoptera littoralis* and *Agrotis ipsilon***.**They indicated that LD50s markedly affected the larval, pupal duration of both insects**.** Also, Salama and Sharaby (1980) found that the green algae *Spirulina geitleri* was less efficient as a partial substitute for kidney beans in a diet for rearing *spodoptera littoralis*; and it caused significant prolonged in the larval duration. This result is contracted some what with that obtained by Aly and Abdou (2010) who reported that duration of larvae and pupae produced from 4th instars larvae fed on leaves treated with *Spirulina platensis* (cell content +cell wall) shortened the larval and pupal life than control at 0.5%,1% and 2.5% concentrations. They may be due to difference of subspecies of Spirulina and its preparation (Nassar *et al*., 1999).

Pupation and adult emergence:

Data in Tables (2and 3) demonstrated that the treatment of the 2nd and 4th instar larvae of *A. ipsilon* with the algal material of the four algae at their LC50 values, caused highly significant (p<0.01) reduction of the pupation percentages, as compared to control. The treatment of 2nd instar with *N. muscorum* and *A. fertilissima*

algae had the most potent in pupation reduction to 44 and 45%, respectively, as compared to that of control (97%).While the 2nd instars fed on *A. flos aquae* and *A. laxa* algae reduced the pupation to50 and 53%, respectively, as compared to that of the check. Whereas, the 4th instars fed on *A. flos aquae, A. laxa, A. fertilissima* and *N. muscorum* reduced the pupation to range from 50.2–70%, as compared to that of control (99.2%).

On the other hand, the treatment of the 2nd and 4th instars larvae of *A. ipsilon* with the four algae at the LC_{50} level caused significant ($p<0.01$) reduction in the adult emergence percentages, as compared to that of control. However, the 2nd larval instars treated with *A. flos aquae* had the most suppressive one on the adult emergence (decrease to average 68.8%, as compared to100 % of control). While, the 2nd instars fed on *A. fertilissima* algae decreased the adult emergence to 77. 8%, as compared to that of control .While the 4th instar larvae fed on *N. muscorum* alga had the highest effect in adult emergence decrease to reach 71%, as compared to that of control (99.7%). And the 4th instar treated with both *A. flos aquae* and *A. laxa* algae reduced the adult emergence to reach80%, as compared to control. Whereas, the treatment of both 2nd instar with *A. laxa* and *N. muscorum* and 4th instar with *A. fertilissima* algae gave none significant decrease in adult emergence to reach 88.9 and87.5%, respectively, as compared to control (100 and99.7%)

This results was agreed with Aly and Abdou (2010) who reported that the treatment of 4th instars larvae of *Spodoptera littoralis* fed on leaves treated with *Spirulina platensis* (cell content +cell wall) at 5% concentration failed to pupate. Also, Abou-Tabl *et al.* (2002) found that the *Spodoptera littoralis* larvae treated with *Sargassum dentifolium,* affected the percentage of pupation.Salama and Sharaby (1980) found that the blue green algae *Spirulina geitleri* was less efficient as a partial substitute for kidney beans in a diet for rearing *Spodoptera littoralis*; and only 52-55% reached the pupal stage compared with 80% on the kidney bean diet.

The pupal weight:

The treatment of 2nd and 4th instars of *A. ipsilon* with the four algae at their LC_{50} levels caused a highly significant reduction in the pupal weight .The 2nd instars larvae treated with the four algae decreased the pupal weight to average 345,343,359and 364mg,respectively, as compared to that of control (462mg). Also, the treatment of 4th instar larvae with *A. flos aquae* algae had the most suppressive one in pupal weight decrease to average 290mg, as compared to 484 mg pupal weight of control, while the 4th instars fed on *A. laxa, A. fertilissima* and *N. muscorum* significantly ($p<0.01$) decreased the pupal weight to average 396,366 and 408mg, as compared to that of control. (Tables 2 and 3).

These results are similar with those obtained by Aly and Abdou (2010) who found that the treatment of *Spodoptera littoralis* larvae with 5% conc. of *Spirulina* cells content and cell wall decreased significantly the larval and pupal weight. In contrary with those obtained by Venkatesh *et al.* (2009) who found that *Spirulina* increased the weight of pupa and shell of *Bombyx mori*, which produced by the treated larvae.

Morphogenetic effects:

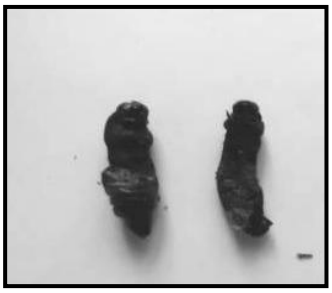
Data presented in Tables (2&3) showed that the larval treatment of 4th instars of *A. ipsilon* with *A. flos aquae* and *N. muscorum* algae at the LC50 values induced the noticeable percentage of malformed pupae reached 20 and 21.3%,as compared to 0% of control. Whereas, the 2nd instars treated with the four algae induced none significant increase in pupal malformations ranged from10 to12.5%.

With regard to the adult malformations (Tables 2 &3), it was found that the larval treatment of both 2nd instars of *A. ipsilon* with *A. flos aquae* and 4th instar with *N. muscorum* algae at the LC50 values induced the highest percent of adult malformations, it reached to31.3 and 30%,respectively,as compared to 0% of control. While the treatment of 2nd instar with *A. fertilissima* and 4th instar with both *A. flos aquae* and *A. laxa* increased the adult malformations to reach to22.5 and 20%, respectively, as compared to control. While the larval treatment of 2nd instar with both *A. laxa* and *N. muscorum* and 4th instar with *A. fertilissima* algae induced none significant increase in the adult malformations reached11 and 12.5%, respectively, as compared to that of control (0%).

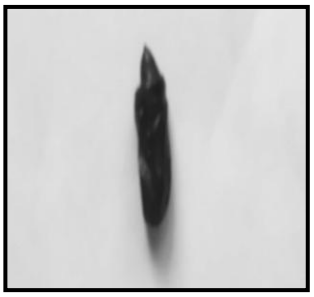
Malformations of *A.ipsilon* pupae resulting from the larval treatment of the 2nd and 4th instars of *A.ipsilon* with the algal material of the four *via* feeding on the leaf discs showed prepupae failed to pupate with blacking in the body color(Fig.1)or larval-pupal intermediates (Fig2,3), or undersized pupae (Fig.4) or monstrosital pupae (Fig. 5) or pupal with larval skin (Fig.6). Malformed adults appeared as adults without or had weakly wings (Fig.7, 9) or adult with pupal body in the posterior end (Fig.8) or adults with slight twisting in the wings (Fig.10) or adults with strongly malformation in the wings or body (Figs.11 and 12), as compared to pupae and adults of control (Fig.13 and14).

These results are similar with those obtained by Antonious *et al.* (1992) who recorded that morphological aberrations were obtained in pupae and adults of *S.littoralis* emerging from larvae fed on food plant leaves treated with the plant extracts of *D.maculata* and *A.vasica*, i.e. pupae retaining larval thoracic legs, and adults with abnormal abdomens, legs and wings. Also, Aly and Abdou (2010) reported that the treatment of 4th instars larvae of *Spodoptera littoralis* fed on leaves treated with *Spirulina platensis* (cell content +cell wall) at 5% concentration increased the malformation.

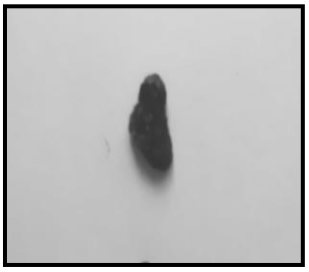
(Fig.1): prepupae failed to pupate with blacking in the body



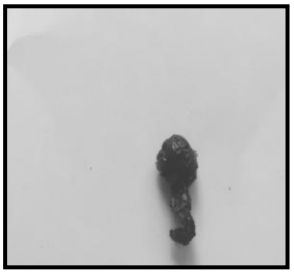
(Fig.2, 3): larval-pupal



(Fig.4): undersized pupae.



(Fig.5): monstrosital pupae.



(Fig.6) pupal with larval skin.

Malformed adults without or had weakly wings (Fig.7, 9) or adult with pupal body in the posterior end (Fig.8).

(Fig.10): Adults with slight twisting in the wings.

(Fig.11, 12): Adults with strongly malformation in the wings or body.

(Fig.13 and14): pupae and adults of control

Table 4. Biological activities of *Anabaena flos aquae, Anabanea laxa*, *Anabanea fertilissima* and *Nostoc muscorum* at their LC_{50} values against the 4th instar larvae of *A. ipsilon*

Treatments	Fecundity eggs/ f	Eggs hatching%	Longevity (days)	Adult sex ratio (%)	
Anabaena flos aqua	zero**	zero	7.3±1.5*	66.7	33.3
Anabanea laxa	50±13**	92.5	7.2±1.7*	60	40
Anabanea fertilissim	90±8.2**	100	10±1.5n.s	50	50
Nostoc muscorum	zero**	zero	7.4±0.8*	60	40
Control	505±76	100	11±3.4	50	50
P value	0.00653		0.0294		
L.S.D. at 0.05	95.3		3.2		

** = Highly Significant (p<0.01) * Significant (p<0.05)
S.D.=Standard deviation Malfo.= Malformation%
L.S.D. = Least significant difference Lab**.** =Laboratory strain
n.s=none Significant (p>0.05)

Adult fecundity and fertility:

Data in Table (4) indicated that the treatment of the 4th instars of *A. ipsilon* with the four algae significantly (p<0.01) reduced the adult fecundity. And the 4th instars larvae treated with both *A. flos aquae* and *N. muscorum* algae had the strongest effect in adult fecundity inhibition to reach zero, as compared to505 eggs/f of control. While the 4th instars treated with *A. laxa* and *A. fertilissima* decreased the adult fecundity to an average50 and 90 eggs/f, respectively, as compared to that of control (505eggs/f).

Therefore, the treatment of the 4th of *A. ipsilon* larvae with both *A. flos aquae* and *N. muscorum* algae complete inhibited the eggs fecundity and fertility to reach zero, as compared to that of control. And the eggs laid by adults treated as 4th instars larvae with *A. laxa* alga had 92.5% of hatching, as compared to that of control (100%).While, the treatment of 4th instars with *A. fertilissima* alga recorded eggs hatching similar to that of control (100%).

These results are in agreement with those obtained by Nassar *et al.* (1999) who proved that at the obtained LD50s of 4th larval stages of *Spodoptera littoralis* and *Agrotis ipsilon***,** the algal treatment of cyanobacteria (blue-green algae) suppressed the oviposition of the survivor adults. Also, Sarkar *et al.* (1995) reported that the P_{7a} fraction derived from petroleum ether extract of *Chara zeylanica* alga induced maximum sterility and reduced average fecundity significantly in the cotton pest. Salama and Sharaby (1980) found that the blue green algae *Spirulina geitleri* was less efficient as a partial substitute for kidney beans in a diet for rearing *spodoptera littoralis* and the total egg production was low on the *Spirulina* containing diet.

Adult longevity:

Data obtained in Table (4) showed that the treatment of the 4th instars of *A. ipsilon* with *A. flos aquae, A. laxa* and *N. muscorum* algae reduced significantly ($p<0.03$) the adult longevity to average7.3±1.5, 7.2±1.7 and 7.4±0.8 days, respectively, as compared to11±3.4 days of control. While, the larval treatment with *A. fertilissima* alga gave none significant decrease in the adult longevity, as compared to that of control.

These results are in agreement with that obtained by *Nassar et al. (*1999) who studied the toxic activity of the cyanobacteria (blue-green algae) against 4th larval stages of *Spodoptera littoralis* and *Agrotis ipsilon* **.**The LD_{50}s markedly affected the adult longevity of both insects**.**

Adult sex ratio:

Data obtained in Table (4) demonstrated that the larval treatment of the fourth instars of *A. ipsilon* with *A. flos aquae, A. laxa* and *N. muscorum* algae at the LC_{50}values had the highest effect on the sex ratio disorder of adult males and females, as compared to that of control. While the larval treatment with *A. flos aquae* alga had the strongest effect in adult males increase to reach 66.7%, as compared to50%adult male of control, and it decrease the adult females to reach 33.3%, as compared to 50% of adult females of control.Also, the larval treatment with both *A. laxa* and *N. muscorum* algae increased the adult males to reach 60%, and decreased the adult females to reach 40%, as compared to that of control (50:50%).While the larval treatment with *A. fertilissima* alga gave the same sex ratios that of control.

CONCLUSION

The results of the present work demonstrated that the treatment of the 2nd and 4thinstar larvae of *A.ipsilon* via feeding on leaf discs treated with the four tested algae were effective against the survival and biological of *A.ipsilon*. Thus, the algal material of both *N. muscorum and A. flos aquae* induced maximum sterility and reduced average fecundity significantly to zero and disorder the sex ratios of males and females and shorted the adult longevity, thus its affect the various biological activities of this insect. Therefore, the algae are a large and diverse group of microorganisms and used often as biofertilizer and soil stabilizers (Abdel-Raouf *et al.,* 2012) and its were effective if applies as biopesticides within the cutworm baits or spray around the aerial parts (roots) that invade by pest as replacement means for the used synthetic insecticides (carbaryl e.g.Sevin or permethrin, chlorpyrifos or diazinon) for control of the mentioned pest that caused effects on the environment

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مقارنة سمية أربع من الطحالب المختبرة أنابينا فلوس اكو،أنابينا لكسا ، أنابينا فيرتليزميا والنوستوك ضد العمر اليرقي الثاني والرابع للسلالة المعملية للدودة القارضة

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أجريت هذه الدراسة بغرض مقارنة التأثير السام لأربع من الطحالب الخضراء المزرقة وهى أنابينا فلوس اكو و أنابينا لاكسا و أنابينا فيرتليزميا والنوستوك مسكوروم وقيمت معمليا كمبيدات حيوية ضد يرقات العمر الثاني والرابع للدودة القارضة تحت الظروف المعملية. غذيت يرقات العمر الثاني والرابع لمدة ٢٤ ساعة على شرائح ورقيه من الخروع تم دهنها بالمادة الطحلبية في سلسلة تركيزات لكل طحلب من الطحالب الأربعة المختبرة لتحديد قيم التركيز النصفي لكل طحلب. أوضحت النتائج أن معاملة النوستوك كان له التأثير الأقوى والغالب ضد كل من العمرين الثاني والرابع حيث بلغت قيمة التركيز النصفي القاتل له ٤٥، 150,ملجم/ملي. ومعاملة أنابينا فلوس اكو كان له التأثير الثاني حيث بلغت قيم التركيز النصفي٦٢,٥ و ٢٠٠ملجم/ملي وكان لمركب أنابينا لكسا التأثير الثالث حيث بلغت قيمة التركيز النصفي له١٨٠و٥٠٠ ملجم/ملي و كان لمعاملة أنابينا فيرتليزميا التأثير الأقل حيث بلغت قيم التركيز لنصفي له ٦٦٠،٢٥٠ ملجم/ملي لكل من العمرين الثاني والرابع على التوالي. تأثرت المعايير البيولوجية لليرقات بعد المعاملة لكل من للعمرين الثاني والرابع بالطحالب الأربعة. التأثير تنوع مع اختلاف العمر اليرقى و مع الطحلب المختبر وبناء على ذلك كان معاملة العمرين بالطحالب الأربعة خفضت نسب التعذير وكانت لمعامله النوستوك الجهد الأكبر في خفضها إلي ٤٤% بالمقارنة ٩٧% للكنترول. كما أن معاملة العمرين خفضت معنويا من الوزن العذري وكانت لمعاملة العمر الرابع بالأنا بينا فلوس اكو التأثير الأعلى في إنقاص الوزن العذري الي٢٩٠ملجم مقارنة ٤٨٤ملجم للكنترول. كما أن معاملة العمرين ذودت العمر العذري والمعاملة بكل من انابيينا فلوس اكوا وانابينا لاكسا أعطت الفترة الأطول في هذا الشأن.كما أن معاملة العمرين زودت نسب التشوه العذري والمعاملة للعمر الرابع بطحلبي النوستوك وانابينا فلوس اكوا أعطت ٢١,٣و٢٠% وأيضا ذودت التشوه الحشري إلي ٣٠و٢٠% بالمقارنة بالكنترول.بينما كان لمعاملة العمر الثاني بطحلبي النوستوك وانابينا فيرتليزميا التأثير الأقوى في زيادة العمر اليرقي ليتوسط ٢٥،٢٥,٨ يوم بالمقارنة ٢٠,٤يوم للكنترول. كما أن معاملة العمر اليرقي الرابع بالطحالب الأربعه خفضت من عدد البيض الموضوع بواسطة كل أنثي (الخصوبة) ونسبة الفقس للبيض ولكن المعامله بالطحلبي النوستوك والأنبينا فلوس اكو كان له التأثير الأعلى في اختزال الخصوبة وفقس البيض إلي الصفر مقارنه ٥٠٥ بيضة/أنثي للكنترول كما أن معاملة العمر الرابع قصرت من العمر الحشري والمعاملة بالنوستوك وانابينا فلوس اكوا وانابينا لكسا أعطت الفترة الأقصر(٧يوم) مقارنة ١١يوم للكنترول. أيضا المعامله للعمر الرابع أخلت بالنسب الجنسية للذكور والإناث بالمقارنة بالكنترول وكان المعاملة بالنوستوك وانابينا فلوس اكوا وانابينا لكسا التأثير الأقوى في هذا الشأن .كما وجد أن المعامله للعمر الرابع بطحلب أنابينا فيرتليزميا لم تعطي تشوه عذري ولم تؤثر علي حيوية البيض والعمر الحشري والنسب الجنسية.