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Effectiveness of the blue-green algae *Anabaena flos-aquae* in controlling *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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Abstract

The blue-green algae are known as *Anabaena flos-aquae*, which is a nutrient-rich food supply. The 2nd and 4th larval instars of the cotton bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) were tested in a lab using a crude extract of the cyanobacterial isolates of the *Anabaena flos-aquae* alga. The algal extract was administered to larvae via contact and feeding techniques. Depending on the larval instars and the applying technique, the algal extract's effects changed. Contact algal treatment was more successful than the feeding treatment. Also, the second larval instar was more vulnerable than the fourth. In terms of contact treatment, the LC_{50} value for the second larval instar was 0.0125 ml/l, while the fourth instar's 0.025 ml/l. In terms of feeding treatment, the LC_{50} value was 0.05 and 0.1 ml/l for the second and fourth instars, respectively. In contrast to controls, where adults emerged at a rate of 100% compared to 85%- 86% adults emerged as second instar and fourth instar in terms of feeding method, respectively. While adults emerged of second instar and fourth instar in terms of contact method was 81%-93%, respectively. The contact method's LC_{50} value for the second larval instar caused a highly significant increase in larval and pupal durations and a drop in adult emergence. Also, the contact method's LC_{50} value for the second larval instar caused the greatest percentage of pupation malformation 37%, and adult malformation (19%). Also, adults that emerged from the contact-treated 4th larval instar showed a significant decrease in egg hatchability to 82.4%. It was found that the proportion of pupation had significantly decreased. Additionally, adult longevity and adult emergence percentage were decreased for contact or feeding treatments on the fourth larval instar.

Introduction

One of the objectives of scientific study is the need for natural toxins. Biotoxins are an important group of naturally occurring, frequently slow-acting crop protection agents that are typically less harmful to people and the environment than traditional pesticides and have fewer long-term side effects. Biological or microbiological agents can produce biotoxins. Toxins from plants (Botanicals) that may inhibit an insect or pest's ability to develop, feed, or reproduce are examples of biochemical toxins (Copping and Menn, 2000). One of the primary reasons

limiting the output of cotton and tomato crops is the cotton bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), which severely damages the crop by feeding as a larva on Seedlings and apical meristematic tissues (Mazurkiewicz *et al.*, 2017). There are few reports on the algae's insecticidal properties, even though algae are the main producers in aquatic systems and are known to excrete some effects that inhibit some members of the aquatic fauna. Allen (1956) documented the toxic effects of blue-green algae on fish and aquatic life. He discovered that *Chlamydomonas* and *Chorella* spp. liberate essential peptides and amino acids to varying degrees. The bioactivity of hapalindoles showed that cyanobacterial biofilms are potentially valuable sources of toxins metabolites for the biocontrol of dipterans (Becher *et al*., 2007). After being purified and chemically characterized, some of the components from the algal extracts revealed that they were either fatty acids or hydrocarbons (Sarkar *et al*., 1995). Chlorella and Scenedesmus, two types of green algae, were also discovered to contain some antimicrobial compounds (Saleh *et al*., 1984 and El-Baz *et al*., 1985). Numerous secondary compounds produced by cyanobacteria have a wide range of bioactivities (Burja *et al*., 2001 and Wiegand and Pflugmacher, 2005). The cyanobacterium, *A. flos-aquae* which has shown to be a reliable source of various bioactive substances, may make a great choice for use as a natural pesticide against lepidopteran pests. Cyanobacteria are unquestionably a safe and effective pest control agent.

The current study sought to determine whether natural toxins found in the microalga *A. flos-aquae* could be used to regulate the cotton bollworm, *H. armigera* in Egypt.

Materials and methods

1. Rearing of *Helicoverpa armigera*:

H. armigera were collected from tomato fields in various locations in tomato production areas to establish a rearing colony in the laboratory at the Bollworm Research Department of the Plant Protection Research Institute (Sharkia branch). The larvae were raised on an artificial diet under controlled conditions (70 ± 5% RH., 26 ± 2°C, and a photoperiod of L: D 12:12) in plastic containers (6.5 × 19.5 cm) until pupation (Douro Kpindou *et al*., 2012). Third-instar caterpillars were moved singly Petri dishes filled with artificial food to avoid cannibalism. According to Dhande and Mohril (2022) ascorbic and sorbic acids, streptomycin, formaldehyde, vitamin complex, agar, distilled water, bean flour, methylparaben, and beer yeast make up the artificial diet. To prevent drying out, the diet was changed every two days, and moistened filter paper was put in each Petri dish (Barrionuevo *et al.*, 2012). Up until adult emergence, pupae were gathered and put in polypropylene containers (6 × 12 cm). For egg laying, the folded paper was placed within the cages. The larvae were raised as previously mentioned after the collected eggs were stored until they hatched.

2. *Anabaena flos-aquae* and culture conditions:

A. flos-aquae is a cyanobacterial (blue-green algae) strain that was frequently present in the North Delta rice fields in Egypt. Obtained the cyanobacterial culture from the Agricultural Microbiology Research Department at Soil, Water and Environment Research Institute (SWERI). According to Allen and Stanier (1968) instructions, B.G110 (Nitrogen-free) medium was prepared. The algal strain was cultivated in 250 ml Erlenmeyer flasks at a constant 28±2 C^{o} under 2500 Lux intensity. After 10 days of incubation, the biomass of the microalgal culture hit 5.7 g/L. After being filtered, air dried, and crushed, the dried algal biomass was put into a Soxhlet extractor that was

equipped with a condenser and a flask with a 500 ml round bottom.

3. Bioassay test and biological parameters:

By diluting the extracted raw materials from *A. flos-aquae* with distilled water, a series of five distinct concentrations (0.2, 0.1, 0.05, 0.025, 0.0125, and 0.00625 ml/l) were created. The control solution was distillate water devoid of any algae components. Effect of LC_{50} on a variety of biological processes, including larval and pupal duration, percentage of pupation and adult emergence, fecundity, egg hatchability, and adult lifespan. Both feeding and contact applications were used to determine the sex ratio of *H. armigera*.

4. Treatment methods:

For the feeding method, the second and fourth instar larvae of *H. armigera* were given tomato leaves that had been soaked in solutions containing various concentrations of the microalga's crude extract for 15 seconds before being allowed to air dry for about an hour. Tomato leaves were used as a control, immersed in distilled water only. For each concentration under test, 40 larvae of the 2nd and 4th instars were used.

For the contact method, diluting the algal crude extract in 99% ethanol, six distinct concentrations were created. One milliliter of the extract solution was added to the 9 cm Petri dish, and it was carefully moved in a circle to spread a thin film from each concentration on the inner surface. As a control, free ethanol was used. Under the temperature of the room, the ethanol vanished. According to Ahmed (1985), ten larvae of the cotton bollworm of the second and fourth instars were exposed for 24 hours in each Petri dish before being moved to clean containers and fed until pupation on fresh tomato leaves. The control and treatment groups 2nd and 4th instar larvae, each numbering about forty, were used.

5. Statistical analysis:

After 24 hrs. from treatment, the percentage of larval mortality in all experiments was noted and adjusted using the Abbott formula (Abbott, 1925). Data were analyzed using the probit analysis (Finney, 1971), and LC_{50} values were estimated for both instars at the two used methods. It was calculated how the LC_{50} affected the biological functions of the larvae that survived. Statistically significant differences between individual means were determined by one-way analysis of variance through the software computer program.

Results and discussion

1. Toxicity of *Anabaena flos-aquae* against *Helicoverpa armigera*:

Data in Table (1) show that the 2nd instar larvae were more sensitive than the 4th instar to contact treatment with the crude extract of *A. flos-aquae* alga. It recorded LC_{50} value for 2nd instar larvae's 0.0125 ml/l as opposed to 4th instar larvae's 0.025 ml/l by contact method. The LC_{50} value by feeding method for 2nd instar larvae was 0.05 ml/l while it was 0.1 ml/l for 4th instar larvae.

Table (1): Toxicity of *Anabaena flos-aquae* against second and fourth larval instars and corrected percentage mortality of *Helicoverpa armigera*.

Percentage mortality	Extracted algae concentration (Milliliter/liter)						LC 50	Slop
	0.2	0.1	0.05	0.025	0.012	0.00625		
Contact method 2nd instar	0	89	82	64	50	35	0.0125	6.48
Contact method 4th instar	0	79	59	50	19	14	0.025	7.7
Feeding method 2nd instar	74	59	50	34	24	20	0.05	13.21
Feeding method 4th instar	59	50	34	25	21	11	0.1	3.3

In both the second and fourth instar, treatment using the contact technique with the crude extract of the alga was more effective than the feeding technique, as the percentages of mortality larvae were higher (Table 1). The findings are in line with those

made by Aly and Abdou, 2010 and Rashwan and Hammad, 2020, who discovered that *Spirulina platensis*, a cyanobacterium, caused 100% death in *S. littoralis* larvae at a concentration of 5%. Also, it was discovered by Saleh *et al*., 1984 and Sharaby *et al.*, 1993, that the green *Scenedesmus acutus* was deadly toxic to *S. littoralis*. Additionally, according to Zaki and Gesraha (2001), algae had a toxic impact on several insect pests, including cutworms *Agrotis ipsilon*. Similar to this, Nassar *et al.* (1999) demonstrated that 4th larval instar of *S. littoralis* and *A. ipsilon* were acutely deadly toxicants of cyanobacteria (Blue-green algae). They noted that the LD_{50}s for *S. littoralis* and *A. ipsilon* were 7.59 and 9.10 g, respectively. A petroleum ether extract of the aquatic alga *Chara zeylanica* Klein ex Wild and its fractions (P1 to P19) was also found to have antimicrobial, ovicidal, and insecticidal effects against pests that affect cotton in Sarkar *et al.* (1995).

2. Biological parameters:

The data in Table (2) showed that the contact method used to treat the second larval instar of *H. armigera* with the crude extract of *A. flos-aquae* alga resulted in a highly significant increase in the larval duration was 26.99±2.9 days when compared to control. Additionally, the mean larval duration increased significantly was 22.8±3.8 days due to the feeding method compared to the control. The contact method used to treat the fourth instar did not affect larval duration because the average larval duration was 15.9 ± 1.9 days with the contact technique, versus 15.5 ± 1.1 days for the control, showing no difference in duration.

Table (2): *Anabaena flos-aquae* toxic activity at its LC_{50} levels against *Helicoverpa armigera* larvae in the 2nd and 4th instars.

***Helicoverpa armigera* stages**	**Feeding method 2nd instar**	**Contact method 2nd instar**	**Feeding method 4th instar**	**Contact method 4th instar**	**Control**
Larval duration (Days)	22.8±3.8**	26.99±2.9**	14.8±0.5**	15.9±1.9 n. s	15.5±1.1
Pupal duration (Days)	26.4±1.99**	28.9±0.6**	9.0±10**	12.3±2.5**	13.9±2.5
Malformed Pupation (%)	20	37	14	7	0
Normal Pupation (%)	80	63	86	93	100
Malformed adult emergence %	15	19	14	7	0
Normal adult emergence %	85	81	86	93	100

S.D.= Standard deviation, Malfo. = Malformation, * Significant (p<0.05), ** = Highly Significant n. s=non-Significant, L.S.D. = Least significant difference.

The pupal duration was considerably prolonged after the 2nd and 4th instar larvae were exposed to the crude extract of the alga using contact and feeding methods at LC_{50} values. The longest pupal duration observed in the second larval instar after the contact method was 28.9±0.6 days compared to the control. While feeding method in the second larval instar gave pupal duration an increase of 26.4±1.99 days. In the fourth larval instar, the feeding and contact method showed that pupal duration is negatively impacted and decreased compared to the control. These findings are consistent with those made by Nassar *et al.* (1999), who determined that cyanobacteria (Blue-green algae) are acutely fatal to the fourth larval instars of *S. littoralis* and *A. ipsilon*. They claimed that the LD_{50} significantly impacted both insect larvae and pupal life cycles. In addition, the green algae *Spirulina geitleri* was discovered by Salama and Sharaby (1980) to be less effective as a partial replacement for kidney beans in a diet for raising *S. littoralis*. The duration of the larva was considerably extended. This finding conflicts with that of Aly and Abdou (2010), who found that *S. platensis* (Cell content + cell wall) treatment of leaves during the 4th instar larval stage resulted in shorter larval and pupal lives than controls at

amounts of 0.5, 1, and 2.5%. They might result from variations in Spirulina subspecies and its processing (Nassar *et al*., 1999).

The data in Table (2) showed that, when the two methods investigated treated the 2nd and 4th instars larvae with the crude extract of the alga at their LC_{50} values, the pupation percentages were significantly reduced when compared to the control. When using the feeding and contact methods, respectively, the pupation rates at the second instar varied from 63 to 80%, as opposed to 100 % pupation at the check. By using the corresponding two methods, it varied between 86 and 93% at the fourth instar in comparison to the control 100 %. Contrarily, treatment of the 2nd and 4th instar larvae with the crude extract of the algae using the contact and feeding techniques at the LC_{50} level resulted in a significant decrease in the adult emergence percentages when compared to the control. However, the most suppressive one in the adult emergence was seen in the treated 2nd larval instar using the contact technique (A drop to an average of 81%, as opposed to 100% in the control). While using the feeding technique, adult emergence was reduced by 85% as compared to 100% of the control. Additionally, when 4th instar larvae were treated using contact or feeding techniques, the average adult emergence was reduced to 93 and 86%, respectively, as opposed to 100 % in the control group. This outcome was consistent with that of Aly and Abdou (2010), who found that *S. platensis* (Cell content + cell wall) at 5% concentration failed to cause the 4th instar larvae of *S. littoralis* to pupate when fed on leaves. Additionally, Abou-Tabl *et al.* (2002) discovered that the proportion of pupation was impacted in *S. littoralis* larvae treated with *Sargassum dentifolium*. The blue-green algae *S. geitleri* was less effective as a partial replacement for kidney beans in a diet for rearing *S. littoralis*, according to Salama and Sharaby (1980), and only 52–55% achieved the pupal stage as opposed to 80% on the kidney bean diet.

The data in Table (2) demonstrated morphogenetic effects at the LC_{50} values, feeding, and contact treatments of larvae of the second and fourth instars with the crude extract of alga caused a noticeably greater rise in pupal malformations than the control. The highest percentage of malformed pupa 37%, as opposed to 0% of the check, was induced in the second instar when using the contact method. Feeding methods caused 20%. The 4th instar larvae were treated by feeding and contact methods, which resulted in 14 and 7%, respectively, as opposed to 0% of the control. The greatest percentage of adult malformation emergence percentage was 19% induced in the second instar after contact treatment with the crude extract. In comparison to 0% at the control, the feeding technique induced 15%. In comparison to the control 0% group, treatment of the 4th instar using feeding and contact techniques resulted in 17 and 7%, respectively. These findings are consistent with those made by Antonious *et al.* (1992), who noted that *S. littoralis* larvae fed on food plant leaves treated with *D. maculata* and *A. vasica* plant extracts developed into pupae and adults with morphological aberrations, including pupae that retained larval thoracic legs and adults with abnormal abdomens, legs, and wings. The findings are also consistent with those made by Aly and Abdou (2010), who found that feeding *S. littoralis* larvae in their fourth instar leaves treated with *S. platensis* (Cell content + cell wall) at a dosage of 5% increased malformation.

Data in Table (3) showed that feeding or contact treatments of the 4th instar larvae of *H. armigera* with the crude extract of the alga significantly decreased adult fecundity. In comparison to the control group 480$\pm$40.1 eggs/female, the contact method-treated 4th instar larvae had the greatest decrease in adult fecundity 33.3+21.2. While the feeding

method reduced adult fecundity by 90±14.2 eggs/ female. Adult egg-hatchability at the contact method achieved 82.4%, as compared to the control, and treatment of the 4th instar also substantially reduced egg fertility. While the eggs hatching rate was reduced by 92.8% using the feeding method in comparison to the control. These findings are in line with those made by Salama and Sharaby (1980), who discovered that the blue-green algae *S. geitleri* was not as effective as kidney beans as a partial replacement in a diet for raising *S. littoralis* and that the total egg production was low. According to Sarkar *et al.* (1995), the P7a fraction made from the petroleum ether extract of the *Chara zeylanica* alga greatly decreased average fecundity and inflicted maximum sterility on the cotton pest. Additionally, Nassar *et al.* (1999) demonstrated that the cyanobacteria (Blue-green algae) treatment of the algal environment inhibited the oviposition of the survivor adults at the obtained LC_{50s} of the 4th larval instar of *S. littoralis* and *A. ipsilon*.

Table (3): Biological activity of *Anabaena flos-aquae* aquae at their LC_{50} levels against *Helicoverpa armigera* 4th instar larvae.

Helicoverpa armigera adult	Feeding method 4th instar	Contact method 4th instar	Control
Adult longevity (Days)	7.7±4.2	5.4±2.0**	8±4.2
Fecundity	90±14.2**	33.3±21.2**	480±40.1
Hatchability %	92.8	82.4	100

Data from Table (3) revealed adult longevity when the fourth larval instar of *H. armigera* was exposed to the crude extract of the alga by feeding or contact methods, adult longevity was significantly decreased to an average of 7.7±4.2 and 5.4±2.0 days, respectively, as opposed to 8±4.2 days in the control. These findings are consistent with those made by Nassar *et al.* (1999), who investigated the toxicity of cyanobacteria (Blue-green algae) against the 4th larval stages of *S. littoralis* and *A. ipsilon* and found that the LD50s significantly reduced adult lifespan in both insects.

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