The effect of Native *Spirulina platensis* on the Developmental Biology of *Spodoptera littoralis* (Boisd)

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**ABSTRACT**

*Spodoptera littoralis* is considered to be one of the most dangerous pests that cause a significant reduction in the yield of different economic crops in both open and greenhouse practices. The need for natural pesticides is one of the scientific research goals. The cyanobacterium, *Spirulina platensis* that proved to be a good source for different bioactive compounds, may be an excellent candidate as a natural pesticide against *Spodoptera littoralis*. *Spirulina* bloom that naturally grows in El-Khadr Lake in Wadi El-Natrun, Egypt was isolated and an axenic culture was obtained. The pure culture was identified as *Spirulina platensis*. The axenic culture was grown in liquid media and maintained at 24±1°C under 3000 Lux light intensity. The *Spirulina* culture yield reached 8.0±1 g wet weight/L after 15 days. Dried material of *Spirulina* culture cell content was obtained by ultrasonication and freeze-drying. Dipping solution was prepared using different concentrations of *Spirulina* culture cell content to test their effect on *Spodoptera littoralis*. The weight, percent mortality and malformation of larva and pupa were determined. Emergency and malformation of the moths were also observed. The cyanobacteria *Spirulina platensis* cell content showed no significant effect on *Spodoptera littoralis* larvae at concentrations of 0.5, 1.0, 2.5 mg cell content dry weight/L dipping solutions. However, significant effect was observed on both pupae and moths at these low concentrations. At 5% concentration larval mortality and malformation increased and 100% mortality was obtained.

**Key Words:** *Spirulina platensis*, *spodoptera littoralis*, cotton leafworm, green algae, cell content, cell wall.

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**INTRODUCTION**

*Spirulina platensis* is a blue-green algae found in warm water and alkaline volcanic lakes. Wild *Spirulina* sustains hunger flocks of filaments in the alkaline East African lakes. The calcium content in the microalga is relatively lower than the other ingredients. Some studies show that a calcium polysaccharide in *Spirulina*, called the Calcium *Spirulina* (Ca-SP), can inhibit the invasion and metastasis of tumor as well as the replication of enveloped virus. Therefore, researchers have paid much attention to the improvement of the calcium content in *Spirulina*.

*Spirulina* has a soft cell wall made of complex sugars and protein (Estrada et al. 2001). Recent studies have demonstrated that in the microalgae *Spirulina platensis* a blue protein called phycocyanin, belonging to the photosynthetic apparatus, has antioxidant and radical scavenging properties both in vivo and in vitro models (Benedetti et al. 2006).

Cotton is considered one of the most important economic crops in Egypt. It is subjected to attacks by many insect pests. The cotton leafworm, *Spodoptera littoralis* (Lepidoptera), could be fairly considered as one of the most important and harmful pests of cotton in south Europe, Africa and the Middle East (Hosny et al. 1986).

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, Angerillli and Beirne (1974) observed that a free floating unicellular *chlorella ellipsoidea* produced certain substances which were lethal to immature stages of mosquitoes. 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*; the larval duration was significantly prolonged and only 52-55% reached the pupal stage compared with 80% on the *Spirulina*-containing diet. Also the total egg production was low on the *Spirulina*-containing diet.
The aim of this work is to use Spirulina as a natural pesticide to control one of the most serious pests (spodoptera littoralis) in Egypt.

MATERIAL AND METHODS

1. Identification of *Spirulina platensis*:
*Spirulina platensis* used in this study has been isolated, characterized and identified by Aly (2000). Harvesting microalgal cells or filaments grown in natural habitats may be done by various methods such as:
- Filtration
- Centrifugation or Separation,
- Flocculation and floating
- Dehydration of microalgal slurry by:
  - Sun- drying.
  - Drum- drying.
  - Fluid- bed drying.
  - Spray drying.

2. Growth Conditions:
*Spirulina platensis* specific medium was used by Vonshack (1986) is composed of:

Solution 1 (g/l): NaHCO₃ 13.61, Na₂CO₃ 10 H₂O, K₂HPO₄ 0.50, completed to 500 distilled water.

Solution 2 (g/l): Na NO₃ 2.50, K₂SO₄ 1.00, Na Cl 3.00, Mg₂SO₄ 7H₂O 0.30, CaCl₂ 2H₂O 0.04, FeSO₄ 7H₂O 0.01, EDTA 0.08, microelement solution 5ml completed to 500 distilled water.

Solution 1 and 2 were autoclaved separately and mixed after cooling. The following microelement solution was added in a concentration of 5× 10⁻⁶ to the solution mixture.

Table 1: Microelement solution.

<table>
<thead>
<tr>
<th>Stock solution (g/L)</th>
<th>Applied solution (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnSO₄·7H₂O</td>
<td>0.1</td>
</tr>
<tr>
<td>MnSO₄·4H₂O</td>
<td>0.1</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>0.2</td>
</tr>
<tr>
<td>Co(NO₃)₂·6H₂O</td>
<td>0.02</td>
</tr>
<tr>
<td>Na₂MoO₄·2H₂O</td>
<td>0.02</td>
</tr>
<tr>
<td>CuSO₄·5H₂O</td>
<td>0.0005</td>
</tr>
<tr>
<td>Na₂SeO₃</td>
<td>0.0005</td>
</tr>
<tr>
<td>Distilled water</td>
<td>981</td>
</tr>
<tr>
<td>FeSO₄·7H₂O</td>
<td>0.01</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.8g</td>
</tr>
</tbody>
</table>

3. Purification of *Spirulina*
Cultures of *Spirulina* grown to an OD₄₈₀ of 1.0 to 1.5 were filtered through sterilized Whatman (Clifton. NJ,USA) 41 filter paper and washed three times with Zarrouk medium (Gòdia et al. 2002) whose composition is shown in the following Table.

Table 2: Zarrouk Medium.

<table>
<thead>
<tr>
<th>Stock solution (g/L)</th>
<th>Applied solution (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaHCO₃</td>
<td>18.0 g/L</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>2.5 g/L</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>0.5 g/L</td>
</tr>
<tr>
<td>K₂SO₄</td>
<td>1.0 g/L</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.0 g/L</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.04 g/L</td>
</tr>
<tr>
<td>Na₂EDTA</td>
<td>0.08 g/L</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>0.2 g/L</td>
</tr>
<tr>
<td>FeSO₄·7H₂O</td>
<td>0.1 g/L</td>
</tr>
</tbody>
</table>

The washed filaments were suspended in Zarrouk medium covered with aluminum foil and kept in the dark at 35°C for 2.5 h to this suspension, the following ingredients (final concentration) were added: Glucose (1%), peptone (0.5%), yeast extract (0.3%), NaCl (0.5%), ampicillin (100 µg/mL), cefoxitin (100 µg/mL) and imipenem (100 µg/mL). This culture was incubated at 35°C for 48h in the dark filtered and washed six times and suspended in Zarrouk medium. Algal filament and heterotrophic bacterial counts of this suspension were obtained.

The antibiotic treated cultures were diluted to five *Spirulina* filaments per mL and 0.2 mL of these diluted cultures was added to 3 mL of Zarrouk medium dispensed in 15 x 150-mm tubes. These cultures were incubated at 30°C and subjected to a 14-h light and 10-h dark cycle for 3 to 4 weeks. By which time *Spirulina* growth became apparent, according to Ferris (1991).

Culturing and drying process of *Spirulina platensis* biomass:
*Spirulina platensis* that was isolated from El-Khadra Lake at Wadi El-Natrun, Egypt was cultivated in 500 ml flask containing 300 ml Zarrouk medium. After 20 days, 250 ml flasks containing 100 ml sterile Zarrouk medium were inoculated with 1 ml of the mentioned 20 days old-*spirulina* culture under aseptic conditions. The cultures were again incubated at room temperature (22°C ± 2°C). The cultures were placed on the bench, exposed to light
through a window and additional fluctuating laboratory lighting for a simple mass production. After 3 months *Spirulina* suspension was filtered through 22.2 µm filter, washed with sterile distilled water and filtered biomass yield was re suspended in distilled water, frozen at – 80°C and then lyophilized. Each 100 ml culture gave yield about 0.3 g dry biomass.

**Production of Cell wall and Cell Content:**
0.5 g of washed sea sand was added to 10 ml of *Spirulina* culture and centrifuged at 4000 µ for 20 min then a few drops of ethanol were added and centrifuged again for 10 min. The sediment of the culture at the bottom is the inside cell and the top is the cell wall. Siphoning system is used to get the cell and the flowing washed and dried as the beginning of this method.

**Insect cultures and rearing:**
Laboratory conditions under which the colony cultures of insect experimented upon in this study was maintained at 27± 2°C and 60 -70% RH, the larvae of *S. littoralis* was laboratory-reared according to El-Ibrashy and Cheneuda (1970). The larvae then were starved for 4hrs before being tested.

**Preparation of test Solutions:**
Five g of *Spirulina* (cell wall) powder was dissolved in 100ml of distilled water to obtain 5% solution this was appropriately diluted with water to prepare 1%, 2.5% and 0.5% concentration. The same steps made with *Spirulina* (cell content).

**Effect on egg stage:**
The newly-laid eggs of the same batch size of eggs (500-800) were sprayed with different concentrations of *spirulina* (cell wall and cell content) and after dryness the egg mass were transferred to cups and incubated at 28±2°C until hatching. Each experiment was repeated 5 times.

**Insect treatment:**
Ten 4th instar larvae were left to feed for 24 hrs on discs of Castor oil plant leaves treated with different concentrations of the tested extract. After 24 hrs feeding, the larvae were kept in clean glass jars provided with fresh untreated Castor leaves. Daily records were taken for the larval weight, percentage of larval mortality, pupation and pupal malformation.

**Statistical analysis:**
The data were subjected to statistical analysis of variance for identifying significant differences among the treatments using standard method under MS Excel software. Significant test were carried out using Dunnett’s comparison method.

**RESULTS**

**Weight rate as influenced by *Spirulina* (cell content) treatment to larvae of the 4th instars:**
The treatment of larvae by (cell content) of *spirulina* are not significantly differ from low concentrations (0.5%,1%,2.5%) and control in weight except for the larvae that were fed on the 5% conc., they died in two days after treatment and their weight drops to 0.0435.

**Weight rate as influenced by *spirulina* (cell wall) treatment to larvae of the 4th instar:**
In case of *Spirulina* cell wall (Figure 2), the larval weight was highest (3.966g) at 0.5% conc., (0.4626 g) at 1%, (0.3995 g) at 2.5% and at 5% concentration cell wall showed drop in larval weight 0.3926 g.

**Duration rate as influenced by *spirulina* (cell content) treatment to larvae of the 4th instar:**
The mean duration of the larval instars was markedly shorted when treated with *spirulina* cell content (Figure 4). The larvae that were treated with 0.5% were 11±0 days and when treated with 1% and 2.5% were 7.75±0.16 and 6±0, respectively, but were 3±0 when treated with 5% concentration.

**The pupal duration rate as influenced by *spirulina* (cell content) treatment to larvae of the 4th instar:**
In the treatment with *spirulina* (cell wall) the larval duration also showed shorter duration period than control. In case of cell wall treatment, larvae that were treated  with 1% *Spirulina* cell wall were 8±0 days and when treated with 2.5% and 5% were 7.0±0.316 and 7.33+/-. 42 days, respectively.

**Duration rate as influenced by *spirulina* (cell wall) to larvae of the 4th instar:**
Pupal duration of pupae that resulted from the larvae treated with *spirulina* cell wall showed shortage in the pupal duration at 0.5%,1%,2.5% concentrations, but at 5% conc. larvae failed to pupate.

**Eggs hatchability as influenced by *spirulina* (cell wall) and (cell content) treatments:**
Study of the effect of *Spirulina* (cell wall and cell content) on egg hatchability (Table 1) revealed that no significant effect at 0.5, 1, 2.5% concentrations and light effect at 5% concentration.
DISCUSSION

**Spirulina as an insecticide:**

Algae are reported to have insecticidal effect on several insect groups like chrysoperla caranea (steph.) (Zaki and Gesraha 2001), house flies, cotton leafworm and rice weevil. Saleh et al. (1984) and Sharaby et al. (1993) found that the green Scenedesmus acutus have antifeedant and insecticidal activity against *S. littoralis*. Our work showed that *S. littoralis* lethally affected at high concentration 5% and the larvae were prevented to continue to the pupal instar in both cell content and cell wall treatments. Larval mortality could be due to many factors deterrent and repellent or toxic effect of algal extract, but low doses are not lethal to Spodoptera. Egg also, are not influenced by *Spirulina* cell wall and cell content at all concentrations.

The treatment with 5% conc. of *Spirulina* cell content and cell wall was significantly decrease larval and pupal weight.

When compared with control 0.393 at p<0.05, the effect of the treatment with other concentrations exhibit significant increase in the larval weight and pupal weight, but there

### Table 3: Effect of different concentrations of *Spirulina* (cell wall + cell content) on egg stage of Spodoptera littoralis.

<table>
<thead>
<tr>
<th>Concentration %</th>
<th>Number of tested eggs</th>
<th>Average hatchability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cell content</td>
</tr>
<tr>
<td>5</td>
<td>500-800</td>
<td>85.8</td>
</tr>
<tr>
<td>2.5</td>
<td>500-800</td>
<td>95</td>
</tr>
<tr>
<td>1</td>
<td>500-800</td>
<td>96</td>
</tr>
<tr>
<td>0.5</td>
<td>500-800</td>
<td>92</td>
</tr>
<tr>
<td>Control</td>
<td>500-800</td>
<td>99.4</td>
</tr>
</tbody>
</table>

Figure 1: Effect of spirulina (cell content) on average weight of *Spodotera littoralis* (larva).

Figure 2: Effect of spirulina (cell wall and cell content) on average weight of *Spodotera littoralis* (larva).

Figure 3: Effect of spirulina (cell content) on larval duration of *Spodoptera littoralis*.

Figure 4: Effect of Spirulina (cell wall) on larval duration of *spodoptera littoralis*.

Figure 5: Effect of spirulina (cell content) on pupal duration of *Spodoptera littoralis*.
were no significant difference between 1％ and 2.5％ conc. Both concentrations are not significant in cell wall and cell content treatments. The duration of larva at treatment with *Spirulina* cell content showed significant difference when compared with control, but there were no significant difference between 0.5％, 2.5％ and 1.0％ treatments.

**Larval response to different algal concentrations:**
A negative relationship between the concentration of *Spirulina* (in and out) and larval weight (Figure 3) was observed. This result correlates with Aboutabl et al (2002) who found that there is a positive relationship between the concentration of brown algae *Sargassum dentifolium* (Agerdh) and antifeedant activity was observed and contrast of that result get by Venkatesh Kumar et al. (2009). He found that *Spirulina* increase the weight of pupa and shell of Bombyx mori which produced by the treated larvae.

In case of duration in our study *Spirulina* (cell content + cell wall) shorted the larval life than control (Figure 5). This result is perfect to use *Spirulina* in the field it reduced the time that larvae spend in the plant so, it reduce the amount of damage which caused by eating the plant leaves. This result is contrasted with Salama and Sharaby (1980) who found that the larval duration was significantly prolonged when treated with *Spirulina* geitleri. This may be due to difference of subspecies of *Spirulina* and its preparation.

Duration of pupa showed prolongation in pupal period than control in case of *Spirulina* cell content and there is a negative relationship between concentration and pupal period until concentration reached 5％. All larvae failed to pupate and no pupa was obtained and the same results were obtained in case of *Spirulina* cell wall. This result may be due to presence of insect antifeeding derivatives from substance Like Azulene which present on brown algae Dictyota dichotoma, the crude mixture was insecticidal to house flies, cotton leaf worm. Saleh et al. (1984) and El-Baz et al. (1985) found some antimicrobial substances in green algae Chlorella and Scenedesmus.

The total percentages of mortality among the larvae of Spodoptera littoralis were increase with the increase of concentration in case of *Spirulina* cell content and cell wall this result was agreed with Aboutabl et al (2002), treatment of the larvae affected with the percentage of pupation.

Egg hatchability was not affected by spirulina both cell wall and cell content in all concentrations, these results contrast with Aboutabl et al. (2002). This may be due to the aquas solutions of spirulina not dissolve certain materials that petroleum ether can dissolve.

From the previous findings, it can be concluded that application of 5％ concentration of water solution of the green algae, *Spirulina platensis*, can protect the host plants from being attacked by *S. littoralis* with the other means of integrated pest management.

**REFERENCES**


