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Assessment of Cyanobacteria as Biocontrol Agents against the Fall Armyworm, *(Spodoptera frugiperda)*

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ABSTRACT

The pests' growing resistance to chemical pesticides necessitates the search for alternative pest management strategies. The toxicity of cyanobacterial microalgal strains *Nostoc muscorum, Arthrospira*, *Anabanea fertilissima* and *Anabanea laxa* were examined in in vitro assay as a bio pesticide against 4th instars of fall armyworm (*Spodoptera frugiperda)* Larvae fed on leaf discs of castor leaf oil painted with several concentrations of algal solution. The mortality percentage of treated 4th instar larvae fed on the 4 algal treatments shows that *Arthrospira* was the most potent, exhibiting the highest toxicity against the 4th larval instars. Where, its LC50 value was 83.5 ppm while, *Anabanea laxa* was the least one, its LC50 value was 380.2ppm, the moderate effect 177.8ppm and 243.4ppm for *Arthrospira* and *Anabanea fertilissima* respectively all algal treatments show observed effect on carbohydrates hydrolyzing enzymes and biochemical aspects of the pest it was noticed that noticeable decrease in enzymatic activity of invertase, amylase, and trehalase also there was a great disturbance in total carbohydrate, total protein, and total lipid content of larvae which indicate the efficacy of microalgal strains of cyanobacteria as bio pesticide for control fall armyworm.

INTRODUCTION

The fall armyworm, *Spodoptera frugiperda*, is a significant pest that causes substantial damage to crops worldwide, particularly in the Americas and Africa (Sagar *et al*., 2020). Traditional pest control often relies on chemical pesticides, which pose risks to ecosystems, human health, and non-target organisms (Tudi *et al*., 2021). Adult *Spodoptera frugiperda* are nocturnally active, engaging in mating and dispersing to new regions (Togola *et al.,* 2025).

Research indicates that within a single generation, fall armyworm (*S. frugiperda*) adults can migrate over 500 km before reaching reproductive maturity (Asimakis *et al* .,2022). Both males and females exhibit this long-distance movement.

In recent years, researchers have explored alternative approaches to pest management, including the use of green algae (Ingle *et al*., 2018).

Green algae have been shown to possess insecticidal properties, making them a promising tool for biopesticide development (Aioub *et al.,* 2024). Studies have demonstrated that certain species of green algae can inhibit the survival and progression of various insect pests, including the fall armyworm (Gonçalves *et al.,* 2021). For example, a study published in the Journal of Functional Foods in Health and Disease found that extracts from the green alga Chlorella vulgaris exhibited significant insecticidal activity against fall armyworm larvae (Mohammad *et al.,* 2024).

The use of green algae as biopesticides offers several advantages, including reduced environmental impact, biodegradability, and potential for targeted control (Mol *et al.,* 2020). Further research studies are required to optimize the utilization of green algae for fall armyworm control and to understand the underlying mechanisms of action.

The bioactive compounds present in green algae, such as alkaloids, terpenes, and phenolics, are thought to contribute to their insecticidal properties (Casanova *et al.,*2023). These compounds can interact with insect physiological systems, disrupting normal functioning and ultimately leading to mortality (Petchidurai *et al.,* 2023*)*. The use of green algae as biopesticides offers several advantages, including reduced environmental impact, biodegradability, and potential for targeted control (Costa *et al.,* 2019*).* Further investigation is required to enhance the application of green algae for fall armyworm control, including the identification of the most effective algal species and extracts, as well as the development of efficient delivery methods (Singh *et al .,*2022*).* Additionally, understanding the underlying mechanisms of action and potential interactions with other pest management strategies will be crucial for the successful integration of green algae-based biopesticides into agricultural practices.

The methanol extract derived from the seaweed *Ulva lactuca* demonstrated significant pest-killing and antifungal effects. These bioactive properties appear linked to its rich composition of sesquiterpenes and fatty acids, which are well-documented for their antifungal capabilities (Abbassy *et al.,* 2014).

MATERIALS AND METHODS

1. Insect Collection and Rearing:

Spodoptera frugiperda (fall armyworm) specimens, originally collected from multiple locations in Bani-Suef, were maintained in a controlled lab environment (28 ± 2°C, 60 ± 5% RH) for three consecutive generations. Following the protocol established by (Kruger *et al.,* 2012), larvae were reared on fresh *Ricinus communis* (castor oil plant) leaves inside large glass containers until pupation. Upon emerging as adults, moths were transferred to mating chambers glass jars containing sugar-soaked cotton (10% solution) for nourishment and castor leaves for egg deposition. The resulting egg clusters were kept in plastic containers until larvae hatched (Kruger *et al.,* 2012).

2. Microalgae and Growth Medium:

Nostoc muscorum, Arthrospira, Anabanea fertilissima and *Anabanea laxa* are a cyanobacteria strains frequently found in rice fields across Egypt's northern delta region. An axenic culture of these strains was acquired from the Agricultural Microbiology Research Department's cyanobacteria collection, part of the Soils, Water, and Environment Research Institute at the Agricultural Research Center in Giza, Egypt. The strain was cultivated in nitrogen-free B.G11$_0$ medium, following the formulation described by (Allen *et al.,* 1968). Cultivation was carried out in 250 ml Erlenmeyer flasks at a temperature of 28.0±2°C under continuous light exposure at an intensity of 2500 lux.

3. Growth Conditions:

The cyanobacterial strain was grown in 5 L Erlenmeyer bottles, containing 3 L of

nitrogen-free B.G11$_0$ medium, inoculated with 30 ml of exponentially pre-cultured isolates. Mixotrophic conditions were established by supplementing the medium with 1% (w/v) glucose. Cultures were aerated with ambient air at a steady flow rate of 200 ml/min (50 Hz), using rubber-stoppered flasks fitted with a narrow glass outlet tube. The setup relied on atmospheric CO_2 (0.03%).

4. Preparation of Algal Solution:

Upon reaching the stationary growth phase, the cultivated microalgae were harvested, filtered, and dehydrated under ambient conditions. The dried biomass was mechanically pulverized to produce a fine algal powder. Serial dilutions of this powder from four microalgal species were prepared in 99% ethanol, yielding nine test concentrations (2000, 1500, 1000, 500, 250, 125, 62.25, 31.25 and 15.5 mg/ml). An algal-free ethanol solution served as the experimental control.

5. Bioactivity Tests:

The larvicidal activity of the algal extracts was evaluated through leaf disc bioassay (Rani *et al*., 2009). Fresh *Ricinus communis* leaves were sectioned into 9 cm^2 discs using a sharp cutter. Control discs were coated uniformly on both surfaces with ethanol alone, while treatment discs received equal applications of *Nostoc muscorum*, *Arthrospira*, *Anabanea laxa*, or *A. fertilissima* extracts at concentrations of 2000-15.5 mg/ml/cm^2. All discs were air-dried and allowed to larvae for feeding daily along the experiment period till pupation.

6. Analysis of Carbohydrate Hydrolyzing Enzymes Activities:

The activities of three carbohydrate-hydrolyzing enzymes amylase, invertase, and trehalase were assessed. This study aimed to evaluate the impact of the tested materials on the digestive system's physiological functions in affected larvae. The enzymatic activity was measured following the procedure of (Ishaaya *et al.,* 1976). which involved quantifying glucose produced from starch and sucrose digestion using 3, 5-dinitrosalicylic acid reagent. Specific reaction mixtures were prepared for each enzyme, and the enzymatic activity was expressed as mg glucose released/min/g body weight

7. Processing Tissue Homogenates for Biochemical Analysis:

Whole larvae were homogenized in chilled insect physiological saline (0.5 g tissue in 5 mL saline) using a Teflon homogenizer, with samples immediately placed on ice. The homogenate was centrifuged at 4500 rpm for 5 min at 4°C to pellet cellular debris. The resulting supernatant was aliquoted (0.5 mL portions) and preserved at -20°C for subsequent biochemical assays.

8. Determination of Total Lipid Content:

The homogenate's total lipid content was measured utilizing the phosphovanillin assay as described by (Baronos *et al.,* 1973). with results reported mg/ml.

9. Determination of Total Carbohydrate Content:

The insect homogenate's total carbohydrate content was measured following (Singh *et al.,* 1977). With results expressed as mg/ml.

10. Determination of Total Protein Content:

The homogenate's protein content was measured utilizing the folin phenol reagent (Peterson *et al.,* 1977). With results expressed as mg/m.

Statistical Analysis:

After 24 hours, the mortality rates of 4th instar larvae exposed to the four algal treatments at varying concentrations were assessed and adjusted using Abbott's formula (Abbott *et al*., 1925). Probit analysis (Finney *et al*., 1971). Was employed to calculate LC_{50} values for each treatment, and the impact of these concentrations on the biological functions of surviving larvae was evaluated. Statistical analysis, including P-values and LSD (least significant difference) at a 0.05 significance level, was performed using Microsoft Excel.

RESULTS AND DISCUSSION

1. Insecticidal Activity:

Table 1, demonstrates the insecticidal activity of four cyanobacterial species (*Nostoc muscorum, Arthrospira, Anabanea laxa, A. fertilissima*) *against* the 4th larval instars of. *S. frugiperda.* Larvae were fed leaf discs treated with each algal extract. Among the tested species, *Nostoc muscorum* demonstrated the highest toxicity against the 4th larval instars. The LC_{50}values were 83.5 ppm, While, *Arthrospira* was the second effect, with LC_{50}values were 177.8 ppm, whereas *A. fertilissima* was the third effect, with LC_{50} values 243.4 ppm, While, *Anabaena laxa* was the least one, its LC_{50} values were 380.2ppm. These results agree (Saber *et al.,* 2018) who demonstrated that cotton leafworm treated with algal and cyanobacterial extract showed pest mortality (Elela *et al.,* 2024). Reported that the treatment of second-instar larvae of *A. ipsilon* with *N. muscorum* and *A. flos aquae* had highly insecticidal properties.

Not only did chemical pesticides cause mortality for all S. *littoralis populations* but also *Parachorella kessleri* and *N. carneum* exhibited significant toxicity against, *S. littoralis* (Eldessouky *et al.,* 2024). According to (Rawi *et al.,* 2011a) 7 % ethanol extract of *S. platensis* has the highest larvicidal activity against *S. littoralis*.

Lantana camara essential oil, fractionated using three different solvents, demonstrated direct repellent and toxic effects against maize grain weevils. The insect mortality and repellency were primarily attributed to its bioactive and phytochemical constituents (Ayalew *et al.,* 2020**).**

Table 1: Toxic effects of the four microalgae, *N. muscorum, A., A. laxa, A. fertilissima against* the 4th larval instars of *S. frugiperda.*

Treatment	*Spodoptera frugiperda Larvae* 4nd instar			
	LC50 values PPm	Slope function	95% confidence limit	
			Upper	Lower
Nostoc muscorum	83.5	0.78	627	11.0
Spirolina	177.8	0.86	603	52.5
Anabanea fertilissima	243.4	1.89	683.0	80.4
Anabaena laxa	380.2	1.10	1230.0	117.5

2. Enzymatic Activity:

The biochemical analysis of enzymatic activity in *Spodoptera frugiperda* following treatment with various cyanobacterial species revealed notable differences in digestive enzyme levels relative to the control group. The impacts of *Nostoc muscorum, Arthrospira, Anabanea fertilissima* and *A. laxa* on the carbohydrate-digesting enzymes of the 4th instar larvae of *S. frugiperda* are listed in Table (2). Exposure to the LC50 of each treatment significantly influenced the digestive enzymatic activity of *S. frugiperda*. The control group exhibited the highest invertase activity (55.7 ± 1.71 µg glucose/min/g), while treatment with *A. fertilissima* showed moderately high activity (51.3 ± 0.75), In contrast, *N. muscorum* , *Arthrospira and A. laxa* exhibited significantly lower activity (33.9 ± 0.96 and 37.9 ± 0.70 and 42.6 ± 0.98 respectively), indicating potential suppression of sucrose hydrolysis. This suggests that certain cyanobacterial compounds may disrupt carbohydrate metabolism, potentially impairing energy availability. On the other hand Amylase activity followed a similar pattern, with control insects showing 34.8 ± 1.29 µg glucose/min/g. The highest activity among treated groups was seen with *Anabaena fertilissima* (35.0 ± 0.68), close to the control, indicating a mild effect. *Anabaena laxa* and *Nostoc muscorum* significantly

suppressed amylase activity (29.6 ± 0.72 and 30.4 ± 0.32, respectively), which may impair starch digestion in larvae. While the tested Trehalase shows highest enzyme activity (54.3 ± 0.90), essential for trehalose metabolism (a major insect blood sugar). Treatments with cyanobacteria significantly reduced this activity, especially in *Arthrospira* (34.4 ± 0.53) and *N. muscorum* (38.2 ± 0.70). Only *A. laxa* maintained a relatively high activity (41.7 ± 0.46), suggesting less impact on trehalose regulation.

Sharanappa *et al* (2024), reported that the *n*-Hexane extracts of *Epaltes divaricata*(NH-EDx) demonstrated notable insecticidal effects against *Aedes aegypti*, a dengue vector, and *Spodoptera litura*. The extract exhibited larvicidal properties and, upon analysis of enzymatic responses, a decline in both α- and β-carboxylesterase activity was observed in the two insect species. In contrast, there was a marked increase in GST and CYP450 levels. Additionally, midgut tissue analysis revealed strong larvicidal effects, growth inhibition, and suppression of key metabolic enzymes.

Table 2: The effects of *N. muscorum, Arthrospira, A. fertilissima* and *A. laxa* on the carbohydrates digestive enzymes of the *S. frugiperda* fourth instar larvae.

Enzymes	**Treatments compound**				
	Nostoc muscorum	***Arthrospira***	***Anabanea fertilissima***	***Anabanea laxa***	**Control**
Invertase (mg glucose released /min/b.w)	33.9±**0.6**	37.9±**0.70**	51.3±**0.75**	42.6±**0.98**	**55.7 ± 1.71**
Amylase (mg glucose released /min/b.w)	30.4±**0.32**	33.9±**0.70**	35.0±**0.68**	29.6±**0.72**	34.8±**1.29**
Trehalase (mg glucose released /min/b.w)	38.2±**0.70**	34.4±**0.53**	40.7±**0.58**	41.7±**0.46**	54.3±**0.90**

3. The Effects of *Nostoc muscorum Arthrospira, Anabanea fertilissima* and *Anabanea laxa* on Key Insect Metabolites:

Data in Table (3), showed the effects of *N. muscorum Arthrospira, A. fertilissima and A. laxa* on critical biochemical constituents (total protein, total carbohydrate and total lipid) after treatment of the 4th instar larvae of *S. frugiperda* for six generations. All tested treatments resulted in a highly significant reduction in total carbohydrate levels, with recorded values of 40.7, 54.3, 61.2, and 64.2 mg/g tissue, respectively, compared to 71.7 for the control. Similarly, treating 4th-instar larvae of *S. frugiperda* with the four tested green algae for six generations, significantly decreased the total protein content, with Mean values of 40.7, 54.3, 61.2, and 64.2, respectively, as compared to 54 for control. The four tested compounds led to a markedly significant decrease in lipid contents were 27.1, 33.5, 41.9, and 44.6 as compared to 46 % of control.

Lipids, total proteins, and carbohydrates are the main constituents vital for pest to develop, grow, and carry out its essential roles. Any defect in the main constituent synthesis will affect all the pest life cycles.

The insect's sensitivity to insecticides is attributed to Alterations in energy stores, including carbohydrates, lipids, and proteins, which lead to variations in its function (Ibraheem *et al* 2002). In agreement results were obtained by. Spinosad treatments significantly reduced the total protein and carbohydrate levels in the *S. littoralis* 6th-instar larvae (Piri *et al.,* 2014). In addition, the total protein level significantly decreased in imidacloprid-treated larvae of *S. littoralis* when compared with control (El-Sheikh, 2012). In addition, the lower protein level may result from the degradation of protein into amino acids, entering the tricarboxylic acid (TCA) cycle as keto acid derivatives to support the insect's energy production. This tissue protein depletion appears to be a physiological

adaptation, potentially functioning as a compensatory response under insecticidal stress by maintaining free amino acid levels in the hemolymph, thereby supplying essential intermediates for the TCA cycle (Tawfik *et al SSRN 4429152).* A mechanical formation of l organs could be the cause of the decrease in protein content (Nath *et al* 1977, Bhavan *et al*., 2001). Also, the reduction of protein level might be due to the destructive effect on some of the cerebral neurosecretory cells of the brain responsible for secretion of the protein of the treated larval instars of *S. littoralis* (Mosleh *et al*., 2003) who proved that a neurotoxic effect had occur due to spintoram *S. littoralis* treatments has manifested as defined in histopathological changes in nerve and neurosecretory cells of *S. littoralis*. Lipids are an important structural component of the cell membrane and cuticle. They supplied a rich source of metabolic energy. The obtained results declare that the four tested insecticides caused a highly significant decrease in lipid contents as compared with susceptible strain. The breakdown of lipids into simpler moieties that could be utilized as a carbon source for growth may be the cause of the significant decrease in total lipids. According to (Hamouda *et al*., 2008). Infected larvae may generate an enzyme that uses lipids as a source of energy. Fatty acids such as oleic, stearic, and linoleic acids demonstrated high toxicity against Earias insulana (Boisd.), with all treatments at their LC50 levels significantly prolonging both larval and pupal durations **(**Bennett *et al*., 1972). Additionally, these treatments led to a reduction in body weight and altered the insect's lipid, carbohydrate, and protein contents.
That crude extracts of *Spirulina platensis* contain hydrophobic and hydrophilic bioactive compounds that show antibacterial activity against (*Staphylococcus aureus, Salmonella typhimurium*) by affecting their cell membrane (Moustsfs *et al*.,2018).

Table 3: The effects of *N. muscorum Arthrospira, A. fertilissima* and *A. laxa* on the carbohydrates digestive enzymes on the fourth instar larvae of *S. frugiperda*

The biochemical components	**Treatments compound**				
	Nostoc muscorum	***Arthrospira***	***Anabanea fertilissima***	***Anabanea laxa***	**Control**
Total carbohydrate mg glucose /larva	40.70±0.55	54.3±0.95	61.2±0.96	64.7±1.24	71.73±0.75
Total protein mg/larva	36.1±1.16	53.6±1.33	45.2±1.02	44.4±0.96	54.0±0.98
Total lipid mg oleic/larva	27.4±1.32	33.5±1.11	41.9±0.70	44.0±1.04	46.0±1.11

Conclusion

Cyanobacterial microalgal strains *Nostoc muscorum, Arthrospira, Anabanea fertilissima* and *Anabanea laxa* have had great larvicidal effect on fall armyworm, all algal treatments show highly effect on insect biology and life cycle more over the data revealed that cyanobacteria effect the pest body and constituent as total carbohydrate, total protein and total lipids also all treatments cause disturbance in carbohydrate digestive enzymes which assure the efficiency of cynobacterial strains in management of fall army worm *S. frugiperda*

Declarations

Ethical Approval: Not applicable.

Competing Interests: The authors declare that they have no competing interests.

Authors' Contributions: AMS, RAS, and AMM did the conceptualization. AMS, and AMM contributed to the formal analysis. AMS, and AMM took part in the investigation.

AMS wrote the original draft. AMM did the writing–review and approved the final manuscript. All authors read and approved the final manuscript.

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Availability of Data and Materials: All datasets analyzed and described during the present study are available.

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