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Biocidal Effect of Some Algae Against the Cotton Leafworm *Spodoptera littoralis* Under Laboratory Conditions

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ABSTRACT

Traditional agricultural practices, such as the use of synthetic pesticides, lead to numerous problems including environmental contamination, generating pest resistance and harmful effects on non-target organisms. Consequently, these negative effects have emphasized the need to develop new efficient safe and eco-friendly natural alternatives to synthetic insecticides. Algal products play a significant role as chemical substitutes in agricultural applications. The current study to bioassay biocide effects of ten commercial algal products as an natural and safe bio-pesticides on 4th larval instar of cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). A high level of corrected mortality was found for the algal product (Biocontra formulation) through exposure period of 96 hours under laboratory conditions. The toxicity against 4th larval instar of cotton leafworm insect increased as exposure time and concentrations increasing. Biocontra formulation caused comparatively the highest potential toxic activities >90% mortality and achieved the same value (92.5%) after 72 and 96 hours. exposure time. The highest mortality of Biocontra (80.0, 82.5 and 93.0%) were recorded with the highest concentration (10 mg\ml) after 48, 72 and 96 hours exposure times, respectively compared to control treatments. The least mortality percentages were observed with other concentrations 2, 4, 6 and 8 mg\ml after 48, 72 and 96 hours from exposure. Biocontra formulation was found to be the most toxic with LC_{50}= 27.8 mg/ml to 4th larval instar of cotton leafworm after 96 hours from exposure. Results of treated larvae with LC_{50} of Biocontra formulation indicated that, the larval duration, female life span, fecundity and fertility are significantly affected by algae treatment. Moreover, protein content, Chitinase, β-esterase and GST significantly decreased compared to untreated larvae. Generally, algae are an important in agriculture system that they are been as biocontrol agent in the integrated crop managements, especially under modern climatic changes.

Keywords: Cotton leafworm, *Spodoptera littoralis*, Commercial algae products, Biological features, Protein content, Chitinase, β-esterase and GST enzymes.

1. Introduction

One of Egypt's most destructive insect pests is the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) (Kandil *et al.,* 2003). The larval stages are very destructive, causing economic losses in many crops (Hosny *et al.,* 1986). Although, chemical insecticides are used as an effective tool to combat and prevent serious harmful impacts that were done by insect, their use has harmful effects on the insect resistance, human health and environmental balance (Dahi *et al.,* 2017). Globally, there were 954 pest species that had effects on pest resistance (Tabashnik *et al.,* 2014). Thus, it should create alternative methods (El-Naggar and Jehan, 2013).

Using of marine algae is one of the most promising methods of pest control without using insecticides. Algae have the ability to reduce crop pests by acting as pesticides (Asimakis *et al*., 2022). It could serve as a biopesticide (Cheung *et al*., 2014). Moreover, Asimakis *et al*., (2022) pointed to the increasing human numbers must be necessary to produce more food. Algae produce substances that may serve humans in different biotechnological areas. The potential of algal

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metabolites had bio-pesticides. Biotoxins are a crucial class of crop protectants since they often have fewer residual effects than conventional pesticides and are safer for people and the environment. (Copping and Menn, 2000). Algae showed some aquatic faunal component-inhibiting effects, but there aren't many reports on the algae's insecticidal capabilities. Hapalalindoles' bioactivity produce toxic compounds that may be helpful for dipteran biocontrol (Becher *et. al*., 2007). Cyanobacteria produce some metabolites that exhibiting diverse bioactivities (Wiegand and Pflugmacher, 2005). The cyanobacterium aquae that gave insecticidal effects on lepidopteran insects and had polysaccharides which were gave biological properties against some pests (Philippe, 2018).

Proteins have been associated to population dynamics, life histories, and even biological diversification at higher levels of organization. They are essential for individual-level witness associated features including body size, growth rate, and fecundity (Abd El-Kareem *et al.,* 2022).

Chitin, carbohydrates, and protein make up the majority of the chemical components of insect epidermis. Chitin, which makes up to 20–50% of the weight of the insect stratum corneum, is the main scaffold component among them. In order to degrade old epidermis, chitinase enzyme is necessary (Chapman, 2013).

The wide family of enzymes known as glutathione transferases (GSTs) is present in all aerobic organisms. They are essential for both endogenous and exogenous chemical detoxification, as well as intracellular transport, hormone production, and defense against oxidative stress. In insects, GSTs have a very important role in insecticide resistance. Insects contain numerous esterase enzymes with differing substrate spectra. It is a fact that developments of more active hydrolytic detoxification systems by resistant insects are the most probable explanation of resistance (Casida, 1958 and (Oppenoorth and Van-Asperen, 1960).

Esterases are necessary in the detoxification of insecticides in all insect and arthropod species; they hydrolyze enzymes that break down ester molecules when water is added, producing alcohol and acids (Rashwan, 2013). The detoxifying enzymes, which include the esterase enzymes, are responsible for detoxifying any foreign substances from an insect's body (Abd El-Kareem *et al.*, 2022).

The objective of this study to evaluate the toxicity of some algae formulations and its effects on some biological features and biochemical components against 4th instar larvae of *S. littoralis*.

2. Materials and Methods

2.1. Insects rearing technique

Freshly collected egg masses supplied by the culture of the laboratory in the Plant Protection Department, Desert Research Centre, Cairo, Egypt formed the basis of the culture planned to provide cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), for use in the current study. To be provided daily, the larvae were reared on caster bean leaves (*Ricinus communis*). The formed pupae were collected and placed in clean jars with moist saw dust placed at the base to provide the pupation site. Adults were provided with 10% sugar solution. They were left to deposit their egg-mass on the lower surfaces of oleander leaves. The deposited egg-masses were collected daily and left till hatching. The newly hatched egg-masses were transferred to fresh castor leaves. All stages of *S. littoralis* were cultured and tested at 26±2°C and 70± 5 % R.H (Shalaby *et al*., 2013 and Sallam, 2008 & 2017).

2.2. Algae formulations sources

Ten commercial algae formulations were investigated under laboratory conditions to select the most effective samples on the biotic potential of *S. littoralis*. The commercial algae that used were shown in Table (1) as the following:

Table 1: Different algae formulations

	Name	Case	Source
1	**Nemabiokey**	Liquid	Egyptian Algae Technology Company
2	**Biogreen Power**	Liquid	
3	**Biocontra**	Liquid	
4	**Power Set**	Liquid	
5	**Biosizer**	Liquid	

6	**Freesaline**	Liquid	
7	**Veggie**	Powder	Anatour Egypt Company
8	**Spirulina**	Powder	Traditional product
9	**Stimu Grow 600**	Powder	Leili Marine Biohoustry inc.
10	**G6**	Liquid	Green Power company

2.3. Bioassay of algae treatments

The leaf-dipping technique was the method used to assay the algae treatment against larvae of *S. littoralis*, the 4th instar (Ali *et al*., 2017; Rashwan and Hammad, 2020 and Ali *et al*., 2021). To determine the potential of different algae formulations on larvae of cotton leafworm, the 4th larval instar was fed on fresh castor leaves that were dipped in concentrations from different algae treatments (ten formulations). Algal formulations were carried out by soaking the fresh and clean castor leaves in each algae treatments for one minute and drying in air at room temperature. Feeding on the treated castor leaves was carried out for 48 hrs.. Thereafter, larvae fed on normal fresh castor leaves. Each treatment was replicated four times using ten larvae for each replicate as well as control. After that, the mortality of counts was recorded. Mortality percentages were calculated after 48, 72 and 96 hours of treatment. Algae formulations were preliminarily assayed against cotton leafworm for their toxicity. The formulations that gave potential results in these preliminary tests were subjected to use as serial concentrations as follows 2, 4, 6, 8 and 10 mg/ml. All the LC_{50} values of the tested treatments were calculated as mg/ml. The LC_{50} values were calculated with the technique of Finney, (1971). Depending on LC_{50} values, the most effective algae formulations were determined for biological and biochemical tests.

2.4. Biological effects

LC_{50} of the most effective treatment was applied on the 4th instar larvae of *S. littoralis*. Insects resulting from treatment were maintained under constant temperature (25±2°C) and relative humidity (75±5%R.H.). Larval and pupal duration, adult longevity, number of eggs per female, percent of hatchability and incubation period were calculated (Sallam, 2017, and Rashwan and Hammad, 2020).

2.5. Biochemical effects

Larvae samples were taken after 48, 72 and 96 hours of 4th instar larvae of *S. littoralis* that was the most effective treated by algae formulations, for the biochemical tests.

2.5.1. Determination of total protein content

The total soluble proteins of treated and untreated larvae were estimated using a standard of Bovine serum albumin according to the method described by Bradford, (1976).

2.5.2. Determination of Chitinase activity

Chitinase was assayed using 3,5-dinitrosalicylic acid reagent to determine the free aldehydic groups of hexosamine liberated on chitin digestion according to the method described by Ishaaya and Casida, (1974).

2.5.3. Determination of Glutathione S- transferases (GST) activities

The activity of Glutathione S transeferase was determined according to the method described by Habig *et al*., (1974) using 1-chloro- 2,4- dinitrobenzene (CDNB) as a substrate.

2.5.4. Determination of beta-Esterases activities:

Beta esterases (β E) activities were determined according to the method of Van, (1962) using β -naphthyl acetate as a substrate.

The increasing or decreasing in the enzymes activities were calculated as follow:

$$\% \text{ Increase or decrease than control} = \frac{\text{Treated - Control}}{\text{Control}} \times 100$$

2.6. Statistical analysis

2.6.1. Bio-Statistical analysis

The statistical analysis of data on mortality was subjected to the Abbott formula (Abbott, 1925) for correction wherever required. Probit analysis was determined to calculate LC_{50} (Finney, 1971), through software computer program by using Ldp line software according to Baker, (2000). The Homogeneity ratio was determined by LC_{90}/LC_{50} to show the ability of algae formulation (Gamil, 2012; Rasheed *et al*., 2015 and 2016).

2.6.2. Statistical analysis

The data set of studied traits was collected and subjected to univariate statistical analysis as a one way ANOVA according to (Gomez and Gomez, 1984). LSD test as post hoc test to compare the treatment for significant difference was used.

3. Results and Discussion

3.1.1. Bio efficacy of some algae formulations against *S. littoralis*

3.1.1.1. Toxic effects evaluation of some algae formulations (Primary test)

The data given in Table (2) showed all algae formulations caused different mortalities percentage against the 4th larval instar of *S. littoralis* after 96 hours treatment. The toxicity against the 4th larval instar insect increased as exposure time after treatment increased. Biocontra formulation caused comparatively the highest potential toxic activities more than 90% mortality and recorded 92.5% after 72 and 96 hours from exposure time. It was observed that Stimu Grow 600 and G6 formulations caused the median mortality percentage with 55.0 and 50.0% for 4th instar larvae after 96 hrs from treatment exposure time. The algal formulations (Nemabiokey, Biosizer, Biogreen Power, Freesaline, Biosizer, Power Set and Spirulina) caused mortality ranging from 60-73% and were considered over moderately virulent to the 4th larval instar of cotton leafworm after the same time (96 hrs). The Veggie formulation resulted in < 15% mortality percentage and was given poorly virulent.

Table 2: Toxicity of algae formulations against 4th larval instar of cotton leafworm *S. littoralis* under laboratory conditions.

Formulations (mg\ml)	Mortality (%)		
	Exposure time (hours)		
	48	72	96
Spirulina	20.0	30.0	60.0
Biosizer	13.3	36.6	66.3
Freesaline	10.0	53.3	66.0
Power Set	46.6	50.5	65.0
G6	26.6	46.6	50.0
Biogreen Power	20.0	63.3	67.0
Stimu Grow 600	35.0	47.5	55.0
Biocontra	47.5	92.5	92.5
Nemabiokey	16.6	33.3	73.3
Veggie	7.5	7.5	12.5

Our findings were closest to those obtained by Aly and Abdou, (2010) who found that cyanobacterium, *Spirulina platensis* gave 100% mortality of cotton leafworm preadult at 5% water extract concentration. In recent studies, Asimakis *et al*., (2022) provided that algae and extracts display as biopesticides properties against crop pests. This observation is agreement with the data of Saber *et al*., (2018) who found all the crude ethanolic extracts of the coccoid green alga Parachlorella kessleri and the *heterocytous cyanobacterium* exhibited toxic and effective activity against the different stages (2nd and 4th larval instars) of the cotton leafworm *S. littoralis*, while *Chara vulgaris* specimens had the lowest insecticidal effect. Priya *et al*., (2022) also pointed out that the toxic effects of crude aqueous and methanol extracts of brown *Macroalgae, Padina tetrastromatica, Sargassum wightii and Turbinaria conoides* had potential effects on the second instar larvae in different concentrations. Manilal *et al*., (2012) and Vieira *et al*., (2017) claimed that the brown *macroalga Lobophora variegata's* methanolic extract may have substantial synergistic effects with fatty acids like -linolenic, oleic, myristic, and hexadecatrienoic acids. Moreover, Delaney and wilkins, (1995)

pointed to the efficacy of the *cyanobacterial cyclic* metabolite hepatotoxin 'microcystin-LR' as a potent insecticide against the third instars of the cotton leafworm and confirmed that it yielded 24 h LD_{50} values of 4.7 and 13.1 $mg.kg^{-1}$, respectively. Several studies were exhibited that different strains of algae have biopesticidal activity on *S. littoralis* and some economic pests, such as blue-green alga anabaena flosaquae (Abdel-Rahim and Hamed, 2013), brown seaweed *Sargassum dentifolium* (Aboutabl *et al*., 2002 and Matloub *et al*., 2012), *Hheterocytous cyanobacterium* Nostoc strain ATCC 53789, (Biondi *et al*., 2004), green micro alga *Scenedesmus acutus* (Saleh *et al.,* 1984 and Sharaby *et al*., 1993), algae and several types of marine algae (Zaki and Gesraha's, 2001 and Asharaja and Sahayaraj, 2013).

Based on the previous test, our results suggest that the Biocontra formulation is the most effective against larval instars of *S. littoralis*. The data proved that the larval stage was the most sensitive towards the Biocontra formulation. So the Biocontra formulation was chosen for detailed studies for a series of concentrations (2, 4, 6, 8 and 10 mg/ml) to calculate different toxicological parameters, biological aspects and biochemical effects.

3.1.1.2. Toxic effect of Biocontra formulations against cotton leafworm.

Results in Tables (3 and 4) and figs. (1, 2 and 3) revealed that leaf deep technique of algae formulation (Biocontra) after 48, 72 and 96 hrs. All different concentrations (2, 4, 6, 8 and 10 mg/ml) caused different corrected mortality percentages against larval instars of *S. littoralis* insect. Furthermore, resistance of the instar to Biocontra formulation depended on their concentrations and exposure time. Percentages of larval mortality of *S. littoralis* were distinctly low resistance to Biocontra formulation than other formulations. The highest mortality of Biocontra formulation was observed with the highest concentration (10 mg/ml) and achieved 80.0, 82.5 and 93.0% mortality percentage after 48, 72 and 96 hrs. exposure times compared to control treatments. Another concentrations 2, 4, 6 and 8 mg/ml were recorded the least mortality percentage that ranged from 5 to 12.5 % after 48, 72 and 96 hrs.

The lethal concentrations (LC_{50}) is presented in Table (3) and based on the adulticidal activity, Biocontra formulation was found to be the most toxic (LC_{50}= 27.8 mg/ml to the larvae of cotton leafworm after 96 hrs followed by 72 hrs with LC_{50} 33.8 mg/ml but after 48 hrs was the least lethal concentration with 48.1 mg/ml. The data revealed that the homogeneity ratio of LC_{50} and LC_{90} were 18.4, 13.1 and 11.0 after 48, 72 and 96 hrs exposure time, respectively. Our findings were likely to be related to the synergistic effects by Rashwan and Hammad, (2020) of the toxic effect of two algal species; *Spirulina* and *Sargassaum* vulgar in three concentrations (3, 5 and 7 %) gave higher toxicity against 2nd and 4th larval instars *Spodoptera littoralis*. Wahidah, (2021) reported that, overall *Spirulina* at 10% concentration had 40% mortality against larvae of the red palm weevil, *Rhynchophorus ferrugineus* and gave mortality in higher concentrations (20%) in two weeks. Low concentration (0.5% to 5%) had a acceptable effect on red palm weevil larval growth. Also, Rashwan and Hammad, (2020) performed that, water and ethanol extracts of *Sargassaum vulgar* exhibited higher toxicity against 2nd and 4th larval instars of cotton leafworm, In even better agreement with our results, Asharaja and Sahayaraj, (2013) illustrates that the marine alga *Sargassum wightii* and *Padina pavonica* are effective against some cotton pests. This study may provide a biopesticide effects of *macroalgal* algae formulation and can be operationally used for pest control.

Table 3: Mortality of Biocontra formulation against 4th larval instar of cotton leafworm *S. littoralis* at different concentrations.

Concentrations (mg/ml)	Mortality (%)		
	After 48 hrs	After 72 hrs	After 96 hrs
Control	0.0	0.0	0.0
2	5.0	5.0	5.0
4	7.5	7.5	7.5
6	10.0	12.5	12.5
8	12.5	12.5	12.5
10	80.0	82.5	93.0
LC_{50}	48.1	33. 8	27.8
Slope	1.01	1.18	1.26

Table 4: Toxicity values of algae-Biocontra formulation against *S. littoralis* 4th larval instar.

Exposure time	LC_{10}	LC_{30}	LC_{50}	LC_{90}	Homogeneity LC_{90}/LC_{50} ratio
After 48 hrs	2.6	14.6	48.12	887.3	18.4
After 72 hrs	2.56	11.77	33.78	444.2	13.14
After 96 hrs	2.5	10.4	27.8	305.9	11.0

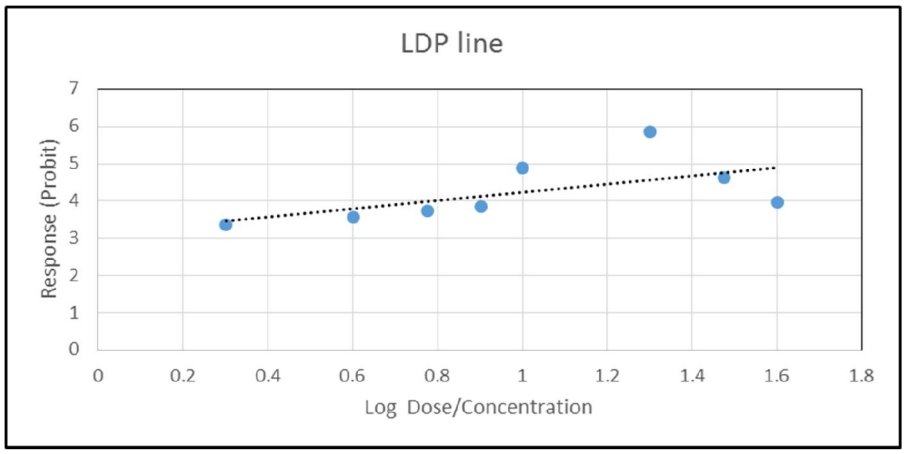

Fig. 1: Toxicity line of Biocontra formulation against *S. littoralis* 4th instar larvae after 48 hrs of treatment.

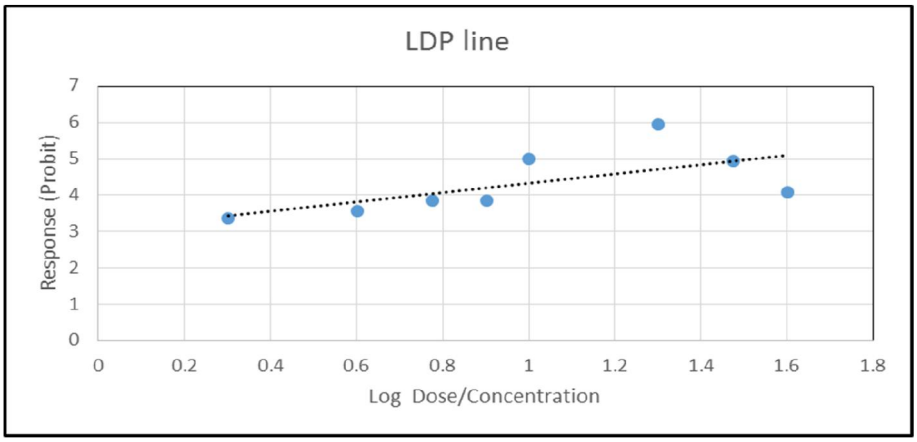

Fig. 2: Toxicity line of Biocontra formulation against *S. littoralis* 4th instar larvae after 72 hrs of treatment.

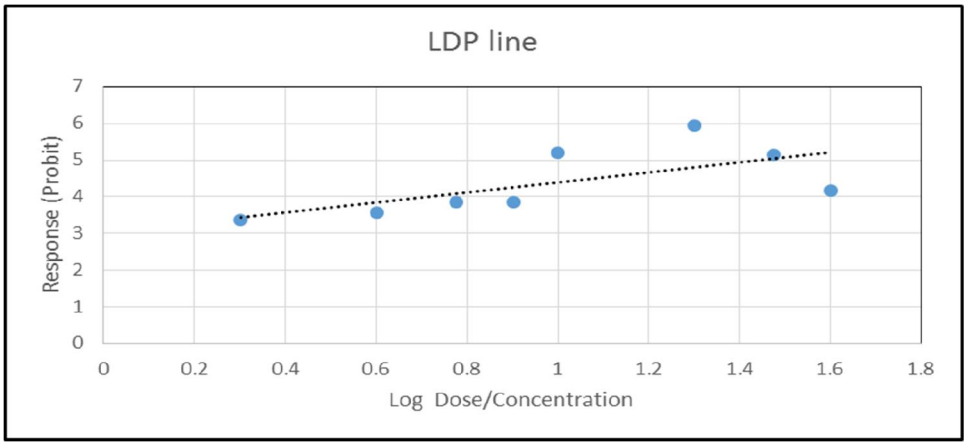

Fig. 3: Toxicity line of Biocontra formulation against *S. littoralis* 4th instar larvae after 96 hrs of treatment.

3.2. Biological Studies

Biological features of *S. littoralis* treated with the LC_{50} of algae-Biocontra formulation are showed in Table (5). Results indicated that; the larval duration from initial treated instar up to pupation significantly decreased to 4.30 days compared with 7.65 days for the untreated. On the other hand, the pupal duration exhibited non-significant values between control (10.70 days) and treated (11.19 days) of 4th instar larvae by LC_{50} of algae-Biocontra formulation. As shown in Table (5), the adult female moth life span emerged from the treated larva was decreased significantly to 7.2 days compared to 10.50 days with untreated larva, respectively. While, the results displayed that, the values of adult male life span were not affected by LC_{50} of algae-Biocontra formulation. The reproduction potential of moths emerging from treated larvae by LC_{50} was recorded, where total number of deposited eggs per female was 426.80 eggs with 71.45% egg hatchability. In comparison, untreated females deposited 678.70 eggs per female with 92.73% egg viability. Also, significance increase in incubation period was observed between eggs produced from treated larvae (4.30 days) as compared with those obtained from untreated larvae by 3.30days. The obtained result of larval duration was in agreement with Abbassy *et al.,* (2014), Rashwan and Morsi, (2021) and Asimakis *et al*., (2022) they recorded highly inhibition effect of different algae species on larval growth of *S. littoralis*, where they attributed the inhibition effect of algae to their chemical contents or composition from phenols, tannins and alkaloids. This interpretation supported by Helmi and Mohamed, (2016) who reported that phenolic component of algae have insecticidal activity through inhibition development of insect stages, where the detoxification mechanism of insect was affected by phenolic component. While, Zayed *et al.,* (2022) attributed the decreasing in biological aspects (larval duration, female life span, fecundity and fertility) of *S. littoralis* to the breakdown of protein into amino acids that provides energy during many processes i.e., egg production, development stages and formation of larval and adult tissues, specially the cuticle.

Table 5: Biological aspects of *S. littoralis* treated with LC_{50} value of algae Biocontra formulation.

Biological aspect	Untreated	Treated
Larval stage duration (days ± S.E)	$7.65^{a} \pm 0.20$	$4.30^{b} \pm 0.17$
Total pupal duration (days ± S.E)	$10.7^{a} \pm 0.46$	$11.19^{a} \pm 0.10$
Female moth life span (days ± S.E)	$10.5^{a} \pm 0.46$	$7.2^{b} \pm 0.51$
Male moth life span (days ± S.E)	$7.8^{a} \pm 0.29$	$8^{a} \pm 0.58$
Mean no. deposited (egg / ♀ ± S.E)	$678.7^{a} \pm 72.34$	$426.8^{b} \pm 104.56$
Egg incubation period (days ± S.E)	$3.3^{a} \pm 0.15$	$4.3^{b} \pm 0.18$
% Egg hatchability	92.73%	71.45%

Additionally, the decreasing in adult lifespan, fecundity and hatchability of *S. littoralis* are in agreement with Asharaja and Sahayaraj, (2013), Hamed *et al*., (2018), Saber *et al.,* (2018) and Asimakis *et al*., (2022) they observed the same trend on cotton insect pests as affected by various

species of algae. Also, Yu *et al.*, 2014 and Asimakis *et al*., (2022) attributed the reduction in biological aspects to the insecticidal activity of algae as a biopesticides, where insecticidal activity of algal extracts are related to the bioactive component such as polysaccharides, phenolics, proteins, terpenes, lipids and halogenated compounds.

3.3. Biochemical Studies

Data in Table (6) showed the effect of LC_{50} value Biocontra formulation on protein content (mg/ml) of 4th instar larvae of *S. littoralis* larvae after 48, 72 and 96 hours. It's clear that, protein content was sharply decreased with all different periods of treatments (48, 72 and 96 hours). The protein content of untreated larvae was 50.21 mg/ml, while the corresponding values after 48, 72 and 96 hours were 27.59, 26.79 and 26.31 mg/ml with a percentage decrease 45.05, 46.64 and 47.60% than control, respectively. The reduction in protein content could be attributed to insect detoxification process caused by insecticidal activity of algae.

Didair *et al.,* (2018) mentioned that reduction of protein content may be one of the reasons of insect death. The deficiency of protein content delayed various physiological processes in insects, where adult insects require protein to promote ovulation and egg development, which supports this conclusion. Our findings supported by Rashwan and Morsi (2021) they found that a significant decrease in total soluble protein of bean seed beetle (*Bruchidius incarnates*) treated by LD_{50} of two algae tested (S*pirulina platensis* and *Fucus vesiculosus*), where they attributed the reduction in total soluble protein to the insecticidal stress of algae. The authors also mentioned that, the reduction in larval protein content may be due to the consumption of amino acids in synthetic protein which consumed in recovering insecticidal stress. Also, they attributed the reduction in protein synthesizing to reduction in the levels of nucleic acids. Also, according to Zayed *et al*., (2022), the loss in protein during intoxication is brought on by either protein being converted into amino acids, protein being broken down to provide energy, or direct impacts on the amino acid transport system of the cell. Additionally, the synthesis, storage, transport, and degradation of structural and functional contents, as well as the response to physiological conditions, are all negatively impacted by a drop in protein content. Abd El-Kareem *et al.,* (2022) who referred that the depletion in total protein may be due to the breakdown of protein into amino acids, thus, the entrance of these amino acids to tricarboxylic acid cycle (TCA) as a keto acid, will help supply insect with energy. Therefore, protein deficiency may constitute a physiological mechanism and might play a role in compensatory mechanisms under insecticidal stress to provide intermediates to the TCA cycle by retaining free amino acid in hemolymph.

Table 6: Protein content in 4th instar larvae of *S. littoralis* treated with the LC_{50} value of algae Biocontra formulation.

	Protein (mg/ml)	**% Increase or decrease than control**
After 48 hrs.	$27.59^{b} \pm 2.76$	- 45.05
After 72 hrs.	$26.79^{b} \pm 1.32$	- 46.64
After 96 hrs.	$26.31^{b} \pm 1.51$	- 47.6
Untreated	$50.21^{a} \pm 5.58$	-

Data in Table (7) showed the effect of LC_{50} value of Biocontra formulation on enzymes activity (Chitinase, Glutathione S-transeferase and β-esterase) applied at 4th instar larvae of *S. littoralis* after 48, 72 and 96 hours, respectively. It is seen that, the measured enzymes activity significantly decreased after 48, 72 and 96 hours compared to untreated, respectively.

The activity of Chitinase exhibited highly significant reduction after 48, 72 and 96 hours by 168.14, 113.97 and 188.44 *µg N-acetylglucosamine /min/ g.b.wt* compared with control by 962.76 *µg N-acetylglucosamine/min/g.b.wt* which caused significant decrease in enzyme activity by -82.53%, -88.16% and -80.42% than control after 48, 72 and 96 hours, respectively. The same reduction trend was recorded with GST activity, where the values were 18.14, 4.96 and 17.48 *m mole sub.conjugated/min/g.b.wt* after 48, 72 and 96 hours from treatment, respectively, compared with control by 66.96 *m mole sub.conjugated/min/g.b.wt*. Obtained data also showed marked decrease in GST activity after 48, 72 and 96 hours by -72.9%, -92.6% and -73.9% than control, respectively.

While, β-esterase was significantly increased after 48 hours from applied algae by (150.30 *µg* β-*naphthol /min / g.b.wt*) and then significantly decreased after 72 and 96 hours compared to control treatment by (12.79 and 69.28 *µg* β-*naphthol /min / g.b.wt*), respectively. The β-esterase activity increased than control by 28.3% after 48 hrs and then significantly decrease by -89.08% and -40.85% after 76 and 96 hrs from treated larvae with Biocontra formulation, respectively.

The outer epidermis forms as the larvae stop growing after hatching from the egg. The larvae must now molt, which means that the old epidermis must be removed and a larger, new epidermis must be grown. Chitin plays a crucial part in the structure, growth, and development of insects; hence new pesticides are being developed with a focus on how they affect chitin production and breakdown (Oyeleye and Normi, (2018); Zhang *et al.,* (2021) and Wang *et al.,* (2022). Chitinase is very important enzymes during insect ecdysis, where the main role of chitinase is the digestion of old cuticle of larvae. As regard to the data in Table (7) the chitinase activity was sharply decreased after fed on algae compared to control. Moreover, failure of molting of some larvae has been observed and then caused larval death without complete their metamorphosis. This decreasing of chitinase activity may be attributed to the reduction in protein content that main structure of enzyme synthesis. This result is in agreement with Ismail and Shaker, (2015) who observed that the differences in enzyme activity due to the result of varying protein production in response to various treatments. It is clear that, the effect of algae on chitinase activity gave similar effect of IGR on *S. littoralis* larvae. In this concern, Lee *et al.,* (1990) and Ismail and Shaker, (2015) reported that, IGR caused highly reduction of chitinase activity in *S. littoralis* larvae, and this led to moulting perturbation, metamorphosis and finally death of larvae. Fetoh and Asiry, (2013) ascribed the decrease in chitinase activity in *S. littoralis* larvae treated with lufenuron (IGR) to chitin production inhibition; as a result, the larvae failed to ecdysis into the next instar.

A major family of detoxification enzymes is Glutathione *S*-transferases (GSTs EC. 2.5.1.18). They help increase the solubility of lipophilic substances and facilitate their excretion from the cell by catalyzing the conjugation of the tripeptide glutathione to the electrophilic centers of those molecules. One of GST's main roles is to catalyze the removal of harmful substances from the body by xenobiotics, such as pesticides, through the mercapturic acid route (Hayes and Pulford, 1995). By their reductive dehydrochlorination or by conjugation events with reduced glutathione, GSTs can metabolize insecticides to produce water-soluble metabolites that are more easily eliminated. In addition, they help in the removal of toxic oxygen free radical species as a result of pesticide impact (Enayati *et al.,* 2005). This group of enzymes has been linked to one of the main mechanisms in insects that counteract the damaging effects of insecticides. (Grant *et al.,* 1991; Syvanen *et al.,* 1994; Ranson *et al.,* 1997 and Huang *et al*., 1998). Moreover, a group of multifunctional proteins known as GSTs play a variety of roles in detoxification. (Grant and Matsumura, 1989). It has been proposed that the function of this enzyme is to defend physiological nucleophiles from conjugated electrophilic foreign substances such as pesticides, medicines, and carcinogens (Abdel-Halim *et al.*, (2019) and Koirala *et al.,* (2022).

Our finding exhibited that, the glutathione-S-transferase (GST) activity was decreased after larvae fed on Biocontra formulation compared to unfed larvae, this reduction may be due to one of two reasons, the first one, related to the effect of algae antimetabolites, where antimetabolites produced by algae are small molecules that inhibit enzymes activities by mimicking physiological substrates (Brilisauer *et al*., 2019). In this concern, the antimetabolite may attach to an enzyme in the same way that the native substrate does, but it is not transformed into a functional product. Antimetabolites may so compete with the natural substrates and may block an enzyme's catalytic activity (Asimakis *et al*., 2022). The second one related to the effect of algae (Biocontra formulation) on GST activity which exhibited the same effect of IGR as a bioinsecticides. In this concern, Abd-El Aziz, (2014) found significant reduction of GST activity after treatment of the 4th larval instar of *S. littoralis* with emamectin benzoate. Badawy *et al*., (2013) recorded decline of GST activity after treated earthworms *Aporrectodea caligniosa* by lufenuron. Also, Abou-Taleb *et al*., (2015) and Shenouda *et al.,* (2019) found that the GST activity was decreased after *S. littoralis* larvae were treated with lufenuron (IGR).

A large and varied group of hydrolases known as general esterases hydrolyzes a wide range of substrates, including esters and certain non-ester compounds. Several studies have shown that esterases are crucial in assisting the detoxification of insecticides in various insect and arthropod

species. Esterases are hydrolyzing enzymes that break down ester molecules when water is added, producing alcohol and acids (Rashwan, 2013). Also, Abd El-Kareem *et al*., (2022) referred to the detoxifying enzymes, which include the esterase enzymes, are in responsible of detoxifying any foreign substances from an insect's body. Moreover, it hydrolyzes any toxicant's esteric bond and responds most strongly to environmental stimulus.

The same observations for GST activity were observed with β-esterase activity which decreased after treated 4th larval instar of *S. littoralis* by Biocontra formulation. This reduction may be due to (1) algae Biocontra formulation antimetabolites effect, (2) effect of algae Biocontra formulation as IGR or bioinsecticides, (3) reduction of total protein content affected on enzyme production.

Our results agreed with Anwar and Abdel-Mageed, (2005) who declared that *S. littoralis* laboratory and field strains treated by six IGR achieved the reduction in β -esterase. Omar *et al.* (2006), showed that the use of Spintor (Spinosad) on larvae of *Pec. gossypiella* and *E. insulana* caused decrease in beta -esterase in larvae of pink and spiny bollworms compared to the control. Also, Fahmy and Dahi, (2009), Wang *et al.,* (2009) and Baker, (2000) referred that the use of rynaxypyr and Spinetoram on 4th larval instar of *S. littoralis* decreased alpha-esterase, beta- esterase and acetylcholinesterase (AChE) activity. El-Kawas *et al.,* (2009) demonstrated that, in comparison to control, diflubenzuron (IGR) inhibited the activity of non-specific esterases (α and β-esterases) in immature stages of *Tetranychus urticae*. Rashwan, (2013) found a significant decrease in β -esterases activity when 4th instar of *S. littoralis* larvae was treated with LC_{50} of Spinetoram. When *S. littoralis* larvae was treated with emamectin benzoate, a high significant reduction in β-esterase was observed by Abd-El Aziz, (2014). In the same trend, Abdel-Halim, (2019) recorded reduction in β-esterases after *S. littoralis* was treated with bioinsecticides. Although there is a deficit of information about the mode of action of algal metabolites against agricultural pest insects; limited work performed with other insects might provide more details (Watanabe *et al*., 1990 and Asimakis *et al*., 2022).

Table 7: Chitinase, Glutathione S-transeferase and β-esterase, activities in 4th instar *S. littoralis* larvae affected by treatment with the Alga LC_{50} Biocontra formulation.

Treatments	Chitinase	% Increase or decrease than control	GST	% Increase or decrease than control	β-esterases	% Increase or decrease than control
After 48 hrs.	168.14^{b}±34.31	-82.53	18.14^{b}±4.80	-72.9	150.30^{a} ±7.48	28.3
After 72 hrs.	113.97^{b}±12.74	-88.16	4.96^{c}±0.96	-92.6	12.79^{d} ±2.18	-89.08
After 96 hrs.	188.44^{b}±13.55	-80.42	17.48^{b}±2.33	-73.9	69.28^{c} ±2.82	-40.85
Untreated	962.76^{a}±141.04	-	66.96^{a}±4.46	-	117.14^{b} ±11.22	-

The decrease in activities of detoxifying enzymes (GST and β-esterase) explains that the insect cannot tolerate the toxic effects of the algae-Biocontra formulation, which have a strong insecticidal effect, so their enzymatic defense systems break down and the insect dies as a result. Based on the above discussion, the reduction in estimated enzyme activity in our study could be attributed to the reduction in total soluble protein as a result of the insecticidal activity and stress of the algae.

4. Conclusion

Globally, there is nowadays a growing demand for the application of plant and natural products as insecticides due to very low health risks, biodiversity conservation, an increasing shift in consumer demand for safer food, and finally, the current trend towards increasing organic farming under modern climatic changes. In conclusion, this work revealed that the Biocontra formulation could be considered as having potential effects as an eco-friendly bioactive component for the integrated crop management of the cotton leafworm *S. littoralis*. Moreover, natural insecticidal products, such as those identified in this study, are considered safer for the environment. Therefore, it can be recommended that, the use of algae as environmentally friendly trend in the integrated pest management for insect control.

It is important to study the mode of action of Biocontra formulation as an algal product on

larvae instar and the results indicated that the larval duration, female life span, fecundity, and fertility are significantly affected by algae treatment. Moreover, protein content, chitinase, β-esterase and GST significantly decreased compared to untreated larvae.

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