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The Double Face Effect Of Algae Extracts As Bio-Pesticides Against Fall Armyworm, Spodoptera Frugiperda, And Root-Knot Nematodes, Meloidogyne Incognita

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Abstract
Background *The fall armyworm (FAW), Spodoptera frugiperda, and root-knot nematode, Meloidogyne incognita, are the most harmful agricultural pests, as they can persist in many environmental conditions and have a broad host range. Therefore, it is necessary to employ several control strategies, such as using biopesticides to reduce their population. This study aimed to designate and evaluate the effects of different concentrations of two methanolic algal extracts, Nostoc muscorum (Cyanobacteria) and Chlorella vulgaris (Chlorophyta), on 3rd instar FAW larvae and 2nd instar juvenile nematode stage (J2s) as a biological environmental strategy.*
Results *Two algal extracts were evaluated against two dangerous plant pests. Subsequently, the expression profiles of acetylcholinesterase and cytochrome P450 genes will be studied. Both algal extracts had clear insecticide and nematicide effects, as shown in the results. Both extracts' highest concentration (100%) resulted in significantly higher mortality rates, which recorded 73% and 67% for FAW larvae and 94% and 88% for J2s, respectively. The expression levels of SfAChE, SfCYP9A60, MiAChE1, and MiCYP were lowered significantly when both target pests were treated with 75% N. muscorum after 24 hours post-treatment. The laboratory bioassay demonstrated that the extracts were highly biopesticide-active at 100% and 75% concentrations, which suppressed the immune response of FAW larvae and M. incognita to invade the host.*
Conclusions *The two algal extracts resulted in various deficiencies in the normal growth and reproduction of insects and root-knot nematode larvae. This caused several issues, including a high mortality rate among FAW larvae, a reduced pupation percentage, and the production of abnormal pupae and moths, as well as juvenile mortality and a decrease in egg hatching rates in nematodes. Given that these algae are easily accessible and extensively cultivated, they can be effectively used by small-scale farmers to control fall armyworms and root-knot nematodes without relying on harmful chemical pesticides, thereby minimizing environmental pollution.*

Keywords *Spodoptera frugiperda, Meloidogyne incognita, In-vitro assay, Quantitative expression genes, eco-friendly pesticide*

Introduction

Globally, insect pests and root-knot nematodes significantly threaten to agricultural productivity, crop quality, and food security. The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), stands out as a significant pest globally, leading to substantial yield reductions in maize crops. FAW was initially recorded in 1797 as a heavy pest native to subtropical and tropical areas of America [1]. When FAW larvae appears in significant quantities, particularly with an aggressive impact, it is poised to create a sustained and detrimental risk to numerous vital crops. The surrounding conditions offer an ideal setting for a range of host plant species, coupled with favorable weather conditions for reproduction across different regions [2].

Recently, there have been reports of a significant occurrence of FAW larvae in 44 African nations, including Egypt. In May 2019, a report from the Agricultural Pesticide Committee (APC) of the Ministry of Agriculture in Egypt documented the initial detection of FAW larvae in a maize field located in a village within Aswan Governorate, Upper Egypt [3]. The first record for FAW in Egypt was reported by [4] who revealed that it became clear that the migration to the north occurred rapidly, affecting Luxor, Qena, and Sohag Governorates. Subsequently, the productivity yield of crops can be significantly

damaged by FAW larvae, resulting in yearly yield losses of US$2.5–6.2 billion [5] [6]. Small-scale maize farmers, who mostly depend on the crop to prevent hunger and poverty, are the ones most affected by these losses [7] [8].

Although insecticides have been used for FAW management in maize and other crops, it is still crucial to explore alternative management strategies, such as biological control. The effectiveness of entomopathogenic nematodes of *Heterorhabditis* as novel species targeting FAW has demonstrated their potential as a powerful and safe biocontrol agent, suitable for integration into biological control strategies against this invasive corn insect pest in Egypt [9]. Fuarthermore, FAW larvae were treated by jojoba oil as a botanical extract, *Bacillus thuringiensis* as a bioinsecticide, and lufenuron as an insect growth regulator. As a result, the jojoba oil recorded the maximum toxicity on FAW and negatively affected on all biological parameters [10].

Furthermore, one of the most destructive root-knot nematode species is *Meloidogyne incognita* (Chitwood) (Secernentea: Heteroderidae). *M. incognita* is the most widely distributed and harmful species worldwide. These highly polyphagous nematodes participate in complex interactions with host plants that lead to the establishment of a permanent feeding site, referred to as giant-cells, which facilitates nematode development and maturation [11]. Plant-parasitic nematodes result in annual losses of US$100 billion in worldwide agriculture [12].

Managing insects and root-knot nematodes poses a significant challenge for modern agriculture worldwide. The widespread use of chemical control classes has caused numerous ecological and environmental issues. As a result, there is an urgent need for safer and more effective alternatives. Biopesticides can lessen the need for dangerous chemical pesticides and significantly contribute to sustainable agriculture[13]. Biopesticides are strategies that originate mainly from microorganisms, including bacteria, fungi, nematodes, and algae [1]. Biological pesticides offer promising solutions because they are more specific, can still be effective in pest management, and have lower risks of harming the environment [14].

Algae are microscopic photosynthetic organisms found primarily in freshwater and marine habitats. Microalgae have been proposed as a safe alternative to pesticides due to their excellent biodegradability, minimal residuality, and enhanced specificity [15]. They are less likely to acquire resistance due to the numerous algae action modes that can be used to protect plants from pests sustainable farming practices. Microalgae can be either prokaryotic (Cyanobacteria) or eukaryotic (Chlorophyta) [16]. Cyanobacterium strains such as *Anabaena flos aquae*, *A. laxa,* and *A. fertilissima* demonstrated efficacy as biopesticides against 2nd and 4th larval instars of *Spodoptera littoralis* [13].

The two most widely recognized algae genera are *Nostoc muscorum* (C.Agardh ex Bornet et Flahault) (Cyanobacteria) and *Chlorella vulgaris* (Beijerinck, 1890) (Chlorophyta). *N. muscorum* cells are filamentous, green gram-negative algal cells that play significant roles in the soil ecosystem's nutrient cycle, specifically in the carbon and nitrogen fixation processes. There may be a relationship between the pesticidal activity of *N. muscorum* extracts and various biological parameters, including the mortality and malformations of FAW larvae [17][18]. *C. vulgaris* has bioactive compounds; it is utilized in a wide range of applications [19] ; for instance, C. *vulgaris* organic extracts caused significant mortality in third larval instars of *Aedes aegypti* [20]. The utilization of algae for the biological control of root-knot nematodes is advisable, particularly in sustainable agriculture, to preserve soil health and enhance fertility. The findings demonstrated that soil treated with *Spirulina* or *Amphora* extracts resulted in notable increases in vegetative growth, yield, and fruit quality. The integration of these two applications significantly improved plant resistance against nematodes, as evidenced by various nematode parameters[21]. The eco-nematicidal efficacy of the aqueous extract of the freshwater green algae, *Cladophora glomerata* and its silver nanoparticles (Ag-NPs) affected on *Meloidogyne javanica*. The *C. glomerata* extract may stimulate the immune system to combat nematode infection [22].

Cyanobacteria and Chlorophyta have been identified as new and rich sources of bioactive compounds and possess a noteworthy antioxidant system that is considered more effective [23]. The most potent and significant antioxidants found in algae include pigments, vitamins, phenols, and flavonoids [24][25].

Transaminases are more important enzymes and have been demonstrated in several insect tissues, which produce non-essential amino acids, gluconeogenesis, nitrogen waste metabolism, and protein anabolism and catabolism[26]. Transaminases such as aspartate transferase (AST), also known as glutamic oxaloacetic transaminase (GOT), and alanine transaminase (ALT), also known as glutamine pyruvic transaminase (GPT). ALT is an essential enzyme for gluconeogenesis. In insects, the AST and ALT enzymes, and their mammalian counterparts are utilized as markers of the healthy operation of the fat body [27].

Fundamental to cellular metabolism in insects, alkaline phosphatases (ALP) as antioxidative enzymes are mostly found in insect tissues with extensive membrane transport, such as hemolymph, Malpighian tubules, and midgut epithelium [28]. ALPs are engaged various insect biological processes and respond to infection, pathogenesis, and stress. The physiology of the insect gut will be impacted by any reduction in its activity [29]. ALP is one significant enzyme that influences the levels of insect developmental hormones [30]. Furthermore, acetylcholinesterase (AChE) has been an effective target for insecticide development, essential aspect of defense and stress [31]. Certain pesticides block AChE action, which causes ACh accumulation in synapses. This overstimulation of ACh receptors produces nerve impulses, which kill insects and nematodes [32]. AChE might be crucial in detoxifying different pesticide classes33. The high percentage of mortality in *Spodoptera litura*

resulting from the downregulation of AChE expression reported by [34] who suggested that AChE plays a critical role in the survival and immune response of insects. Moreover, it has been widely demonstrated that the metabolic detoxification of xenobiotics, including biological compounds, insecticides, and harmful substances, is facilitated by insect cytochrome P450 (CYP) [35].

The study's objective was to evaluate the methanolic extract effects of *N. muscorum* and *C. vulgaris* on the developmental biological parameters of 3rd FAW larval instars in parallel with the impact on the 2nd juvenile stage (J2s) nematode mortality. Subsequently, we investigated whether algal extracts affected different molecular parameters of FAW larvae and *M. incognita* along with high-performance liquid chromatography (HPLC) analysis of potentially bioactive constituents of *N. muscorum* and *C. vulgaris*.

Materials and methods

1-Microalgae

1.1. Collection and culturing of microalgae isolates

Samplings were carried out from El-Nubaria, El Beheira Governorate, Egypt, at latitude 31.1851°N and longitude 30.5246°E (March to April 2022). Samples of algal mats were crushed using glass beads that had been sterilized, and the resulting mixture was diluted with pre-sterilized double-distilled water. The resulting mixture was then spread solidified agar plates[36] containing BG-11 medium. The plates were then incubated at a light intensity of 4000 lux until colonies appeared under a 12:12 h light/dark cycle with constant aeration. The algae isolates were preliminarily identified based on their morphology[37] at the Plant Protection and Biomolecular Diagnosis Department, Arid Lands Cultivation Research Institute (ALCRI), City of Scientific Research and Technological Applications (SARTA, City), Alexandria, Egypt. For additional validation based on its morphological features, a light microscope study of the isolated species was conducted using a light microscope (Olympus BX40 microscope, Japan). Ocular dimensions and photographs were captured using a Nikon E5000 digital camera at the Biomedical Lab of SARTA, City.

1. 2. Extract preparation of *N. muscorum* and *C. vulgaris*

Algal extracts were prepared following the methodology outlined by[38]. In preparing algae, ~21-day-old *N. muscorum* and *C. vulgaris* biomass were harvested during the stationary phase of algal growth. The biomass was collected by centrifugation at 4,000 × g for 15 minutes, and the aqueous phase was discarded. The resulting pellets were then dried in an oven at 45 ± 2°C until a constant weight was achieved. After drying, the pellets were subjected to extraction.

For extraction, 10 g/L of each *N. muscorum* and *C. vulgaris* dry weight was separately extracted using either 500 ml of methanol solvent or water at 55 ± 5°C. Each *N. muscorum* and *C. vulgaris* powder was immersed in two different flasks, and an ultrasonic micro-tip probe of 100 W was used for extraction for 15 minutes. The two extracts were then incubated at 37°C with constant shaking for one week at 150 rpm to ensure all cell content was released and thawed out. Sonication was used to break down and blast the extract. The methanolic extract was concentrated to dryness using a rotary evaporator at a temperature of 35±2°C. Centrifugation at 8000 × g for 20 minutes was performed for the water extracts. The combined supernatant from the centrifugation step was evaporated to dryness at a temperature of 40°C using an oven for 7-10 days or until complete dryness was observed. Finally, the two dried extracts were collected and stored in labeled sterile vials in pre-weighed test tubes and then preserved at a temperature of 4°C [37]. For the biological experiments, a series of concentrations was prepared by diluting the stock of each algal extract (i.e., 100%) with distilled water [39][40] as follows:

1. N1: *N. muscorum* at 25% concentration.
2. N2: *N. muscorum* at 50% concentration.
3. N3: *N. muscorum* at 75% concentration.
4. N4: *N. muscorum* at 100% concentration.
5. C1: *C. vulgaris* at 25% concentration.
6. C2: *C. vulgaris* at 50% concentration.
7. C3: *C. vulgaris* at 75% concentration.
8. C4: *C. vulgaris* at 100% concentration.

2. HPLC analysis for *N. muscorum* and *C. vulgaris* methanolic extracts

The quantification of polyphenolics (phenolic acids and flavonoids) in the extracts of *N. muscorum* and *C. vulgaris* was conducted using an HPLC system [41]. The HPLC system consisted of a Waters 515 reciprocating pump, a variable photodiode array (PDA) detector (Waters 2996), and a system controller with Empower TM software for data acquisition, integration, and analysis. The separation of phenolic extracts was performed using reverse phase liquid chromatography under the following conditions: room temperature, injection volume of 10 µl per sample, isocratic separation mode, C-18 column (250 × 4.6 mm i.d., particle size 5 µm), mobile phase consisting of methanol: 0.4% acetic acid in water (55:45%,

v/v), flow rate of 1 mL/min, and detection at wavelengths of 254 nm and 280 nm 52 with slightest modifications. Standard compounds, including gallic acid, chlorogenic acid, caffeic acid, vanillic acid, ferulic acid, rutin, quercetin, and kaempferol from Sigma-Aldrich, were used for comparison. Before injection into the HPLC column, the samples were filtered through a membrane with a pore size of 0.45 µm. The identification of compounds in the samples was achieved by comparing their retention times with those of the standard compounds and by co-injection. Quantification was performed by comparing the peak areas of the identified compounds in the samples with those of the standard compounds.

3. Fall armyworm insect

3.1. Insect rearing

FAW larvae have been collected from infested maize fields (early whorl stage) close to West Nubariyah during June- July 2022, El Beheria governorate, Egypt, 30°43'46.5"N, 29°57'27.9"E. The larvae were identified and confirmed using morphological characteristics at Egypt's Plant Protection Research Institute, Agricultural Research Center (ARC). The larvae were reared in plastic containers that measured 25 cm long, 15 cm wide, and 12 cm high with fine muslin and daily fresh castor leaves as feeding at the laboratory. From the third instar onward, larvae were kept separated from one another until pupation to prevent cannibalism [42]. Pupae were observed and collected daily inside the ovipositional cage jars (20 cm in height and 15 cm in diameter). Adult moths were given a 10% sugar solution and fresh castor leaves for egg deposition when they emerged. Every day, egg masses were collected and kept in different containers. To ensure progressive culture continuity, the insects were maintained in carefully controlled laboratory settings (temperature of 27±1°C, relative humidity of 70±5%, and photoperiod L12:D12). Before being employed in an experiment, FAW culture was raised for five generations to develop an effective chemical contamination-free culture.

3.2. Biological experiment of FAW larvae

The leaf dip method was used to experiment[43]. Cleaned castor leaves of the same size were immersed in each algal concentration prepared previously for 60 seconds. The treated leaves were dried under laboratory conditions and transferred to separate jars. A control group of ten 3rd larval instars was fed on clean castor leaves. Another ten larvae were fed for 48 hours on treated castor leaves, followed by fresh leaves until pupation for each algal extract and concentration. Five replicates were performed for either the control or treated groups. The study evaluated the following biological parameters: percentage of larval mortality, pupation and adult emergence, and developmental period of larvae, pupae, and adults. The observations were recorded at each 24-hour interval [44]. The mortality percentages were recorded by touching FAW larvae with a tiny needle and showed no movement [45]. The lethal concentration assay of both algal extracts was calculated after 72 hours post-treatment of FAW larvae.

3.3. Hemolymph sample preparation

Using fine scissors to cut the prolegs of survival larvae at their bases carefully, hemolymph was collected from control and treated third larval instars of FAW after 24-, 48-, and 72-hours without collecting any other organs[46]. Hemolymph was collected from 10 larvae fed clean or treated castor leaves with 75% and 100% of *N. muscorum* and *C. vulgaris* extracts for each biochemical parameter. Hemolymph was drawn out using calibrated capillaries and placed immediately into vials maintained at -20°C for subsequent analyses. For each biochemical parameter, three replicates were carried out.

4. Biochemical parameters

4.1. Transaminase determination

AST and ALT were determined calorimetrically in FAW hemolymph [47]. AST transfers the amino group from L-aspartate to α-keto acid (α-ketoglutaric acid), producing a new amino acid (L-glutamate) and a new keto acid (oxaloacetic acid). ALT transfers the amino group from D, L alanine to α-keto acid (α-ketoglutaric acid), resulting in a new amino acid (L-glutamate) and a new keto acid (pyruvic acid). Pyruvate or oxaloacetate reacts with 2,4-dinitrophenylhydrazine, forming pyruvate or oxaloacetate hydrazone, which, in an alkaline medium, forms a brown color that can be measured spectrophotometrically. The reaction mixture consisted of 1 ml of phosphate buffer (pH 7.2), 0.2 mM α-ketoglutaric, and 200 mM D,L alanine or L-aspartate. Incubate for precisely 30 minutes. Add 1 ml of 0.001 M 2, 4-dinitrophenylhydrazine. Wait for at least 30 minutes. Then, 10 ml of 0.4 NaOH was added. The optical density of the brown color produced is measured after 5 minutes using a spectrophotometer at 520 nm. The enzyme activity is expressed as U/L.

4.2. Determination of ALP

ALP in FAW hemolymph was determined according to the method described by [48]. The phenol released by the enzymatic hydrolysis of disodium phenyl phosphate reacts with 4-aminoantipyrine, and by the addition of potassium ferricyanide, the characteristic brown color is produced. The reaction mixture consisted of 1 ml of carbonate buffer (PH 10.4), 1 ml of 0.01

M disodium phenyl phosphate (substrate), and a 0.1 ml sample. Mix and incubate for exactly 30 minutes at 37 °C. At the end of the incubation period. 0.8 ml of 0.5 N NaOH was added to stop the reaction. Then, 1.2 ml of 0.5 N $NaHCO_3$ was added, followed by the addition of 1 ml of 4-aminoantipyrine solution (1%) and 1 ml of potassium ferricyanide (0.5). The produced color was measured immediately at 510 nm. The enzyme activity is expressed in units (U). where 1 U will hydrolyze 1 U mole of p-nitrophenyl phosphate per minute at 37°C and pH 10.4.

5. Root-knot nematode

5.1. Chemical nematicide

As a control nematicide, the commercial pesticide Rugby 60% (Cadusafos 20%) was used; it had been purchased from Misr Agriculture Development Company, Egypt.

5.2. Collection, identification, and preparation

A single egg mass was used to inoculate tomato plants. Seedlings were transplanted in 30 cm diameter plastic pots filled with sterilized clay-sand soil (1:2 v/v), and the pots were maintained at 25 ± 3°C for 16 h of photoperiod in the experimental greenhouse, SARTA City. Three months after the infestation of the tomato plant with *M. incognita*, adult females of root-knot nematodes were collected from galled roots for species identification by the characteristics of the perinea pattern technique[49]. Plants were uprooted, and egg masses were collected from the galls of infected tomato roots with a needle. The roots were cut into small pieces (2–3 cm) and then placed in a flask containing 0.5% sodium hypochlorite (NaOCl) solution (Hussey and Barker, 1973). Active juveniles (J2s) of *M. incognita* were obtained using the Baermann plate technique [50].

5.3. Second stage juvenile mortality bioassay of *M. incognita In-vitro*

A laboratory experiment was conducted to investigate the effectiveness of *N. muscorum* and *C. vulgaris* at different concentrations on the J2s mortality rate of *M. incognita*. The eggs were collected and incubated in distilled water for three days to allow them to hatch, and the newly hatched juveniles were collected. Ten culture plates containing nematode egg suspension (approximately 40 eggs/200 µL) and freshly hatched J2s (approximately ten per well) were used for analysis in the treatment. For the mortality rate experiment, all concentrations of *N. muscorum* and *C. vulgaris* methanolic extracts prepared previously were inoculated with freshly hatched J2s (200 µL nematode suspension). One treatment was left untreated as a negative control group (10 mL distilled water + 200 µL nematode suspension), and another positive group was treated only with the nematicide Rugby 60% (10 mL distilled water + 200 µL nematode suspension + 14 µL Rugby 60%) [51].

The number of live J2s was counted under a light microscope at 24-, 48-, and 72-hours post-treatment at room temperature. After treatment, the juveniles were transferred to plain water for observation. Each treatment was replicated five times. Nematodes were considered alive if they exhibited movement or maintained a spiral shape. In contrast, they were considered dead if they did not show any movement after being transferred to tap water and probed with a fine needle [52]. The lethal concentration assay of both algal extracts was calculated after 72 hours post-treatment of J2s. The reduction percentage in nematode mortality was calculated [38] according to the following equation:

$$\text{Reduction \%} = \frac{\text{Total number of J2S in control} - \text{No. of alive J2S in treatment}}{\text{Total number of J2S in control}} \times 100$$

5.4 Hatching of *M. incognita* Eggs Assay

To evaluate the hatchability of nematode eggs, a bioassay was conducted. utilizing a 6-well sterile cell culture plate with roughly 40 eggs in each well. As controls, the plates with dH_2O were used. Five replicates were made. Hatching was noted after 24 and 48h of incubation at 27 ± 2 °C for all treatment plates. Following each count, the eggs were transferred to plates containing new particles of the same concentration after being cleaned in their plates with 1 mL of dH_2O. The hatchability inhibition percentage was calculated.

6. RNA extraction and Quantitative Real-Time PCR to insect and nematode

Whole FAW larvae and J2s of *M. incognita* were collected from control and treated groups by 75% and 100% of both methanolic extracts at 24-, 48-, and 72-hours post-treatment and were promptly processed for RNA extraction. Whole 3rd FAW larvae and J2s nematode specimens were ground into a fine powder using a porcelain mortar and glass beads. RNA was extracted using the TRizol reagent extraction procedure (Cat#15596026, Invitrogen, Germany), assessed for quality via agarose gel electrophoresis, and quantified by measuring absorbance at 260 and 280 nm using a Nano-drop spectrophotometer [22]. RNA extractions were performed on three biological replicates of five pooled FAW larvae and J2s nematodes. Defense genes such as acetylcholinesterase (*SfAChE* and *MiAChE1*) and cytochrome P450 (*SfCYP9A60* and

MiCYP) were detected by running agarose gel electrophoresis of the corresponding PCR amplifications. Subsequently, cDNA synthesis was performed using the RevertAid™ First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA) on one µg of total RNAs reverse transcribed using RNA. PCR mixtures were prepared and incubated before storage for future analysis.

Quantitative real-time PCR was performed with SYBR® Premix Ex Taq™ II (Takara Bio, Japan) and monitored by the Applied Biosystems StepOne™ instrument. The PCR conditions were as follows: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, 95 °C for 10 sec, 40 cycles at 95 °C for 5 sec, 62 °C for 20 sec, and 72 °C for 30 sec. All results were normalized using the mRNA level of two reference genes (*RpL4* for FAW larvae and *q-Actin* for *M. incognita*). Each reaction was performed in duplicate, and the mean of three independent biological replicates was calculated. Primer sequences are listed in **Table 1**. At the end of each qPCR, a melting curve analysis was performed at 95.0°C to check the quality of the used primers. The relative quantification of the target to the reference was determined using the $2^{-\Delta\Delta CT}$ method [53].

Table 1 Primers used in quantitative real-time PCR

Primers	Primer sequence 5`→3`	GenBank accession number	Reference
For *Spodoptera frugiperda* larvae			
SfAChE-F	TCAGCTACCCTGGAATGGTC	KC435023	(Carvalho et al., 2013)
SfAChE-R	AGTTCCGGTCGTTGAGTAGC	KC435023	(Carvalho et al., 2013)
SfCYP9A60-F	CATCGTTTGGCCAGAGAACT	KJ671579	(Giraudo et al., 2015)
SfCYP9A60-R	CAGGGTGTACAGCCAACTCA	KJ671579	(Giraudo et al., 2015)
RpL4-F	CAACAAGAGGGGTTCACGAT		(Giraudo et al., 2015)
RpL4-R	GCACGATCAGTTCGGGTATC		(Giraudo et al., 2015)
For *Meloidogyne incognita* J2s			
MiAChE1-F	ATGATGGATTATTCAATAGAGGACAG	KU366258	(Huang et al., 2016)
MiAChE1-R	CTATTTTATTCCACAAACATCATTATC ACC	KU366258	(Huang et al., 2016)
MiCYP-F	GTGGATCCGTTGACGTTCTTA	A0A914L7A7	(Wram et al., 2022)
MiCYP-R	ACGTCTAACCAAATGAGCAATAAC	A0A914L7A7	(Wram et al., 2022)
q-Actin-F	GGGTATGGAATCTGCTGGTAT		(Huang et al., 2016)
q-Actin-R	AGAAAGGACAGTGTTGGCGTA		(Huang et al., 2016)

7. Statistical analysis

Data was fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp.). Categorical data were represented as numbers and percentages. Quantitative data were expressed as mean and standard error, and the Student t-test was used to compare two groups for normally distributed quantitative variables. In contrast, the One-way ANOVA test was used to compare the different studied groups, followed by the Post Hoc test (Tukey) for pairwise comparison. The significance of the obtained results was judged at the 5% level. Based on concentration-mortality data, the lethal concentration (LC_{50}) value was determined using Origin (Pro), version 2016. OriginLab Corporation, Northampton, MA, USA.

Results

1. Microscopic characterization of the microalgae strain

The two microalgae strains *N. muscorum* (Cyanobacteria) and *C. vulgaris* (Chlorophyta) were employed in this investigation. Morphological examinations were conducted on both strains using a light microscope to measure the samples' widths and heights. Taxonomic identifications were performed, with strain one identified as *N. muscorum*. This strain is characterized by thickly entangled straight trichomes with no branching or tapering; short barrel-shaped or cylindrical vegetative cells without aerotopes; rounded apical cells; single, sub-spherical intercalary heterocyst; and oblong akinetes with yellow smooth wall when visible (**Fig. 1a**). Furthermore, the morphometry data support the identification of strain *C. vulgaris* (MK283687). The morphological features of this algal isolate revealed that its cells were unicellular spherical, measuring only 2 to 10 µm in diameter, either single or forming colonies, containing a single chloroplast with a parietal pyrenoid present and lacking flagella (**Fig. 1b**).

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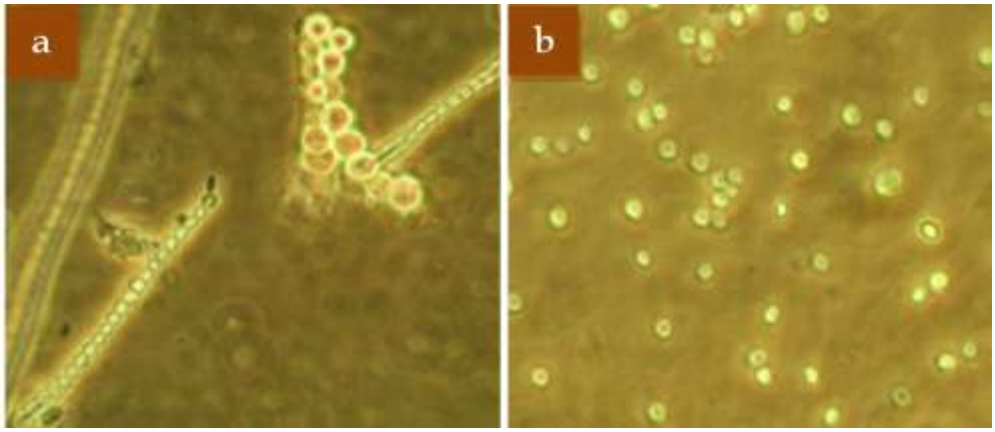

Fig. 1 Microalgae identification. Magnified light micrograph of **(a)** *Nostoc muscorum* and **(b)** *Chlorella vulgaris* methanol extracts (40 ×)

2. Phenol and flavonoid identification by HPLC

The HPLC methanol extract profile results illustrated many peaks at retention times (RT) between 0 and 30 min. After 30 minutes, no extra peaks manifested, revealing that all compounds had been eluted from the column. The cadinene compounds were identified by comparing RT with those for undiluted, pure, original samples of the studied compounds. Moreover, phenol and flavonoid fractions were determined by HPLC (**Fig. 2**). Fourteen different types of phenolic compounds were identified in both extracts. Ten phenolic compounds were present in *N. muscorum* (gallic acid, protocatechuic acid, catechin, esculatin, vanillic acid, pyrocatechol, coumarin, cinnamic acid, 3,4- indol butyl acetic acid, and naphthyl acetic acid) **(Fig. 2a).** Only four were found in *C. vulgaris* (gallic acid, protocatechuic acid, catechin, and esculatin) **(Fig. 2b)**.

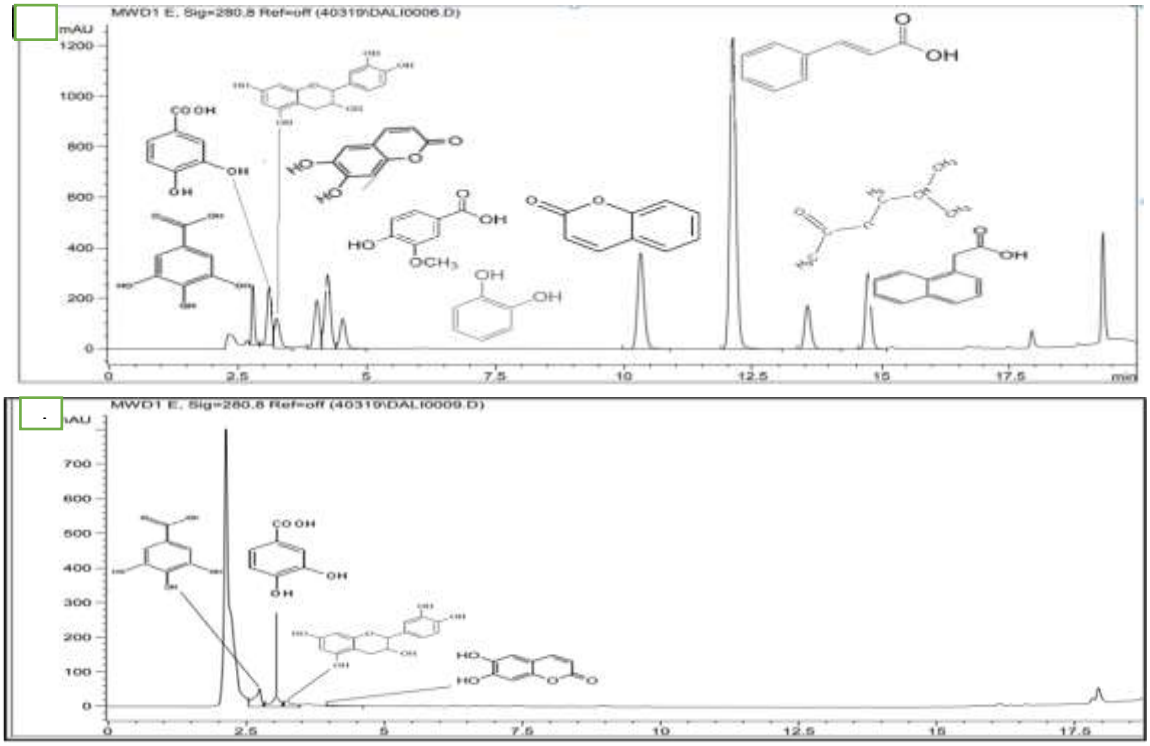

Fig. 2 HPLC chromatogram of methanolic extraction and quantitative content of detected compounds **(a)** in *Nostoc muscorum* extracts and **(b)** in *Chlorella vulgaris* extracts

3. Insecticidal activity of *N. muscorum* and *C. vulgaris* extracts

As shown in **Fig. 3a,** the highest concentration (100%) of the two extracts caused a highly significant larval mortality percentage (73% and 67%) for *N. muscorum* and *C. vulgaris,* respectively, after three days compared to the control group. The percentages of mortality considered good achievement, especially those supported by the decreasing percent of pupation and adult emergence, and even more than that, there is no emergency of moths at all when being treated with *N. muscorum*

extract, as it appears in **Fig. 3b**. Although 100% of *N. muscorum* (N4) caused the highest mortality percent overall (90%), the highest concentration (100%) of *C. vulgaris* extracts (C4) showed the highest mortality percent (23% after 24 hours) **(Fig. 3a)**. This is due to the fact that the latter's extract contains more gallic acid (42 mg/mL) **(Fig. 2b),** which accelerates the inhibition of the insect's metabolism. Low concentrations (25, 50%) of *N. muscorum* methanolic extract (N1 and N2) led to shorter life spans of larvae (5, 4 days). Still, high concentrations (75, 100%) (N3 and N4) led to longer lives (7 and 12 days), respectively, with significant differences to the control group **(Fig. 3c)**. The *C. vulgaris* extract had the same effect on larvae, where C1 and C2 as low concentrations led to shorter lifespans to 5 and 3 days, respectively; however, high concentrations (C3 and C4) brought about elongated larval duration (14 and 16 days), respectively, compared with control with different significant levels, while all concentrations of the two algal extracts led to longer lifespan pupae. The lethal concentration assays of *N. muscorum* and *C. vulgaris* extracts after 72 hours were 60.87% and 70.47% with *N. muscorum* and *C. vulgaris* extracts, respectively, as illustrated in **Fig. 4.**

In addition, deformities appeared in the larvae, pupae, and adults due to treating FAW larvae with 100% *N. muscorum* methanolic extract **(Fig. 5b-d)**. This extract (N4) revealed a strange phenomenon: the old cuticle of larvae was attached to the pupa, and the pupa was stuck on the deformed adult; more overall concentrations resulted in no moth emerging. However, the larvae only deformed due to C4 treatment **(Fig. 5e)** compared to the control larvae **(Fig. 5a)**.

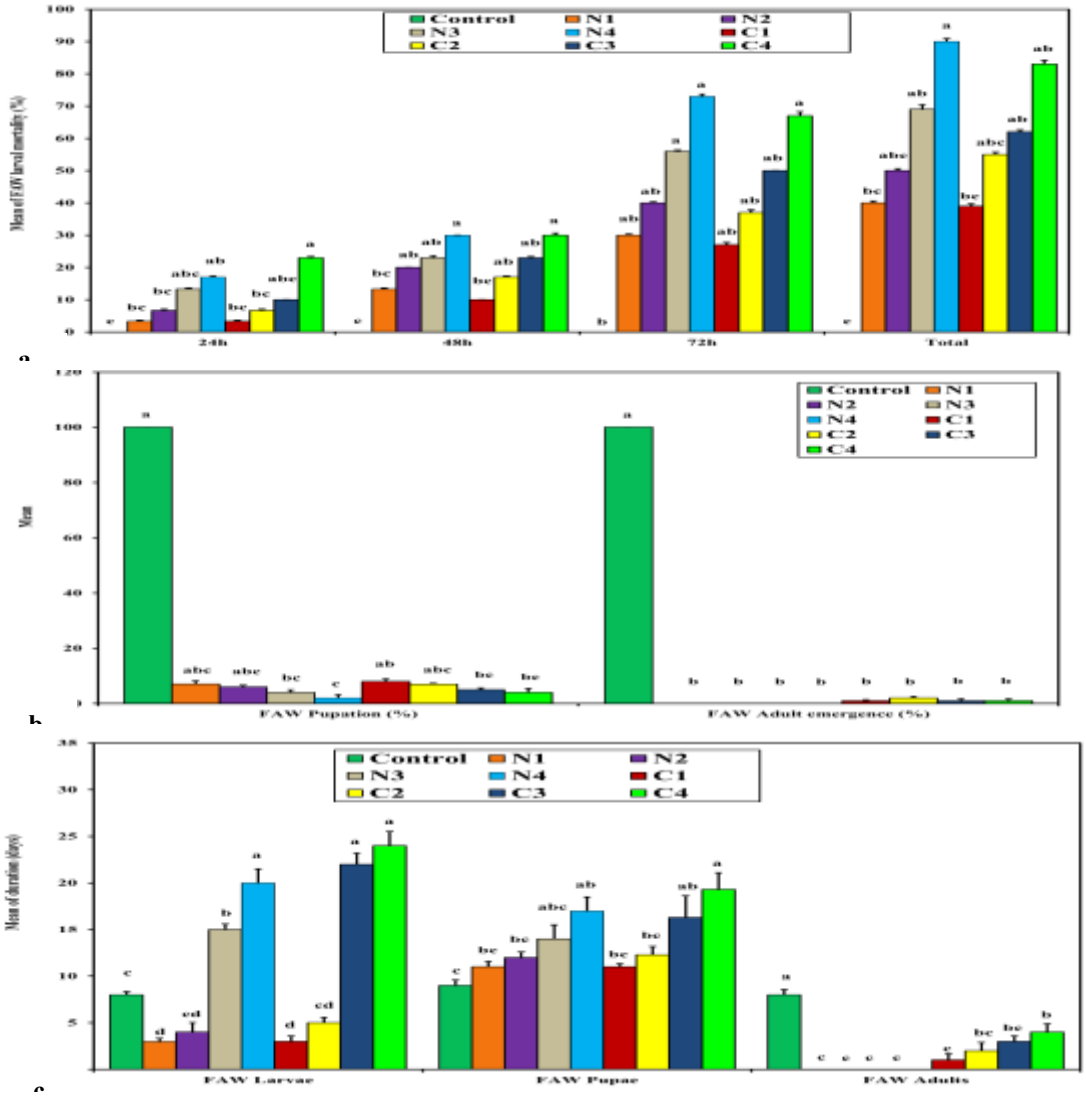

Fig. 3 The lethal effect of *Nostoc muscorum* and *Chlorella vulgaris* methanol extracts on the 3rd larval instar of *Spodoptera frugiperda*. **(a)** Mean percentages (± SE) of larval mortality after 24-, 48-, and 72-hours of treatment; **(b)** mean percentages (± SE) of pupation and adult emergence post-treatment; **(c)** mean (± SE) of duration of larvae, pupae, and adults after algal treatment. Means followed by the different letters of alphabets are significantly different according to Tukey's test at $p \leq 0.001$ level.

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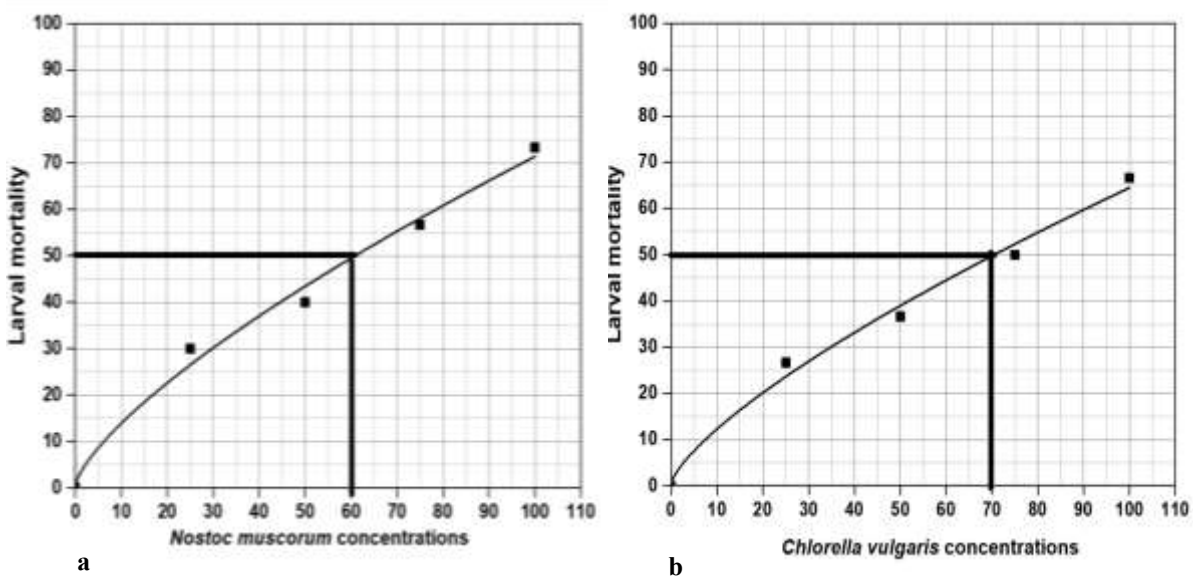

Fig. 4 The lethal concentration assay of **(a)** *Nostoc muscorum* extract with LC_{50} = 60.87% and **(b)** *Chlorella vulgaris* extract with LC_{50} = 70.47% after 72 hours post-treatment of *Spodoptera frugiperda* larvae

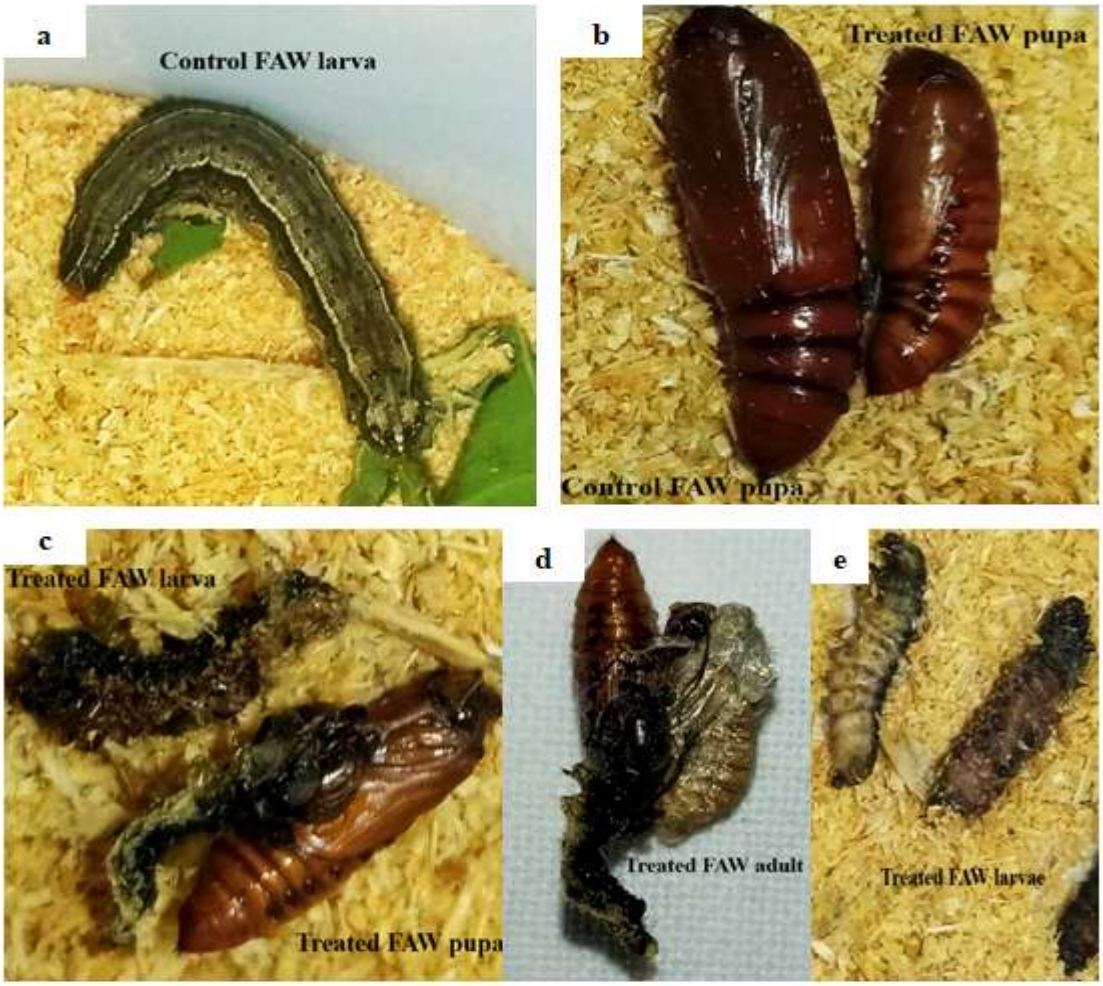

Fig. 5 *Spodoptera frugiperda* malformations after feeding on castor leaves treated by 100% of **(b-d)** *Nostoc muscorum* methanolic extracts and **(e)** by 100% of *Chlorella vulgaris* extract. **(a)** The control FAW larva; **(b)** FAW pupae (control on the left and abnormal treated on the right); **(c)** malformed FAW (larva on the left and pupa with detached old cuticle on the right); **(d)** deformed of three stages (larva, pupa, and adult); **(e)** dead larvae

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Based on this result, concentrations N3, N4, C3, and C4 were determined in both extracts to study the enzymatic activity inside the insect after treatment with them. The results in **Fig. 6a** showed that the enzymatic activity of AST was lower than in hemolymph that of the control, with significant differences after 24 hours of treatment of 3rd FAW larvae. Differences also appeared between the two extracts, as *C. vulgaris* had less AST activity (16U/L ± 1.2) than *N. muscorum* extract (22U/L ± 1.2) after 24 hours post-treatment compared to that in the control (41U/L ± 0.5). In contrast, the activity increased significantly after 48 and 72 hours. When observing the results of the ALT enzyme activity (**Fig. 6b**), it was found that the *C. vulgaris* extract gave the same activity as the previous enzyme, while the *N. muscorum* extract did not differ from the control after 24 hours, it increased significantly after 48 and 72 hours. By continuing to read the results of the enzymatic ALP activity of hemolymph FAW larvae (**Fig. 6c**), it was found that treatment with both methanolic extracts led to a significant increase after 24 hours, then a significant decline after 48 and 72 hours compared to the control. Significant differences were also shown between the highest concentration of *C. vulgaris* extract (C4) after 72 hours and the rest of the treatments, with the latter being more significant and sharply declined (13.3U ± 3.3) when also compared to ALP activity in the hemolymph of the control larvae (69.3U ± 3.5).

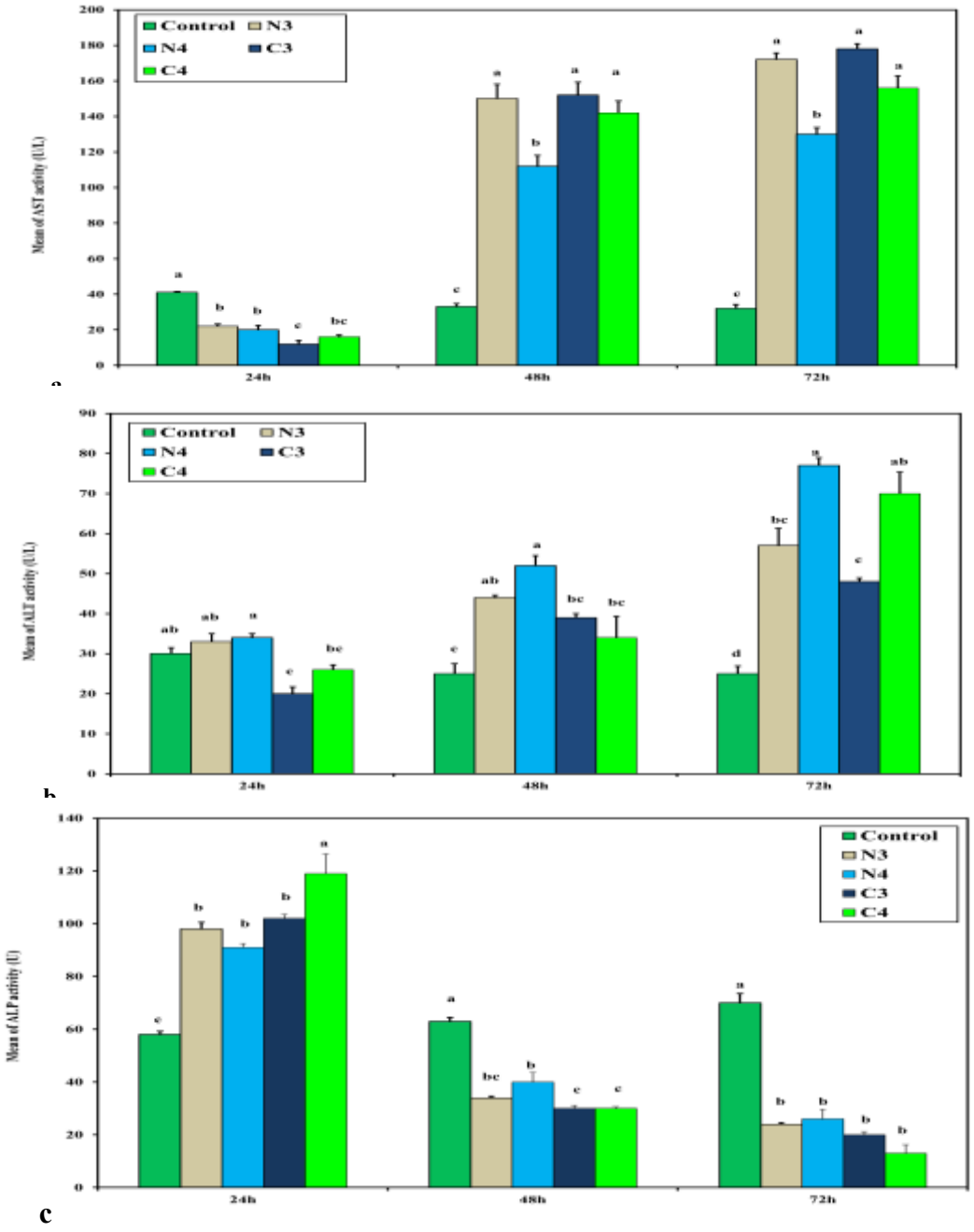
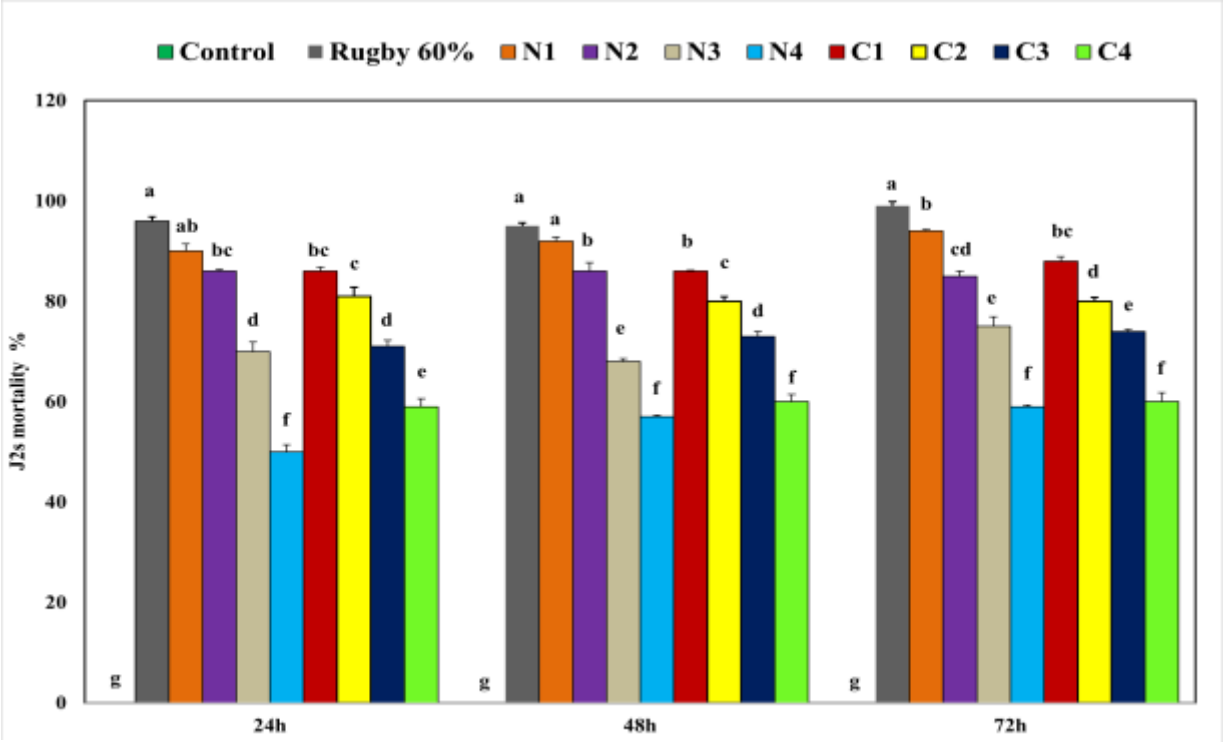


Fig. 6 Effect of (75% and 100%) *Nostoc muscorum* and *Chlorella vulgaris* methanol extracts on *Spodoptera frugiperda* hemolymph activities of **(a)** AST, **(b)** ALT, and **(c)** ALP after 24-, 48-, and 72-hours of treatment. Data was expressed using Mean ± SE. Means followed by the different letters of alphabets are significantly different according to Tukey's test at $p \leq 0.001$ level

4. Nematicidal activity of *N. muscorum* and *C. vulgaris* extracts

The nematicidal activity of both *N. muscorum* and C. *vulgaris* extracts was investigated against the J2s stage of *M. incognita* and compared to nematicide Rugby 60%. The results in **Fig. 7a** showed that the treatment with N1 extract significantly reduced mortality rates, with values of 90, 92, and 94% compared to the negative control (96, 95, 99%) after 24, 48, and 72 hours, respectively. Similarly, the treatment with C1 extract also showed high mortality reduction rates of 86%, 86%, and 88% compared to Rugby. In addition, the results of the nematicidal activity of *N. muscorum* and C. *vulgaris* assays revealed that the algal extract adversely indicated the toxicity of J2s at higher concentrations. The lethal concentration assays of *N. muscorum* and C. *vulgaris* extracts after 72 hours of soaking the nematode larvae were 97.34% and 89.53%, respectively, as illustrated in **Fig. (7b-c)**. Considering the Rugby 60% nematicide potential, both *N. muscorum* and C. *vulgaris* microalgae could be utilized to develop nematocidal products.



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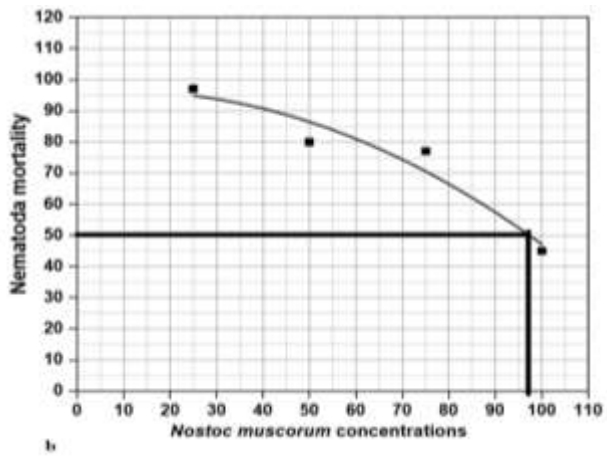

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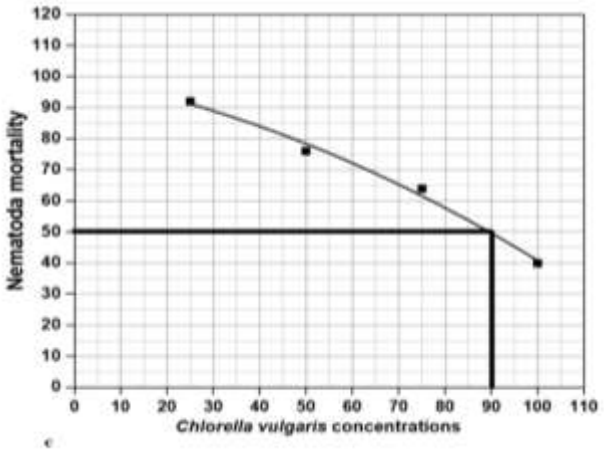

c

Fig. 7 Efficacy of *Nostoc muscorum and Chlorella vulgaris* extracts against J2s of *Meloidogyne incognita.* **(a)** Mean percentages (± SE) of J2s mortality after 24-, 48-, and 72-hours of treatment. Means followed by the different letters of alphabets are significantly different according to Tukey's test at $p \leq 0.001$ level. The lethal concentration assay of **(b)** *N. muscorum* extract with LC_{50} = 97.34%; **(c)** *C. vulgaris* extract with LC_{50} = 89.53% after 72 hours post-treatment of *M. incognita* (J2s)

The hatching percentage of *M. incognita* eggs decreased significantly, and this decrease increased proportionally with increasing concentrations from 25%, 50%, and 75% to 100% at the time of exposure to CuONPs in vitro (Fig. 8).

Whereas immersion of *M. incognita* eggs in Nostoc reduced the egg hatching percentage to 80, 70.7, 66.3, and 65.8%, respectively, after 24 hours of exposure, it also led to a decrease in the number of eggs by 89.1, 83.9, 71.8, and 52.11% after 48 hours of exposure compared to the commercial pesticide (Rugby), when all treatments were compared with the untreated treatment (H_2O).

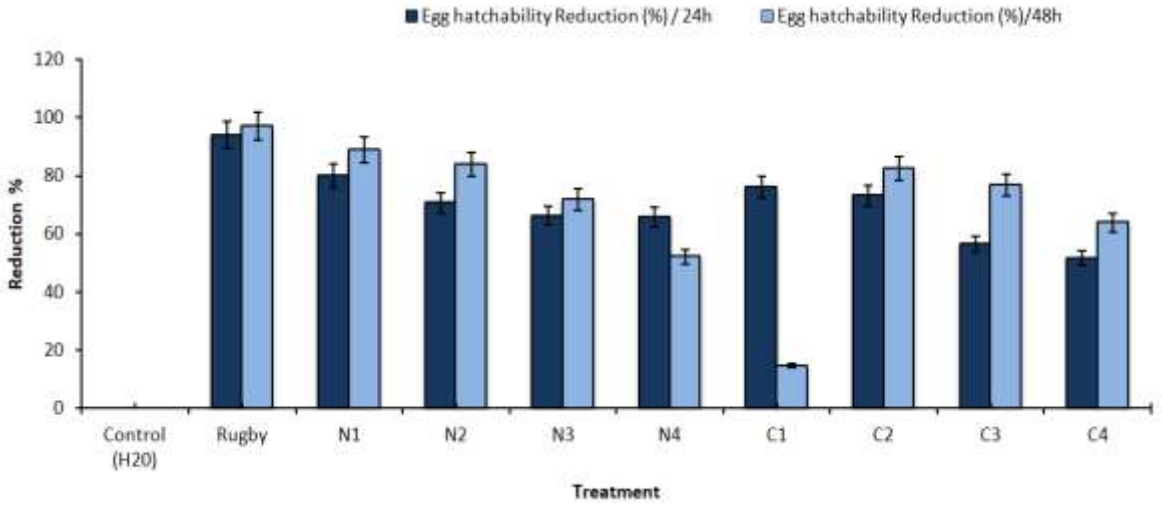

Fig. 8 Efficacy of *Nostoc muscorum and Chlorella vulgaris* extracts against eggs of *Meloidogyne incognita.* **(a)** Mean percentages (± SE) of J2s mortality after 24 and 48 hours of treatment. Abbreviation: (N1) *N. muscorum* at 25% concentration; (N2) *N. muscorum* at 50% concentration; (N3) *N. muscorum* at 75% concentration; (N4) *N. muscorum* at 100% concentration; (C1) *C. vulgaris* at 25% concentration; (C2) *C. vulgaris* at 50% concentration; (C3) *C. vulgaris* at 75% concentration; (C4) *C. vulgaris* at 100% concentration; (S.E) Standard error

5. Defense -related gene expression levels in 3rd FAW larvae and *M. incognita* J2s

To insight into the ability of *N. muscorum* and *C. vulgaris* methanolic extracts (75% and 100%) on the immune system, the levels of expression of AChE and CYP genes were detected in 3rd FAW larvae and J2s of *M. incognita* using quantitative real-time PCR **(Fig. 9)**.

In FAW larvae, the results revealed that the expression level of *SfAChE* had the most significant downregulation when the larvae were treated with N3 after 72 hours post-treatment **(Fig. 9a)**. The highest expression of *SfAChE* mRNA was observed in the C4 treatment after 24 hours. It was shown to be expressed more than five-fold higher than the control group. Furthermore, *SfCYP9A60* showed a similar significant increase in expression levels after 24 hours when 3rd larval instars were treated with C4 **(Fig. 9b)**. In contrast to *SfAChE*, the expression level of *SfCYP9A60* declined sharply when C3 extract was applied to FAW larvae 72 hours post-treatment.

The results of J2s of *M. incognita* indicated that *MiAChE1* showed a specific high expression level when N4 and C4 treated J2s after 48 h and 72 h post-treatment, respectively. The N4 methanolic extract after 24 h and 72 h showed the lowest expression levels of *MiAChE1 and MiCYP,* respectively, compared to untreated samples **(Fig. 9c - d)**.

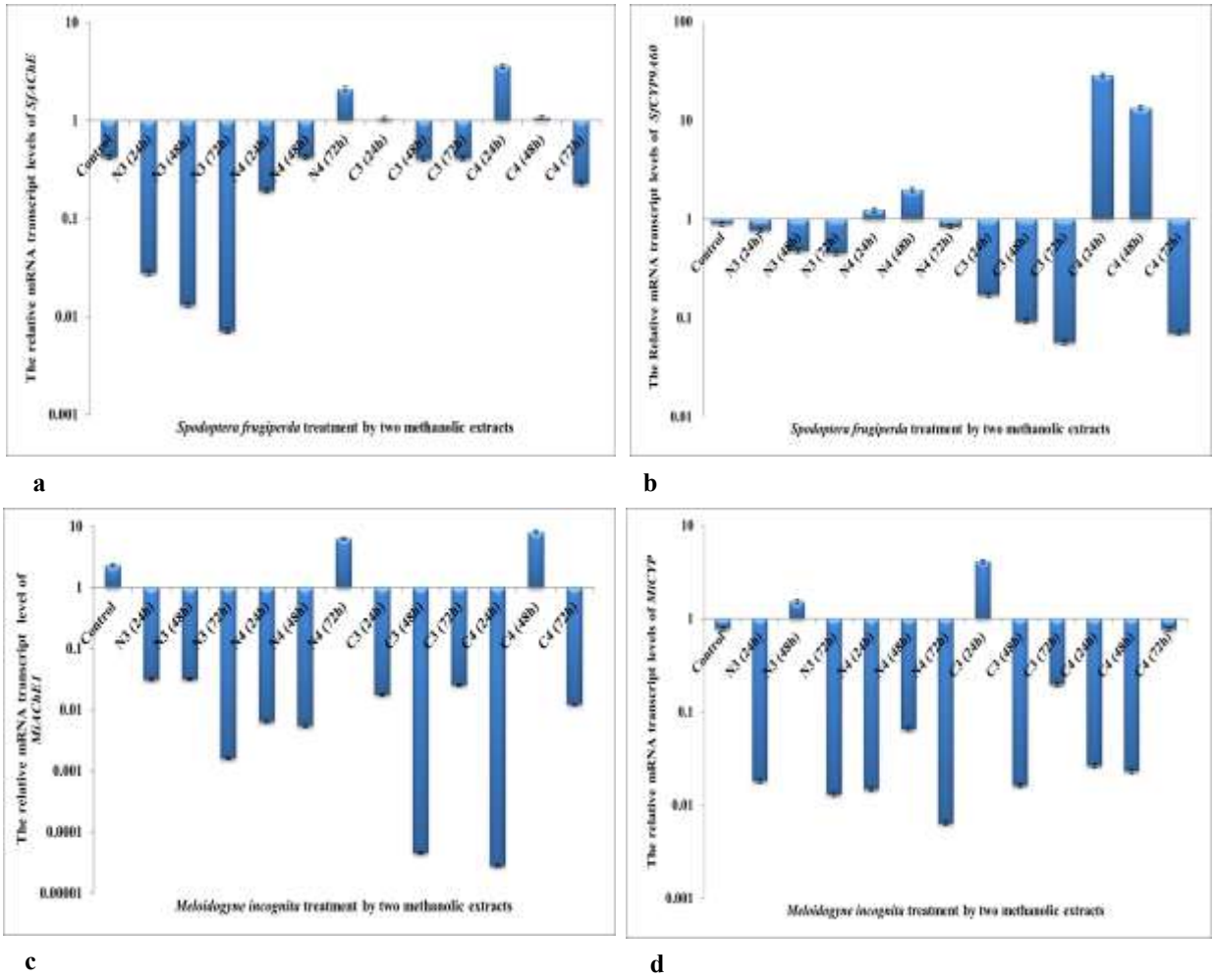

Fig. 9 Relative expression levels of **(a)** *SfAChE*, **(b)** *SfCYP9A60*, **(c)** *MiAChE1* and **(d)** *MiCYP* messenger RNA in *Spodoptera frugiperda* larvae and *Meloidogyne incognita*, respectively following treatment by (75% and 100%) of *Nostoc muscorum* and *Chlorella vulgaris* methanol extracts after 24-, 48-, and 72-hours of treatment. *RpL4* and *q-Actin* were used as the reference genes in quantitative real-time PCR. Reactions from three biological replicates were repeated three times. Error bars represent the standard error of the mean.

(*SfAChE*) *S. frugiperda* acetylcholinesterase; (*SfCYP9A60*) *S. frugiperda* cytochrome P450; (Rugby) rugby commercial nematicide; (*MiAChE1*) *M. incognita* acetylcholinesterase 1; (*MiCYP*) *M. incognita* cytochrome P450 and (*RpL4*) ribosomal protein L4

Discussion

The present study was directed to find a biological control and eco-friendly strategy, such as *N. muscorum* and *C. vulgaris* methanolic extracts to minimize the hazardous effects of FAW larvae and *M. incognita*. The obtained results revealed that the two algal extracts caused high percentages of FAW larval mortality and successfully affected the performance of the FAW development periods and adult emergence (**Fig. 3**). The increase in the larval and pupal periods might be due to the treatment-binding to the midgut epithelium and affecting the digestive system; this might also affect the percent pupation. Algae are considered a rich source of phenolics, among other physiologically active substances (**Fig. 2**), which act as feeding repellents that hinder the growth and survival of larvae [54]. Consequently, the presence of pupal and adult malformation may be due to the effect of the toxicant during metamorphosis [55]. Furthermore, gallic acid, a phenolic compound found in *C. vulgaris* extract in a larger amount than that in *N. muscorum*, might inhibit larval growth even at lower concentrations. This agreed with the previous study, which found that treating *S. litura* with gallic acid affected the fitness and survival of the phytophagous larvae [56]. The same algal extracts were found to impact on *S. littoralis*, as evidenced by the prolonged larval lifetime of the pest [57]. Its effect on the pupae was manifested as a longer pupation duration and a decrease in their percentage. Furthermore, there were deformed adults with an unusual body and slightly bent wings (**Fig. 5**). Deformities observed in FAW larval instars and other developmental stages, were highly compatible with the findings of prior

investigations employing the dry biomass of four cyanobacterial microalgal strains. One is *N. muscorum* conducted with *S. littoralis* [13].

Additionally, the presence of phenolics in algal extracts demonstrated prooxidant activity, primarily in the lumen of the insect gut, where they might have inhibited the absorption of food and caused oxidative destruction of the midgut epithelium [54]. Complicated enzymatic antioxidant systems helped insects combat reactive oxygen produced during stressful situations. The number of oxygen-free radicals generated, and the antioxidant defense enzymes were in balance under normal physiological conditions. The organisms use antioxidant enzymes to deactivate and shield free radicals from harm. The present results propose that algal extracts cause synaptic and metabolic dysfunctions in fall armyworm larvae and change their biochemical physiology in response to oxidative stress. The extracts caused high levels of AST, ALT, and ALP activities in FAW hemolymph after 24 hours of algal treatments. They led to other overlapping changes after 48 and 72 hours (**Fig. 6**). The obtained data agreed with that AST and ALT activities of wax moth larvae, *Galleria mellonella* (L.), as biochemical stress markers, were significantly higher in the larvae whose diet contains high levels of organophosphorus insecticides than in control larvae [58]. The previous results about the effect of two algal extracts on the essential physiology process inside FAW larvae were compatible with the increased ALP activity in diapausing beetles [59].

Moreover, the activity levels of AST, ALT, and ALP as enzymatic components increased in the hemolymph of the sunn pest, *Eurygaster integriceps* (Puton). They caused the dysfunction of two endocrine hormones, juvenile and ecdysone, which had a profound role during the developmental stages of insects, by using insecticides [60]. In addition, using essential oils as insecticides caused a change in AST and ALT levels in the red flour beetle, *Tribolium castaneum* (Herbst) [61]. Whereas in most pyriproxyfen treatments, there was a significant decrease in the quantity of these biological enzymes, but after 120 hours, these enzymes shifted differently in some cases; that happened exactly in the case of the AST and ALP [62]. In *G. mellonella* hemolymph, the variations in metabolic enzyme activity were studied, and the two biomarkers for cell damage were discovered to have elevated AST and ALT activities due to oxfendazole treatment [63]. In the studies applied to *G. mellonella* by four plant growth regulators, disturbed ALP activity was observed [64]. Consequently, phenolics in algal extracts had deterring and insecticidal effects on FAW larvae.

The effectiveness of microalgal extracts on J2s nematodes, *M. incognita*, was summarized in **Fig. 7**. It was important to notice that the result revealed that the nematode number in the microalgal extract treatment was significantly different ($p < 0.05$). For *N. muscorum* extract (N1), nematode J2s inhibition was observed (90%, 92%, and 94%) compared to the negative control after 24, 48, and 72 hours of treatment, respectively, for C1 extract had reduced nematodes J2s by 88% after 72 hours. The results were in harmony with applying algal extracts to seven algae in 60–80% concentrations, and the J2s mortality reached 90% after 96 h of treatment [65]. Moreover, *C. vulgaris* was used to manage the root-knot nematode, and the Bulgarian organic commercial product from the dry extract of *C. vulgaris* also had the highest results [66]. The results revealed that each *N. muscorum* and *C. vulgaris* extract had prominent nematicidal effects due to the presence of the most vital components and impacted nematode juveniles directly or indirectly. In addition, they influenced resistance in nematode tissues, hindering and preventing nematode penetration and feeding after treatment. Many phytochemicals with dynamic biological effects showed efficacy against various pathogens, including nematodes [67]. Exploring natural material-based nematicidal substances has improved safe and sustainable alternative approaches for nematode management [68]. Accordingly, it was suggested that the microalgal extract treatment could aid in nematode suppression and assign these two microalgal metabolites to the secondary metabolites.

It could be understood from the present findings that feeding on *N. muscorum* and *C. vulgaris* methanolic extracts compromised the cellular immune response of FAW larvae and J2s nematodes and adversely affected the essential physiology processes inside the insect. Some key detoxification enzymes were capable of metabolizing plant secondary compounds and insecticides and conferring protection to lepidopteran larvae against stress, among which AChE and cytochrome P450s (or CYP) played a significant role [69][70]. To ascertain a role for the AChE and CYP genes in their immunity, the expression levels of both genes were analyzed by quantitative real-time PCR (**Fig. 9**). After 24 hours of C4 treatment, the gene expression level of *SfAChE* recorded the highest activity. The current investigation supported that the AChE genes in FAW regulate larval development and insecticidal sensitivity in different ways [32]. Furthermore, for J2s, the expression profile of *MiAChE1* was increased after the treatment with N4 and C4. The increased expression level of the AChE enzyme in response to the treatment seems to be a dominant mechanism conferring resistance in the pest [33].

Interestingly, the CYP gene showed higher expression in FAW larvae (*SfCYP9A60*) and J2s nematode (*MiCYP*) after 24 h of treatment with C1 extract. CYP has essential endogenous functions and majorly influences pest adaptations to their external environment [71]. Most CYPs were expressed in the midgut, *Malpigian tubules*, and fat bodies of insects, and in the nematode intestine, constituting the principal organs of detoxification [72]. In the lepidopteran *Helicoverpa zea*, (Boddie) two P450s in particular, *CYP6B8* and *CYP321A1*, had been shown to metabolize plant toxins and insecticides [73]. The overexpression of *CYP6G1* in *Drosophila melanogaster* strains was responsible for multi-insecticide resistance [74]. Furthermore, albendazole (ABZ), as a benzimidazole anthelmintic, induced the expression of *CYP35A2*, *CYP35A5*, and *CYP35C1* in *Caenorhabditis elegans* nematodes [75].

Conclusion

The algal extracts caused several shortages for the normal development and reproduction of insects, in addition to root-knot nematodes larvae, leading to many symptoms in general, which were a high mortality rate of FAW larvae, a low pupation percent, abnormal pupae, and moths, in addition to juvenile mortality and egg hatchability of nematodes. Since these algae are readily available and widely grown, they can be efficaciously employed by small-scale farmers to manage the fall armyworm and root-knot nematodes without resorting to harmful chemical pesticides, given environmental pollution. Finally, the findings from this investigation indicated that the 100% and 75% concentrations of *N. muscorum* (N3, N4) and *C. vulgaris* (C3, C4) extracts enhanced excellent insecticidal and nematicidal activity against fall armyworm, *S. frugiperda,* and root-knot nematode *M. incognita* and might be a possible substitute for synthetic chemically harmful insecticides and nematicides.

Authors' contributions: The idea and design of the methodology for this study were instrumental by [Hanan Alfy]. [Marian Malak] and [Rehab Ghareeb] performed material preparation, and data collection. The analysis and first draft of the manuscript was written by [Hanan Alfy], [Ahmed El-Sabrout], and [Rehab Ghareeb]. Visualization, data curation, manuscript writing, review, and editing were performed by [Marian Malak] and [Hanan Alfy]. All authors read and approved the final manuscript.

Data Availability: The data supporting this study's findings are not openly available due to sensitivity reasons and are available from the corresponding author upon reasonable request.

Reference

1. Guerrero A, Malo EA, Coll J, Quero C. Semiochemical and natural product-based approaches to control Spodoptera spp. (Lepidoptera: Noctuidae). J. Pest Sci. 2014; 87,231-247. DOI: 10.1007/s10340-013-0533-7
2. Midega CA, Pittchar JO, Pickett JA, Hailu GW, Khan ZR. A climate-adapted push-pull system effectively controls fall armyworm, *Spodoptera frugiperda* (JE Smith), in maize in East Africa. Crop Prot. 2018; 105,10-5. https://doi.org/10.1016/j.cropro.2017.11.003
3. Dahi HF, Salem SA, Gamil WE, Mohamed HO. Heat requirements for the fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) as a new invasive pest in Egypt. *EAJBSA* .2020; 13(4),73-85. doi: 10.21608/eajbsa..120603
4. Mohamed HO, El-Heneidy AH, Dahi HF, Awad AA. First record of the fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) on sorghum plants, a new invasive pest in Upper Egypt. *EAJBSA*. 2022; 15(1),15-23. doi: 10.21608/eajbsa.2022.214719
5. Bengyella L, Hetsa BA, Fonmboh DJ, Jose RC. Assessment of damage caused by evolved fall armyworm on native and transgenic maize in South Africa. *Phytoparasitica* . 2021; 49(1): 1-2. https://doi.org/10.1007/s12600-020-00862-z
6. Idrees A, Qadir ZA, Afzal A, Ranran Q, Li J. Laboratory efficacy of selected synthetic insecticides against second instar invasive fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae. *PLoS One* . 2022; 17(5),e0265265. doi: 10.1371/journal.pone.0265265.
7. FAO. Briefing note on FAO 2019. actions on fall armyworm. http://www. fao.org/3/BS183E/bs183e.pdf
8. FAO. (2020). The global action for fall armyworm control: Action framework 2020-2022. Working together to tame the global threat.
9. Shamseldean MSM, Abo-Shady NM, El-Awady MA, Heikal MN. *Heterorhabditis alii* n. sp. (Nematoda: Heterorhabditidae), a novel entomopathogenic nematode from Egypt used against the fall armyworm, *Spodoptera frugiperda* (Smith 1797) (Lepidoptera: Noctuidae). *EJBPC*. 2024; 34(1):13. https://doi.org/10.1186/s41938-024-00778-4
10. Abo Elela MR, El-Gabaly AR. Toxicity and Biological Effects of Jojoba oil, Lufenuron, and Protecto against *Spodoptera frugiperda* (Lepidoptera: Noctuidae), In Egypt. *Egy. Sci. J. Pestic.* 2024); 10 (3):1-6.
11. Escobar C, Barcala M, Cabrera J, Fenoll C. Overview of root-knot nematodes and giant cells. *InAdvances in botanical research*. 2015; (73,1-32). Academic Press. https://doi.org/10.1016/bs.abr.2015.01.001
12. Atkinson HJ, Lilley CJ, Urwin PE. Strategies for transgenic nematode control in developed and developing world crops. *Curr Opin Biotechnol* .2012; (2):251-6. doi: 10.1016/j.copbio.2011.09.004.
13. Abdel-Rahim EF& Mohamed SM. Comparative toxic activity of four algae, against the 2nd and 4th larval instars of black cutworm, *Agrotis ipsilon* (Hufnagel). *Egypt J. Agric. Res.* 2013; 91(4):1303-18. DOI: 10.21608/EJAR.2013.165113
14. Holajjer P, Kamra A, Gaur HS, Manjunath M. Potential of cyanobacteria for biorational management of plant parasitic nematodes: a review. *Crop Prot.* 2013; 53,147-51. https://doi.org/10.1016/j.cropro.2013.07.005
15. Zhao L, Feng C, Wu K, Chen W, Chen Y, Hao X, Wu Y. Advances and prospects in biogenic substances against plant virus: A review. *Pestic Biochem. Physiol.* 2017; 135,15-26. DOI: 10.1016/j.pestbp.2016.07.00

16. Khan MI, Shin JH, Kim JD. The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microb Cell Fact* . 2018; 17(1):36. DOI: 10.1186/s12934-018-0879-x
17. Sharanappa CH, Bheemanna M, Prabhuraj A, Harischandra RN, Nagaraj MN, Saroja NR, Kariyanna B. Toxic effect of cyanobacterial (blue-green algae) extracts as natural pesticides for the control of *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae). *EJBPC* .2023; 33:77. https://doi.org/10.1186/s41938-023-00716-w
18. Sharanappa CH, Bheemanna M, Prabhuraj A, Naik HR, Naik NM, Rao SN, Kariyanna B. Investigation on the insecticidal activities of cyanobacterial extracts as an alternative source for the management of fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae). *Heliyon* .2024; 10(7),e29060. https://doi.org/10.1016/j.heliyon.2024.e29060
19. Ru ITK, Sung YY, Jusoh M, Wahid MEA, Nagappan T. *Chlorella vulgaris*: A perspective on its potential for combining high biomass with high value bioproducts. *Applied Phycology* .2020;1(1):2-11. https://doi.org/10.1080/26388081.2020.1715256
20. Sigamani S, Chinnasamy R, Dharmaraj RK, Ramamurthy D, Devarajan N, Narayanasamy M, Natarajan H. Larvicidal potency of the extracts from *Chlorella* sp. against *Aedes aegypti*. *I S B A B*. 2020; 27:101663. https://doi.org/10.1016/j.bcab.2020.101663
21. El-Eslamboly AASA, Abd El-Wanis MM, Amin AW. Algal application as a biological control method of root-knot nematode *Meloidogyne incognita* on cucumber under protected culture conditions and its impact on yield and fruit quality. *EJBPC*. 2019; 29(1):18.
22. Ghareeb RY, Alfy H, Fahmy AA, Ali HM, Abdelsalam NR. Utilization of *Cladophora glomerata* extract nanoparticles as eco-nematicide and enhancing the defense responses of tomato plants infected by *Meloidogyne javanica*. *Sci. Rep.* 2020; 10(1):19968. doi:10.1038/s41598-020-77005-1
23. Bouyahya A, Bakrim S, Chamkhi I, Taha D, El Omari N, El Mneyiy N, Aanniz T. Bioactive substances of cyanobacteria and microalgae: Sources, metabolism, and anticancer mechanism insights. *Biomed. Pharmacother.* 2024; 170,115989. doi: 10.1016/j.biopha.2023.115989
24. Premkumar C, Divya M, Krishnaveni N, Santhanam P, Pachiappan P. An Estimation of Antimicrobial and Antioxidant Activity of Microalgae. *In: Santhanam, P., Begum, A., Pachiappan, P.* (eds) Basic and Applied Phytoplankton Biology. Springer, Singapore. 2019. 303-314 https://doi.org/10.1007/978-981-10-7938-2_17
25. Rezayian M, Niknam V, Ebrahimzadeh H. Oxidative damage and antioxidative system in algae. *Toxicol. Rep.* 2019; 6:1309-13. DOI: 10.1016/j.toxrep.2019.10.001
26. Teleb SS, Ahmed FA, Mostafa AMEffect of Pyridalyl on alkaline phosphatase and transaminase activities in some tissues of *Schistocerca gregaria* (Orthoptera: Acrididae). *J. Am. Sci.* 2012; 8(12):315-322.
27. Arrese EL, & Soulages JL. Insect fat body: energy, metabolism, and regulation. *Annu. Rev. Entomol.* 2010;55,207-225. doi:10.1146/annurev-ento-112408-085356
28. Matić D, Vlahović M, Grčić A, Filipović A, Ilijin L, Mrdaković M, Perić-Mataruga V. Antioxidative enzymes, alkaline phosphatases and Hsp70 expression in larvae of *Lymantria dispar* (Lepidoptera: Erebidae) from unpolluted and polluted forests after chronic cadmium treatment. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 2023; 273,109721. doi: 10.1016/j.cbpc.2023.109721.
29. Khan RA, Rashid M, Wang D, Zhang YL. Antibody-based detection of alkaline phosphatase in lepidopteran insects (Lepidoptera: Noctuidae). *G M R 1.* 2013;(4):4371-4382. DOI: 10.4238/2013.October.10.3
30. Rauschenbach IY, Chentsova NA, Alekseev AA, Gruntenko NE, Adonyeva NV, Karpova, EK, Bownes M. Dopamine and octopamine regulate 20-hydroxyecdysone level in vivo in *Drosophila*. *Arch. Insect Biochem. Physiol.* 2007; 65(2):95-102. doi: 10.1002/arch.20183.
31. Johnson TO, Ojo OA, Ikiriko S, Ogunkua J, Akinyemi GO, Rotimi DE, Adegboyega A E. Biochemical evaluation and molecular docking assessment of *Cymbopogon citratus* as a natural source of acetylcholine esterase (AChE)- targeting insecticides. *Biochem. Biophys. Rep.* 2021;28,101175. doi: 10.1016/j.bbrep.2021.101175.
32. Gao J, Gong LF, Wang HH, Zhao R, Xiao X, Tian XY, Gu SH. Expression and functional analysis of *ace1* and *ace2* reveal their differential roles in larval growth and insecticide sensitivity in *Spodoptera frugiperda* (JE Smith, 1797). *J. Pest. Sci.* 2023; 96(4):1651-66. https://doi.org/10.1007/s10340-023-01625-5
33. Muthusamy M, Shivakumar S, Karthi K, Ramkumar R. Pesticide detoxifying mechanism in field population of *Spodoptera litura* (Lepidoptera: Noctuidae) from South India. *Egypt Acad. J. Biolog. Sci.* 2011; 3(1):51-7. DOI: 10.21608/eajbsf.2011.17437
34. Salim AM, Shakeel M, Ji J, Kang T, Zhang Y, Ali E, Li J. Cloning, expression, and functional analysis of two acetylcholinesterase genes in *Spodoptera litura* (Lepidoptera: Noctuidae). *Comp. Biochem. Phys. B.* 2017; 206:16–25. https:// doi. org/ 10. 1016/j. cbpb. 2017. 01. 007

35. Mansoor MM, Raza ABM, Abbas N, Aqueel MA, Afzal M. Resistance of green lacewing, *Chrysoperla carnea* Stephens to nitenpyram: Cross-resistance patterns, mechanism, stability, and realized heritability. *Pestic. Biochem. Physiol.* 2017; 135,59-63. doi: 10.1016/j.pestbp.2016.06.004
36. Lee K, Eisterhold ML, Rindi F, Palanisami S, Nam PK. Isolation and screening of microalgae from natural habitats in the midwestern United States of America for biomass and biodiesel sources. *J. Nat. Sci. Biol. Med.* 2014; 5(2):333-9. doi: 10.4103/0976-9668.136178
37. Annadotter H, Cronberg G., Lawton L, Hansson HB, Göthe U, Skulberg O. An extensive outbreak of gastroenteritis associated with the toxic cyanobacterium *Planktothrix agardhii* (Oscillatoriales, Cyanophyceae) in Scania, South Sweden. *Cyanotoxins–occurrence, causes, consequences. Edited by Chorus I. Berlin: Springer–Verlag.* 2001; 200-8.
38. Ghareeb RY, Abdelsalam NR, El Maghraby DM, Ghozlan MH, El-Argawy E, Abou-Shanab RA. *Oscillatoria* sp. as a Potent Anti-phytopathogenic Agent and Plant Immune Stimulator Against Root-Knot Nematode of Soybean cv. Giza 111. *Front Plant Sci.* 2022; 13:870518. doi: 10.3389/fpls.2022.870518.
39. Abd El Moniem EA. & Abd-Allah ASE. Effect of green alga cells extract as foliar spray on vegetative growth, yield and berries quality of superior grapevines. *J. Amer. Eur. Agric. and Environ. Sci.* 2008; 4(4):427-433.
40. Sarkar G, Jatar N, Goswami P, Cyriac R, Suthindhiran K, Jayasri MA. Combination of different marine algal extracts as biostimulant and biofungicide. *J. Plant Nutr.* 2018; 41(9):1163-71. https://doi.org/10.1080/01904167.2018.1434201
41. Singh SP, Tewari D, Patel K, Jain GK. Permeability determination and pharmacokinetic study of nobiletin in rat plasma and brain by validated high-performance liquid chromatography method. *Fitoterapia.* 2011; 82(8):1206-1214. https://doi.org/10.1016/j.fitote.2011.08.010
42. Malak M. Albendazole-induced genotoxicity in the larvae of fall armyworm as a safe environmental tool. *SN Appl. Sci.* 2023;*5*:356 https://doi.org/10.1007/s42452-023-05582-7
43. Naeem-Ullah U, Ramzan M, Saeed S, Iqbal N, Sarwar ZM, Ali M, Ghramh HA. Toxicity of four different insecticides against *Trilocha varians* (Bombycidae: Lepidoptera). *J. King Saud Univ. Sci.* 2020; *32*:1853-1855. https://doi.org/10.1016/j.jksus.2020.01.032
44 . Altaf N, Ullah MI, Arshad M, Afzal M, Al-Shuraym LA, Mehmood N, Sayed SM. The chemical composition and biological activities of plant essential oils against *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae). *JPDP.* 2024; 131(3):705-17. https://doi.org/10.1007/s41348-024-00887-0
45. Mansoor MM. Risk assessment of cyromazine resistance in a field population of *Sesamia inferens* (Walker): Cross-resistance, inheritance, and realized heritability. *Phytoparasitica*. 2023 ;51(3):547-58. https://doi.org/10.1007/s12600-023-01072-z
46. Pantha P, Chalivendra S, Oh DH, Elderd BD, Dassanayake M. A Tale of Two Transcriptomic Responses in Agricultural Pests via Host Defenses and Viral Replication. *Int. J. Mol. Sci.* 2021; 22(7):3568. https://doi.org/10.3390/ijms22073568
47. Reitman S. & Frankel S. Determination of serum alanine and aspartate aminotransferases (ALT and AST). *Clin. Path. Am. J.* 1957; 28:57-63.
48. Powell ME. & Smith MJ. The determination of serum acid and alkaline phosphatase activity with 4-aminoantipyrine (AAP). *J. Clin. Pathol.* 1954; 7(3):245. DOI: 10.1136/jcp.7.3.245
49. Holbrook CC, Knauft DA, Dickson DW. A technique for screening peanut for resistance to *Meloidogyne arenaria*. *Plant Dis.* 1983; 67:957–958.
50. Ayoub SM. Plant nematology: an agricultural training aid. *Nema. Aid.* Publication, Sacramento, California, U.S.A.1980; 195 pp.
51. Aviles-Gomez J, Cristóbal-Alejo J, Andrés MF, González-Coloma A, Carnevali G, Pérez-Brito D, Gamboa-Angulo M. Nematicidal Screening of Aqueous Extracts from Plants of the Yucatan Peninsula and Ecotoxicity. *Plants* (Basel). 2022; 11(16):2138. doi: 10.3390/plants11162138.
52. Aissani N, Urgeghe PP, Oplos C, Saba M, Tocco G, Petretto GL, Caboni P. Nematicidal activity of the volatilome of *Eruca sativa* on *Meloidogyne incognita*. *J. Agric. Food Chem.* 2015; 63(27):6120-5. doi: 10.1021/acs.jafc.5b02425.
53. Livak KJ. & Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods*. 2001; 25(4):402-408. doi: 10.1006/meth.2001.1262.
54. Rashwan RS. & Hammad DM. Toxic effect of *Spirulina platensis* and *Sargassum vulgar* as natural pesticides on survival and biological characteristics of cotton leaf worm *Spodoptera littoralis*. *Sci. Afr.* 2020; 8,e00323. https://doi.org/10.1016/j.sciaf.2020.e00323
55. Kaur M, Saraf I, Kumar R, Singh IP, Kaur S. Bioefficacy of Hexane Extract of *Inula racemosa* (Asteraceae) Against *Spodoptera litura* (Lepidoptera: Noctuidae). *Gesunde. Pflanzen.* 2019; 71,165-174. https://doi.org/10.1007/s10343-019-00462-w
56. Punia A, Chauhan NS, Singh D, Kesavan AK, Kaur S, Sohal SK. Effect of gallic acid on the larvae of *Spodoptera litura* and its parasitoid *Bracon hebetor*. *Sci. Rep.* 2021; 11(1):531. doi: 10.1038/s41598-020-80232-1.

57. Saber AA, Hamed SM, Abdel-Rahim EFM, Cantonati M. Insecticidal prospects of algal and cyanobacterial extracts against the cotton leafworm *Spodoptera littoralis*. *Vie Milieu.* 2018; 68(4):199-212.
58. İçen E, Armutçu F, Büyükgüzel K, Gürel A. Biochemical stress indicators of greater wax moth exposure to organophosphorus insecticides. *J. Econ. Entomol.* 2005; 98(2): 358-366. https://doi.org/10.1093/jee/98.2.358
59. Yi SX. & Adams TS. Age-and diapause-related acid and alkaline phosphatase activities in the intestine and malpighian tubules of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). *Arch. Insect Biochem. Physiol.* 2001; 46(3):152-163. doi: 10.1002/arch.1025.
60. Zibaee A, Zibaee I, Sendi JJ. A juvenile hormone analog, pyriproxifen, affects some biochemical components in the hemolymph and fat bodies of *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae *Pestic Biochem. Physiol.* 2011; 100(3):289-298. DOI: 10.1016/j.pestbp.2011.05.002
61. Hashem AS, Ramadan MM, Abdel-Hady AA, Sut S, Maggi F, Dall'Acqua S. *Pimpinella anisum* essential oil nanoemulsion toxicity against *Tribolium castaneum*? Shedding light on its interactions with aspartate aminotransferase and alanine aminotransferase by molecular docking. *Molecules.* 2020; 25(20):4841. doi: 10.3390/molecules25204841.
62. Etebari K, Bizhannia AR, Sorati R, Matindoost L. Biochemical changes in haemolymph of silkworm larvae due to pyriproxyfen residue. *Pestic. Biochem. Physiol.* 2007; 88(1):14-19. https://doi.org/10.1016/j.pestbp.2006.08.005
63. Sugeçti S. & Büyükgüzel K. Effects of Oxfendazole on Metabolic Enzymes in Hemolymph of *Galleria mellonella* L. (Lepidoptera: Pyralidae) Larvae Reared on Artificial Diet. *Karaelmas Sci Eng J/Karaelmas Fen ve Mühendislik Dergisi*. 2018; 8(2).
64. Ghoneim K, Hamadah K, El-Hela A, Abo Elsoud A. Disturbed activities of the detoxifying enzymes, acid and alkaline phosphatases, of *Galleria mellonella* L. (Lepidoptera: Pyralidae) by four plant growth regulators. *I J B 21*. 2022; (4): 44-60. DOI: 10.12692/ijb/21.4.44-60
65. El-Deen N, & Issa A. Nematicidal properties of some algal aqueous extracts against root-knot nematode, *Meloidogyne incognita* in vitro. *E J A J.* 2016; 15(1):67-78. DOI: 10.21608/ejaj.2016.57484
66. Choleva B, Bileva T, Tzvetkov Y, Barakov P. Preliminary study of the green algae chlorella (*Chlorella vulgaris*) for control on the root-knot nematode (*Meloidogyne arenaria*) in tomato plants and ectoparasite *Xiphinema indexin* grape seedlings. *Commun Agric. Appl. Biol. Sci.* 2005; 70(4):915-26.
67. Akhtar M. Nematicidal potential of the neem tree *Azadirachta indica* (A. Juss). *J. Integr. Pest Manag.* 2000;(5):57-66. https://doi.org/10.1023/A:1009609931223
68. Safdar H, Javed N, Khan SA, ul Haq I, Safdar A, Khan NA. Control of *Meloidogyne incognita* (Kofoid and White) Chitwood by cadusafos (Rugby®) on tomato. *Pakistan J. Zool.* 2012; 44(6):1703-1710.
69. Giordani G, Cattabriga G, Becchimanzi A, Di Lelio I, De Leva G, Gigliotti S, Cavaliere V. Role of neuronal and non-neuronal acetylcholine signaling in *Drosophila* humoral immunity. *Insect Biochem. Mol. Biol.* 2023; 153,103899. doi: 10.1016/j.ibmb.2022.103899
70. Sasabe M, Wen Z, Berenbaum MR, Schuler MA. Molecular analysis of *CYP321A1*, a novel cytochrome P450 involved in metabolism of plant allelochemicals (furanocoumarins) and insecticides (cypermethrin) in *Helicoverpa zea*. *Gene*. 2004;338(2):163-75. doi: 10.1016/j.gene.2004.04.028.
71. Feyereisen R. Insect CYP genes and P450 enzymes. *In Insect molecular biology and biochemistry*. (pp. 236-316). 2012; Academic Press.
72. Laing R, Bartley DJ, Morrison AA, Rezansoff A, Martinelli A, Laing ST, Gilleard JS. The cytochrome P450 family in the parasitic nematode *Haemonchus contortus*. *Int. J. Parasitol.* 2015; 45(4):243-51. doi:10.1016/j.ijpara.2014.12.001
73. Rupasinghe SG, Wen Z, Chiu TL, Schuler MA. *Helicoverpa zea CYP6B8* and *CYP321A1*: different molecular solutions to the problem of metabolizing plant toxins and insecticides. *Protein Eng. Des. Sel.*2007; 20(12):615-24. doi: 10.1093/protein/gzm063.
74. Daborn P, Boundy S, Yen J, Pittendrigh B, Ffrench-Constant R. DDT resistance in *Drosophila* correlates with *Cyp6g1* over-expression and confers cross-resistance to the neonicotinoid imidacloprid. *Mol. Genet. Genomics.* 2001; 266(4): 556-63. doi: 10.1007/s004380100531.
75. Laing ST, Ivens A, Laing R, Ravikumar S, Butler V, Woods DJ, Gilleard JS. Characterization of the xenobiotic response of *Caenorhabditis elegans* to the anthelmintic drug albendazole and the identification of novel drug glucoside metabolites. *Biochem. J.* 2010; 432(3):505-14. doi: 10.1042/BJ20101346.