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Toxicity and enzymatic inhibitory action of *Spirulina* sp. and *Nostoc muscorum* extracts on detoxifying enzymes in *Spodoptera frugiperda* (J. E. Smith) (Noctuidae: Lepidoptera)

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ABSTRACT. The fall armyworm (FAW) *Spodoptera frugiperda* is a destructive insect pest that belongs to the Noctuidae family and Lepidoptera order. It is a polyphagous pest that harms economically significant cereals and many vegetable crops. This pest has developed resistance to most of the synthetic insecticides through increasing enzymatic activity, so there is a need to take action to prevent the resistance development and damage caused by this pest. A laboratory study on the efficacy of *Spirulina* sp. and *Nostoc muscorum* extracts against *S. frugiperda* was tested using four different solvents: hexane, petroleum ether, ethanol and methanol. The results revealed that *N. muscorum* hexane and petroleum ether extract recorded the lowest LC_{50} values of 133.18 and 165.68 ppm, respectively. This was followed by *Spirulina* hexane and petroleum ether extract, with LC_{50} values of 337.28 and 468.05, ppm respectively. The results of the study of enzymatic inhibitory action of cyanobacterial extracts showed that *Nostoc muscorum* hexane and petroleum ether extract significantly decreased the activity of esterase, glutathione S-transferase and cytochrome p450 with a mean of 0.29 and 0.36 µmoles of naphthol min^{-1} $µg^{-1}$ of protein, 1.30 and 1.40 µmoles of CDNB min^{-1} $µg^{-1}$ of protein and 0.28 and 0.38 µmoles of p-nitroanisole min^{-1} $µg^{-1}$ of protein respectively. This was followed by *Spirulina* hexane extract with a mean of 0.69 naphthol min^{-1} $µg^{-1}$ of protein, 1.48 µmoles of CDNB min^{-1} $µg^{-1}$ of protein and 0.46 p-nitroanisole min^{-1} $µg^{-1}$ of protein respectively and control population significantly recorded higher enzymatic activities. *Spirulina* sp. and *N. muscorum* extracts were having a significant effect on larval mortality and inhibiting the detoxifying enzyme activity. These findings demonstrate the potential of cyanobacterial extracts as an innovative strategy against *S. frugiperda* and suggest that this may helpful in managing insecticide resistance.

Keywords: Cyanobacterial extract; *Spirulina* sp.; *Nostoc muscorum; Spodoptera frugiperda;* enzymatic activity.

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Introduction

Environmental pollution is an apprehensive problem worldwide due to the rapid increase in industrialization and urbanization; in particular, the extensive use of chemical pesticides has caused several environmental problems in numerous countries (Aydinalp & Porca, 2004). The need for natural pesticides is one of the scientific research goals. Biopesticides are an important group of naturally occurring, often slow-acting crop protectants that are usually safer to humans and the environment than conventional pesticides and with minimal residual effects.

The fall aymyworm (FAW), *Spodoptera frugiperda* (Noctuidae: Lepidoptera) is a tropical and subtropical American species that was first reported on maize in Shivamogga, Karnataka, India in May and June of 2018 (Sharanabasappa et al., 2018a). The pest was initially spotted in 44 African countries in late 2016 (Goergen, Kumar, Sankung, Togola, & Tamo, 2016; Nagoshi et al., 2019) and it was believed to have moved from Africa (Rwomushana et al., 2018). Among more than 80 other crop species, maize, rice, sorghum, millet, sugarcane, vegetable crops, and cotton are all economically harmed by the polyphagous pest recognized as FAW (Goergen et al., 2018; Day et al., 2017; Prasanna, Huesing, Eddy, & Peschke, 2018; Assefa et al., 2018; Chormule, Shejawal, Nagol, & Brown, 2019). Maize is a more favoured host, with incidence rates in Africa and India

ranging from 6 to 100% (Mallapur, Naik, Hagari, Prabhu, & Patil, 2018). In terms of causing a considerable yield loss in economically significant crops is attributed to its wide host range, strong dispersal ability, high fecundity rate and absence of diapause have all been linked to the severity of FAW (Knipling, 1980).

Several farmers utilize synthetic chemical insecticides to reduce the damage caused by FAW infestation. Nevertheless, (Schmutterer, 1990) and Paragas, Fiegalan, and Cruz (2018) found that the usage of synthetic chemical insecticides had unanticipated negative effects, such as residues in food and water. Also as documented by Abraham et al. (2018) and Blibech, Ksantini, Jardak, and Bouaziz (2015) synthetic pesticides (insecticides and herbicides) also cause the death of beneficial insects. In addition, pesticides like carbamates and organophosphates can have detrimental impacts on human health (Paragas, Fiegalan, & Cruz, 2018; Daniel & Baker, 2013). This requires the development of suitable, efficient and environmentally friendly pesticides.

Cyanobacteria, also known as blue-green algae, are creatures that resemble plants and have photosynthetic pigments in their cells. They are most commonly found in aquatic habitats such as freshwater, saltwater, and wastewater habitats. Algae can range in size from microalgae to macroalgae. According to studies by Seenivasan, Indu, Archana, and Geetha (2010), Chojnacka, Saeid, Witkowska, and Tuhy (2012) and Munirasu, Ramasubramanian, Uthayakumar, and Muthukumar (2013), algae are a rich source of a variety of physiologically active molecules. These algae also create several chemically diverse secondary metabolites, some of which are known insecticides. Secondary metabolites derived from algae have been demonstrated to have strong efficacy against larvae in earlier investigations (Selvin & Lipton, 2004; Alarif, Elnaga, Ayyad, & Al-Lihaibi, 2010). *Spirulina platensis* water extract (5 % concentration), according to the investigation by Aly and Abdou (2010), reported increased *Spodoptera littoralis* (Boisduval) larval mortality and deformity. Rahim and Mohamed (2013) also examined four cyanobacterial microalgal strains, including *Anabaena flos aquae, Anabanea laxa, Anabanea fertilissima* and *Nostoc muscorum* strains proved effective when utilized as biopesticides against the 2nd and 4th instars of the black cutworm *Agrotis ipsilon* (Hufnagel).

Many secondary metabolites produced by cyanobacteria have a wide range of bioactivities (Burja, Banaigs, Abou-Mansour, Grant Burgess, & Wright, 2001; Wiegand & Pflugmacher, 2005). *Anabaena flos aquae*, a cyanobacterium that is a reliable supplier of many bioactive chemicals, maybe a prime option as a natural pesticide against lepidoptera. A known producer of cryptophycin, the cyanobacterium *Nostoc* strain ATCC 53789, was examined for its capacity to serve as a source of natural insecticides (Biondi et al., 2004). Without a doubt, cyanobacteria are a promising and safe pest control tool.

The current study examined the toxic effects of *Spirulina* species and *N. muscorum* extracts as natural alternatives to synthetic pesticides against *S. frugiperda.* It also determined the inhibitory action of cyanobacterial extracts on key detoxifying enzymes in *S. frugiperda.* Since cyanobacterial extracts were also a type of biopesticide and served as a cost-effective and environmentally friendly method to protect various crops from economic pests.

Material and Methods

Cyanobacterial source

The pure culture of *Spirulina* sp. and *Nostoc muscorum* was procured from the National Centre for Industrial Microorganisms (NCIM), Pune, Maharashtra.

Media for mass production of *Spirulina* sp. and *Nostoc muscorum*

For mass production of *Spirulina* sp. *Spirulina* media (NCIM, Pune) and Zarrouk media were used and for *N. muscorum*, fogs media (NCIM, Pune) and BG-11 media (Allen & Stanier, 1968) were used.

Conditions for cyanobacterial growth and multiplication (*Spirulina* sp. and *N. muscorum*)

A loopful of pure culture was inoculated into test tubes with 10 mL of liquid media for sub-culturing purposes in a laminar airflow, plugged with cotton wool and kept in a growth chamber set at 25 ± 2 °C and 75 ± 2 % relative humidity with a steady illumination of a light source. After one month of inoculation, the cultures had reached their maximal growth. Subsequently, 100 mL broth media were made, 1 mL of the culture was added and then kept in a growth chamber. Similarly, the same procedures were followed for 500, 1000, 2000 and 3000 mL broth to obtain an adequate amount of culture according to Rahim and Hamed (2013) with

a little modification. It is necessary to maintain the culture in various quantities of liquid media because the culture's dry weight is quite low.

Harvesting of biomass and powder preparation of *Spirulina* sp. and *N. muscorum*

The fully grown-up culture was filtered through a muslin cloth and shade-dried at room temperature for one week. An electric blender was used to powder cultures. *Spirulina* sp. and *N. muscorum* and dry powder forms were stored until usage in the refrigerator (4°C).

Extraction of *Spirulina* sp. and *N. muscorum*

Five grams of powdered *N. muscorum* and *Spirulina* sp. were used for extraction. Hexane and petroleum ether (PE), two distinct solvents, were utilized to extract each species using the Soxhlet equipment, while ethanol and methanol were used for extraction through the mechanical shaking method.

Soxhlet extraction

Extractions were performed following Rahim and Mohammed (2013). with a slight modification. In an automated Soxhlet system, five grams of the powdered substance were extracted using 120 mL of hexane and petroleum ether. To obtain crude extracts, the extract was concentrated inside of a desiccator. Crude materials were dissolved in dimethyl sulfoxide to obtain standard stock solutions, which were then kept at 4 °C until further use.

Mechanical shaking method of extraction

Five grams of *Spirulina* sp. and *N. muscorum* powder were soaked in 50 mL each of ethanol and methanol solvent for three days to completely extract the bioactive compounds. The extract was then filtered through Whatman n° 1 filter paper and left at room temperature for about one day to allow the solvents to evaporate and to obtain a concentrated form of crude extract. Crude materials were dissolved in dimethyl sulfoxide to obtain standard stock solutions, which were then kept at 4 °C until further use. Extractions were performed following Saber, Hamed, Abdel, and Cantonati (2018) with a little modification.

To study the bioactivity of cyanobacterial extracts against fall armyworm, *S. frugiperda*

Insect culture maintenance

The larvae were reared in an insect breeding circular dish that contained the chopped-up leaves of maize, then covered with a lid, and kept at 25°C, 75°RH, and L12:D12 photoperiod. The adults that had emerged were allowed into cages for oviposition. As an oviposition substrate, paper towels were used to line the cages. Adult male and female pairs were released into each cage. The adults were given daily supplements of the 10% honey solution that was offered to them on cotton pads inside of little plastic caps and kept inside their cages. For the eggs to hatch, eggs were collected and put in a circular dish for incubation. The eggs were checked every 12 hours to see if they had hatched. For the bioactivity test, second larval instars were utilized (Sharanabasappa et al., 2018b).

Bioactivity test

The leaf dip method of bioassay was employed in the bioassay test. A series of six different concentrations, including 0, 100, 200, 400, 800, and 1600 ppm, were prepared from the stock solution obtained out of soxhlet apparatus. Similarly, 0, 500, 1000, 2000, 4000, and 8000 ppm, were prepared from the standard stock solution extracted through mechanical shaker based on bracketing technique.

Leaf dip method

Spodoptera frugiperda second instars were fed on the leaves of a maize plant by dipping the leaves in extracts for 30 seconds and then allowing the leaves to air dry at room temperature (Krishnappa & Elumalai, 2012; Moadeli, Hejazi, & Golmohammadi, 2014). After 24 hours of feeding on treated leaves, fresh maize leaves were given to the larvae for feeding until pupation (Ahmed, 1985). Three replications (10 larvae/replication) of each concentration were performed. Fresh, untreated leaves were substituted for the dried leaves each day. After 96 hours, LC_{50} values were calculated.

Determination of inhibitory action of cyanobacterial extracts on detoxifying enzymes in fall armyworm, *S. frugiperda*

The larvae which survived after the bioassay experiments were used for the enzyme assay studies. The total protein content in the sample insects was studied by using Bradford's method. The activities of three enzymes, *viz.,* carboxylesterase (CarE), glutathione S-transferase (GST) and cytochrome p-450 monooxygenase from the treated and control populations were studied using the standard protocol.

Phosphate buffer preparation

The 1 M of phosphate buffer, pH 6.8 stock solution was prepared by dissolving 1M of sodium phosphate monobasic and 1 M of sodium phosphate dibasic in 100 mL of distilled water. Fifty mM of the working solution of phosphate buffer was prepared from the stock solution.

Preparation of mid-gut homogenate

The five early fifth instar larvae were used for the preparation of mid-gut homogenate. Mid guts were dissected and homogenized with 200 µL of ice-cold 50 mM phosphate buffers, pH 6.8 under cold conditions. The samples were centrifuged at 8000 rpm for 10 min at 4° C. The supernatant obtained was transferred into another tube without disturbing the pellet and used as the enzyme source for the analysis of carboxyl esterase, glutathione S-transferase and cytochrome p450.

Total protein estimation

The total protein of the enzyme sample was determined to quantify the enzyme activity. The total protein in the sample solution was quantified based on the Bradford (1976) method. The principle in this method involves the dye coomassie brilliant blue (CBB). G-250 in an unstable state under acidic conditions bounds with the positively charged amino acids of the protein under basic conditions and gets converted into a stable state changing the color from brown to blue color which leads to an absorbance shift from 465 nm to 595 nm. The intensity of the color is proportionate to the protein content in the sample.

Stock solutions

i. Bradford stock solution**:** 150 mL of stock solution was prepared by dissolving 175 mg of CBB G**-**250 in 50 mL of 95% ethanol and 100 mL of 88% phosphoric acid**.**
ii. Bradford working buffer**:** 500 mL of working solution was prepared by dissolving 425 mL of distilled water, 15 mL of 95% ethanol and 30 mL of 88% phosphoric acid and 30 mL of Bradford stock solution**.**
iii. Standard bovine serum albumin 9 (BSA) stock solution**:** 1 mg of BSA was dissolved in 1 mL of distilled water**.**

Protocol

Pipetted out protein solution (maximum of 100 µL) into test tubes and water was added to make the volume of 300 µL. Three mL of Bradford working solution was added and read absorbance at 595 nm.

Quantitative analysis of the determination of carboxylesterase activity

The carboxylesterase activity of the sample solutions was determined by α-naphthol acetate as substrate. The principle involved is esterase activity estimated based on the quantification of the product formed after the hydrolysis of ester bonds. Here, α-naphthol acetate substrate was used as substrate and the product α-naphthol formed was quantified at 595 nm.

Stock solutions

i. Substrate solution**:** A stock solution of 10 mM of naphthol acetate was prepared in acetone and 1 mL of this stock solution was dissolved in 99 mL of 0**.**1 M phosphate buffer (pH 6**.**8) to prepare 0**.**3 mM of naphthol acetate solution**.**
ii. Staining solution**:** 1% fast blue BB salt (w/v) was prepared freshly in 50 mM phosphate buffer (pH 6**.**8) and 5% fast blue solution was added in 5 parts of 5 % SDS.

Protocol

The assay was performed by following the protocol given by Van (1962). using the spectrophotometer method. Fifteen microliter of enzyme sample was pipetted out in an Eppendorf tube with 3 replications. The

reaction was initiated by adding 800 µL of substrate solution to the enzyme samples in the Eppendorf tube. The Eppendorf tubes were incubated in the dark at 30 °C for 30 min. Later 200 µL of the staining solution was added to stop and stain the reaction. The tubes were set aside for 20 min at room temperature to develop color. The absorbance was read in a spectrophotometer absorbance reader at 595 nm. The enzyme activity was expressed as n-moles of α-naphthol formed min^{-1} μg^{-1} of protein.

Determination of glutathione S-transferase activity

The principle involves the conjugation of the thiol group of reduced glutathione with 1-chloro-2, 4-dinitrobenzene (CDNB) in the presence of the enzyme glutathione S-transferase. Upon conjugation, there was an increase in absorbance at 340 nm.

Stock solutions

i. Reduced glutathione**:** 50 mM of reduced glutathione was prepared in 100 mM phosphate buffer (pH 6**.**5)**.**
ii. CDNB**:** 50 mM of CDNB was prepared in 95 **%** ethanol**.**
iii. Sodium phosphate buffer**:** 100 mM of Sodium phosphate buffer was prepared to contain 1 mM of EDTA and 0**.**1 mM PTU**.**

Protocol

GST activity was measured using CDNB (1-chloro-2, 4-dinitrobenzene) as a substrate (Kao, Hung, & Sun, 1989). Briefly, 50 µL of 50 mmol L^{-1} CDNB and 150 µL of reduced glutathione were added to the enzyme source and the final volume of the assay mixture was made up to 3 mL by using phosphate buffer (0.1 mol L^{-1}, pH 6.5 containing 0.1 mmol L^{-1} PTU). The contents are gently shaken and incubated for 3 min at 37 °C. The increase in absorbance at 340 nm for 5 min was recorded by employing the time scan menu of the Shimadzu UV-VIS double beam spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The enzyme activity was calculated with the formula expressed as µmol min^{-1} mg^{-1} protein (molar extinction coefficient = 9.6 Mm^{-1} cm^{-1}).

$$\text{Enzyme activity} = \frac{\text{ABS (increase in 5 min)}\times 3\times 1000}{9.6\times 5\times \text{protein in mg}}$$

Determination of cytochrome p450 monooxygenase activity

The activity of the enzyme cytochrome p450 monooxygenase was determined by p-nitroanisole o-demethylase assay. The principle involves the o-demethylation of substrate p-nitroanisole in the presence of monooxygenases into the product p-nitrophenol which has an absorbance maximum of 405 nm.

Stock solutions

i. P**-**nitroanisole**:** 1**.**5 mM of p**-**nitroanisole was prepared in 0**.**1 mM sodium phosphate buffer (pH 7**.**5)**.**

Protocol

The assay was performed following the protocol given by Kinoshita et al. (1966), using the spectrophotometer method. Pipetted out 300 µL of enzyme sample in the Eppendorf tube containing 225 µL phosphate buffers with three replicates. Blank was prepared with 525 µL of sodium phosphate buffer instead of the enzyme sample. Then, 525 µL of the substrate solution, p-nitroanisole was added to it and the Eppendorf tubes were incubated at 30 °C for 1 hour with shaking. Later on, 2100 µL of ice-cold acetone and 600 µL of 0.5M glycine NaOH were added to initiate the reaction. The absorbance was read at 410 nm in the spectrophotometer and calculated the enzyme activity from p-nitrophenol.

Statistical analysis

After 96 hours of treatments, the LC_{50} values were determined and corrected using the Abbott formula (Abbott, 1987). The data were analyzed using the probit analysis (Finney, 1971) and for the determination of lethal concentration (LC_{50}) and their 95 % confidence limits (CLs) by using the POLO plus (Leora software 2002, Brkeliy, CA, USA). Detoxification enzyme activities were compared between treated populations and control populations with the analysis of variance (one-way ANOVA) with the Duncan test.

Results and discussion

Bioactivity test

Larvae of *S. frugiperda* fed on maize leaf treated with different cyanobacterial extracts revealed that *N. muscorum* hexane and petroleum ether extracts significantly recorded the lowest LC_{50} values of 133.18 and 165.68 ppm respectively. These treatments were followed by *Spirulina* hexane extract, *Spirulina* petroleum ether extract, *N. muscorum* ethanol extract and *N. muscorum* methanol extract, *Spirulina* ethanol extract and *Spirulina* methanol extract with LC_{50} values of 340.84, 485.69, 720, 890, 1040 and 1180 ppm respectively. Similarly, a study was repeated for the confirmation of the first set of results and the same trend was followed wherein, *N. muscorum* hexane and petroleum ether extract were recorded lowest LC_{50} values of 133.18 and 136.30 ppm. These were followed by *Spirulina* hexane extract, *Spirulina* petroleum ether extract, *N. muscorum* ethanol extract and *N. muscorum* methanol extract, *Spirulina* ethanol extract and *Spirulina* methanol extract with LC_{50} values of 340.84, 485.69, 720, 890, 1040 and 1180 ppm respectively (Table 1).

Results of inhibitory action of cyanobacterial extracts on detoxifying enzymes in *S. frugiperda*

Esterase (EST) Activity

The activity of EST ranged from 0.29 to 3.63 µM of 1-naphthol min^{-1} mg^{-1} of protein. Among different treatments, *N. muscorum* hexane extract and *N. muscorum* petroleum ether extract recorded significantly the lowest activity of EST with a mean value of 0.29 and 0.37 µmoles of 1-naphthol min^{-1} mg^{-1} of protein, followed by *Spirulina* hexane extract (0.71 µmoles of 1-naphthol min^{-1} mg^{-1} of protein) and *Spirulina* petroleum ether extract (0.86 µmoles of 1-naphthol min^{-1} mg^{-1} of protein) and the treatments *N. muscorum* ethanol extract and *N. muscorum* methanol extract were at par with each other by recording 1.41 and 1.51 µmoles of 1-napthol min^{-1} mg^{-1} of protein. Whereas *Spirulina* ethanol extract and *Spirulina* methanol extract were significantly different between the treatments with a mean value of 1.63 and 1.95 µmoles of 1-naphthol $min^{-1}mg^{-1}$ of protein but the control population recorded the significantly higher activity of EST with a mean of 3.63 µmoles of 1 naphthol min^{-1} mg^{-1} of protein (Table 2). Similarly, an experiment was repeated for the confirmation of the first set of results and the results followed a similar trend which was mentioned in Table 2.

Table 1. Insecticidal activity of *Spirulina* sp. and *N. muscorum* extracts against, *S. frugiperda*.

Treatment details	Leaf dip method									
	First set					Second set				
	LC_{50} values (ppm)	Slope Function (± SD)	Chi-square (df:3)	95 % confidence limit		LC_{50} values (ppm)	Slope Function (± SD)	Chi-square (df:3)	95 % confidence limit	
				Upper	Lower				Upper	Lower
Spirulina hexane extract	337.28	0.713 (±0.248)	0.112	739.442	117.393	340.84	0.775 (±0.249)	0.652	688.941	139.852
Spirulina PE ether extract	468.05	0.774 (±0.249)	0.047	1145.393	231.715	485.69	0.836 (±0.250)	0.378	1094.165	260.649
N. muscorum hexane extract	133.18	0.839 (±0.258)	0.148	247.886	22.836	136.30	0.725 (±0.254)	0.266	272.802	11.517
N. muscorum PE ether extract	165.68	0.745 (±0.253)	0.491	317.225	23.911	185.98	0.870 (±0.255)	0.115	324.596	53.933
Spirulina ethanol extract	1070	0.971 (±0.251)	0.083	0.196	0.029	1040	0.895 (±0.255)	0.199	0.178	0.036
Spirulina methanol extract	1090	0.803 (±0.249)	0.054	0.388	0.088	1180	1.052 (±0.258)	0.243	0.187	0.057
N. muscorum ethanol extract	730	1.028 (±0.266)	0.057	0.121	0.024	720	0.865 (±0.258)	0.400	0.131	0.015
N. muscorum methanol extract	800	0.927 (±0.259)	0.056	0.138	0.023	890	0.874 (±0.256)	0.110	0.155	0.025

Ppm = Parts per million; SD = Standard Deviation; Df = degrees of freedom.

The pooled data results revealed that the activity of EST ranged between 0.29 to 3.54 µmoles of 1-naphthol min^{-1} mg^{-1} of protein. Among different treatments, *N. muscorum* hexane extract and *N. muscorum* petroleum ether extract recorded significantly the lowest activity of EST with a mean value of 0.29 and 0.36 µmoles of 1-naphthol min^{-1} mg^{-1} of protein, followed by *Spirulina* hexane extract (0.69 µmoles of 1-naphthol min^{-1} mg^{-01}

of protein) and *Spirulina* petroleum ether extract (0.83 µmoles of 1-naphthol min^{-1} mg^{-1} of protein) and the treatments *N. muscorum* ethanol extract and *N. muscorum* methanol extract were at par with each other by recording 1.34 and 1.42 µmoles of 1-naphthol min^{-1} mg^{-1} of protein. Whereas, *Spirulina* ethanol extract and *Spirulina* methanol extract were significantly different between the treatments and showed moderate effective in reducing the EST activity with a mean value of 1.60 and 1.93 µmoles of 1-naphthol min^{-1} mg^{-1} of protein but the control population recorded the significantly higher activity of EST with a mean of 3.54 µmoles of 1-naphthol min^{-1} mg^{-1} of protein (Table 2) (Figure 1).

Table 2. Effect of *Spirulina* sp. and *N. muscorum* extracts on carboxyl esterase activity in *S. frugiperda*.

Treatments	µmoles of naphthol min^{-1} $µg^{-1}$ of protein			µmoles of CDNB min^{-1} $µg^{-1}$ of protein			µmoles of p-nitroanisole min^{-1} $µg^{-1}$ of protein		
	Mean±SD (1st set)	Mean±SD (2nd set)	Pooled	Mean±SD (1st set)	Mean±SD (2nd set)	Pooled	Mean±SD (1st set)	Mean±SD (2nd set)	Pooled
Spirulina hexane extract	0.71±0.05^{b}	0.67±0.07^{b}	0.69±0.06^{b}	1.51±0.08^{bc}	1.44±0.13^{bc}	1.48±0.04^{c}	0.41±0.13^{bc}	0.50±0.08^{bc}	0.46 ±0.10^{bc}
Spirulina petroleum ether extract	0.86±0.06^{b}	0.79±0.07^{b}	0.83 ±0.06^{b}	1.61±0.11^{c}	1.58±0.11^{c}	1.60±0.06^{d}	0.50±0.14^{cd}	0.59±0.09^{c}	0.54±0.11^{c}
Nostoc muscorum hexane extract	0.29±0.01^{a}	0.29±0.03^{a}	0.29 ±0.01^{a}	1.32±0.14^{a}	1.28±0.14^{a}	1.30±0.07^{a}	0.24±0.09^{a}	0.32±0.09^{a}	0.28±0.09^{a}
Nostoc muscorum petroleum ether extract	0.37±0.03^{a}	0.35±0.03^{a}	0.36±0.03^{a}	1.42±0.13^{ab}	1.38±0.13^{ab}	1.40±0.06^{b}	0.32±0.14^{ab}	0.44±0.09^{b}	0.38±0.11^{b}
Spirulina ethanol extract	1.63±0.09^{d}	1.56±0.12^{c}	1.60 ±0.10^{d}	2.02±0.08^{e}	2.02±0.04^{de}	2.02±0.02^{f}	0.78±0.12^{fg}	0.89±0.03^{e}	0.84±0.07^{e}
Spirulina methanol extract	1.95±0.08^{e}	1.91±0.14^{d}	1.93 ±0.10^{e}	2.08±0.08^{e}	2.15±0.07^{e}	2.12±0.02^{g}	0.93±0.10^{g}	0.93±0.04^{e}	0.93 ±0.06^{e}
Nostoc muscorum ethanol extract	1.41±0.17^{c}	1.28±0.16^{c}	1.34±0.17^{c}	1.77±0.08^{d}	1.90±0.20^{d}	1.84±0.10^{e}	0.61±0.19^{de}	0.69±0.08^{d}	0.65±0.13^{d}
Nostoc muscorum methanol extract	1.51±0.15^{cd}	1.33±0.22^{c}	1.42±0.19^{cd}	1.84±0.11^{d}	1.91±0.04^{d}	1.87±0.04^{e}	0.69±0.19^{ef}	0.76±0.10^{d}	0.73±0.13^{d}
Control	3.63±0.16^{f}	3.45±0.27^{e}	3.54±0.17^{f}	2.89±0.13^{f}	2.95±0.05^{f}	2.92±0.05^{h}	1.23±0.09^{h}	1.30±0.08^{f}	1.26±0.08^{f}
S. Em (±)	0.07	0.10	0.08	0.06	0.07	0.03	0.10	0.06	0.07

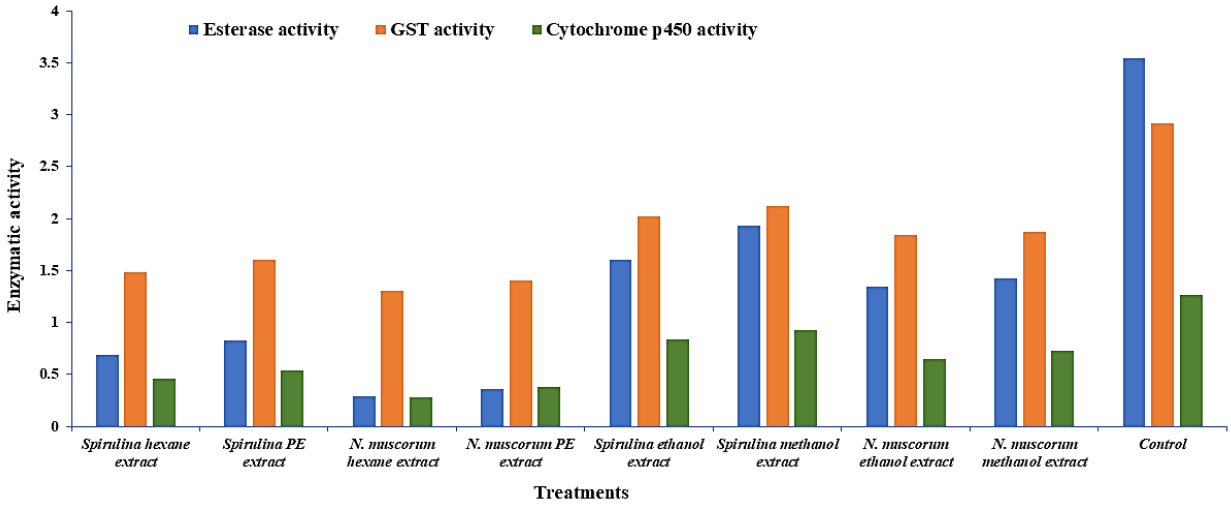

Figure 1. Inhibitory action of *Spirulina* sp. and *N. muscorum* extracts on the carboxyl esterase, glutathione S-transferase and cytochrome p450 activity in *S. frugiperda*.

Glutathione S-transferase (GST) activity

The activity of GST ranged from 1.32 to 2.89 µmoles of CDNB min^{-1} mg^{-1} of protein. Among different treatments, *N. muscorum* hexane extract and *N. muscorum* petroleum ether extract recorded significantly the lowest activity of GST with a mean value of 1.32 and 1.42 µmoles of CDNB min^{-1} mg^{-1} of protein, followed by *Spirulina* hexane extract (1.51 µmoles of CDNB min^{-1} mg^{-1} of protein) and *Spirulina* petroleum ether extract (1.61 µmoles of CDNB min^{-1} mg^{-1} of protein) and the treatments *N. muscorum* ethanol extract and *N. muscorum* methanol extract were at par with each other by recording 1.77 and 1.84 µmoles of CDNB min^{-1} mg^{-1} of protein.

Whereas, *Spirulina* ethanol extract and *Spirulina* methanol extract were also at par with each other and least effective in inhibiting the GST activity with a mean value of 2.02 and 2.08 µmoles of CDNB min^{-1} mg^{-1} of protein but the control population was significantly recorded the higher activity of GST with a mean value of 2.89 µmoles of CDNB min^{-1} mg^{-1} of protein. During the second set of experiments also, a similar trend was followed and the results are mentioned in Table 2.

Pooled data results revealed that the activity of GST ranged between 1.30 to 2.92 µmoles of CDNB min^{-1} mg^{-1} of protein. Among different treatments *N. muscorum* hexane extract and *N. muscorum* petroleum ether extract recorded significantly lowest GST activity with a mean of 1.30 and 1.40 µmoles of CDNB min^{-1} mg^{-1} of protein, followed by *Spirulina* hexane extract (1.48 µmoles of CDNB min^{-1} mg^{-1} of protein) and *Spirulina* petroleum ether extract (1.60 µmoles of CDNB min^{-1} mg^{-1} of protein) and the treatments *N. muscorum* ethanol extract and *N. muscorum* methanol extract were at par with each other in inhibiting the GST activity with a mean value 1.84 and 1.87 µmoles of CDNB min^{-1} mg^{-1} of protein respectively. Whereas, *Spirulina* ethanol extract and *Spirulina* methanol extract were at par with each other and least effective in inhibiting the GST activity with a mean of 2.02 and 2.12 µmoles of CDNB min^{-1} mg^{-1} of protein respectively, but the control population significantly recorded more GST activity with a mean of 2.92 µmoles of CDNB min^{-1} mg^{-1} of protein (Table 2) (Figure 1).

Cytochrome p450 monooxygenase activity

The activity of cytochrome p450 ranges from 0.24 to 1.23 µmoles of p-nitroanisole min^{-1} mg^{-1} of protein. Among different treatments, *N. muscorum* hexane extract and *N. muscorum* petroleum ether extract recorded significantly lowest cytochrome p450 activity with a mean value of 0.24 and 0.32 µmoles of p-nitroanisole min^{-1} mg^{-1} of protein respectively, followed by *Spirulina* hexane extract (0.41 µmoles of p-nitroanisole min^{-1} mg^{-1} of protein) and *Spirulina* petroleum ether extract (0.50 µmoles of p-nitroanisole min^{-1} mg^{-1} of protein) and the treatments *N. muscorum* ethanol extract and *N. muscorum* methanol extract were at par with each other and more effective in reducing the cytochrome p450 activity with a mean value of 0.61 and 0.69 µmoles of p-nitroanisole min^{-1} mg^{-1} of protein respectively. Whereas, *Spirulina* ethanol extract and *Spirulina* methanol extract were least effective in reducing the cytochrome p450 activity with a mean value of 0.78 and 0.93 µmoles of p-nitroanisole min^{-1} mg^{-1} of protein respectively but the control population was significantly recorded higher cytochrome p450 activity with a mean value of 1.23 µmoles of p-nitroanisole min^{-1} mg^{-1} of protein (Table 4). Similarly, the experiment was repeated for the confirmation of previous results and the same trend was followed during the second set of the experiment also and results were mentioned in Table 2.

Pooled data results also followed a similar trend wherein, the activity of cytochrome p450 ranges between 0.28 to 1.26 µmoles of p-nitroanisole min^{-1} mg^{-1} of protein. *Spodoptera frugiperda* larvae treated with *N. muscorum* hexane extract recorded significantly lowest cytochrome p450 activity with a mean value of 0.28 µmoles of p-nitroanisole min^{-1} mg^{-1} of protein, followed by *N. muscorum* petroleum ether extract (0.38 µmoles of p-nitroanisole min^{-1} mg^{-1} of protein) and the treatments *Spirulina* hexane extract and *Spirulina* petroleum ether extract were at par with each other and formed the next best treatments by recording 0.46 and 0.54 µmoles of p-nitroanisole min^{-1} mg^{-1} of protein respectively and the treatments *N. muscorum* ethanol extract and *N. muscorum* methanol extract were at par with each other and showed moderate effect with a mean value of 0.65 and 0.73 µmoles of p-nitroanisole min^{-1} mg^{-1} of protein. Whereas, *Spirulina* ethanol extract and *Spirulina* methanol extract were at par with each other and least effective in inhibiting the cytochrome p450 activity with a mean value of 0.84 and 0.93 µmoles of p-nitroanisole min^{-1} mg^{-1} of protein respectively. Whereas, the control population significantly recorded higher cytochrome p450 activity compared to all the treatments by recording 1.26 µmoles of p-nitroanisole min^{-1} mg^{-1} of protein (Table 2) (Figure 1).

The toxic effect of *N. muscorum* and *Spirulina* sp. on *S. frugiperda* in the current study is in line with studies of the other cyanobacteria and algae are reported by other authors such as *N. muscorum* and *A. flos aquae* were having more insecticidal activity against second-instar larvae of *A. ipsilon* (Rahim and Mohamed, 2013), *N. carneum* and *Parachorella kessleri* exhibited greater toxicity against cotton leaf worm, *S. littoralis* (Saber et al. 2018), *S. platensis* caused 100 per cent mortality of *S. littoralis* larvae (Aly & Abdou, 2010) and *S. platensis* and brown macro alga, *Fucus vesiculosus* were considered an effective bio-control agent against broad bean beetle (Maissa, Doaa, & Rania, 2016). *Nostoc muscorum* hexane and petroleum ether extracts exhibited greater insecticidal activity and recorded lowest LC50 values followed by *Spirulina hexane* and petroleum ether extract

(Sharanappa et al., 2023; 2024), *N. carneum* and *P. kessleri* exhibited greater mortality of *S. littoralis* (Saber et al., 2018), ethanol extract of *A. flos aquae* caused higher mortality of *S. littoralis* (Rahim and Mohamed, 2013), ethanol extract of *S. platensis* (7 %) was the most toxic to the larvae of *S. littoralis* (Rania, & Doaa, 2020) and chloroform extracts of green algae, *Bryopsis pennata* exhibited the strongest larvicidal activity followed by methanol extract of *B. pennata* against *Aedes aegypti* (Linnaeus, 1962; Yu, Wong, Ahmad, & Jantan, 2015).

However, hexane and petroleum ether extracts of other algal and plant extracts having insecticidal properties against other crop pests are similar to the studies such as, hexane extracts of narrow-leaf, *Epaltes divaricate* (Asteraceae) showed significant mortality of *A. aegypti* and *Spodoptera litura* (Fabricius) larvae (Amala et al., 2021), petroleum ether extract of green algae, *Scenedesmus acutus* showed greater toxicity against *S. littoralis* (Sharaby, Salama, Maged, & Din, 1993), and hexane extract of an Asian plant, *Inula racemose* (Asteraceae) induced significantly higher mortality in *S. litura* larvae as compared to control (Mandeep, Isha, Rakesh, Inder, & Kaur, 2019).

The present study relevant to the inhibitory action of cyanobacterial extracts on the enzymatic activity of *S. frugiperda* was not reported and as it is of the first report, so as mentioned earlier, biopesticides of plant origin involved in inhibiting the detoxifying enzymes were used as a reference for discussing the results of the experiment.

The results are similar to the previous reports such as hexane extracts of *E. divaricata* were most toxic to *A. aegypti* and *S. litura* and α and β-carboxylesterase levels were decreased significantly in both the insect pests (Amala et al., 2021), petroleum ether extract of *Euphorbia prostrata* caused a maximum reduction in the activity carboxyl esterase enzymes (Riaz et al., 2018), ethanolic extracts of four essential oils of *Basilicum ocimum*, *Cuminum cyminum*, *Origanum marjorana* and *Matricaria chamomilla* against *Aphis craccivora*, Koch, indicated significant reduction in GST, esterase and mixed function oxidase as compared to the control group (Abdelaal et al., 2021), phenols and ȧ, β-unsaturated carbonyl compounds are potent inhibitors of GSTs in fall armyworm (Yu & Elghar, 2000) and plant phenolics were potent inhibitors of GST in cabbage looper, *Trichoplusia ni* (Hubner) and black swallowtail, *Papilio polyxenes* (Lee, 1991).

Similarly, ethanol extract of *Acalypha wilkesiana* caused a significant decrease carboxyl esterase activity and glutathione S-transferase activity in *Collasobruchus maculates* (Fabricius) (GST) (Mercy et al., 2019) Petroleum ether extract of *Illicium verum* inhibited the activity of GST in *Sitophilus zeamais* (Motschulsjy) (Li et al., 2013) and bio-pesticides such as azadirachtin, plumbagin, tagitinin C and pyridalyl were inhibited carboxyl esterase in *Helicoverpa armigera* (Hubner) (Sewali & Sanjayan, 2016). This indicates cyanobacterial extracts were also a type of bio-pesticides and some of the compounds present in the extracts may inhibit the carboxyl esterase activity, GST and cytochrome p450 monooxygenase.

Conclusion

Spirulina sp. and *N. muscorum* extracts had potential toxicity against *S. frugiperda.* The cyanobacterial extracts particularly, *N. muscorum* and *Spirulina* hexane extract were more toxic in causing larval mortality and inhibiting the activity of detoxifying enzymes *viz.*, carboxyl esterase, glutathione S-transferase and cytochrome p450 enzyme in *S. frugiperda* followed by *N. muscorum* and *Spirulina* petroleum ether extract. This indicated that cyanobacterial extracts could control insecticide-resistant populations. Since cyanobacterial extracts were also a kind of biopesticide, utilizing these types of extracts served to protect the environment from synthetic pesticides and was safer for human beings.

List of Abbreviations

FAW: Fall Army Worm
NCIML: National Centre for Industrial Microorganisms, Pune, Maharashtra
PE: Petroleum Ether
LC_{50}: Lethal Concentration
CLs: Confidence Limits
ANOVA: Analysis of Variance
CD @ 1%: Critical Difference at 1 percent level
S. Em: Standard error mean

Nm: Nanometer
Ng μL^{-1}: Nanogram per microliter
L: Liter
M: Mole
µmol: Micromole
µL: microliter
mL: milliliter
mg: microgram
Kg: Kilogram
G: gram
SD: Standard deviation

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