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Toxic effect of cyanobacterial (blue–green algae) extracts as natural pesticides for the control of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae)

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Abstract

Background The *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is a cosmopolitan polyphagous pest that feeds on nearly 80 species of plants The pest can cause upto 100% damage to crops if neglected. It is also resistant to the most of the chemical molecules available in the market, so it is necessary to identify alternate technology to combat this pest.

Results The crude cyanobacterial extracts of hexane, petroleum ether, ethanol and methanol extracts of *Spirulina* sp. and *Nostoc muscorum* were evaluated against second instar larvae of *S. frugiperda* through diet overlay method of bioassay. The results revealed that *N. muscorum* hexane extract and *N. muscorum* petroleum ether extract recorded the lowest LC_{50} value of 49.09 and 61.37 ppm, respectively. This was followed by *Spirulina* hexane extract, *Spirulina* petroleum ether extract, *N. muscorum* ethanol extract, *N. muscorum* methanol extract, *Spirulina* ethanol and methanol extract which were recorded 173.16, 227.22, 530, 730, 970 and 1000 ppm, respectively. Further, their potential effects on different biological parameters of *S. frugiperda,* viz. larval duration, pupal duration, percent pupation, pupal weight, pupal malformation, adult malformation, percent adult emergence, fecundity, and male and female adult longevity, were studied. The significant effects on different biological parameters were recorded by *N. muscorum* hexane and petroleum ether extract, followed by *Spirulina* hexane and petroleum ether extract. Similarly, an experiment was repeated for the confirmation of the first set of results, which followed by a similar trend. The first-ever novel study on *S. frugiperda* concluded that crude extracts of *Spirulina* sp. and *N. muscorum* extracts showed significant effects in causing larval mortality and affecting different biological parameters.

Conclusions The experimental results can be recommended as a potential source of natural pesticides for the control of *S. frugiperda*.

Keywords *Spodoptera frugiperda*, *Spirulina* sp. and *Nostoc muscorum*, Biological parameters, Natural pesticide

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Background

Cyanobacteria also called as blue–green algae are morphologically diverse groups of prokaryotic, photosynthetic organisms that flourish in diverse types of habitats. The cyanobacterial species, namely *Spirulina* sp. and *N.ostoc muscorum*, are free-living microorganism that inhabits both terrestrial and freshwater aquatic environments. Most species of cyanobacteria are live in freshwater, marine, or terrestrial habitat (Cameron 1960). *Spirulina* is a freshwater spiral unicellular prokaryote (Mitchell et al. 1990). *Spirulina* thrives at a pH of 8 and above and a temperature of around 25–30 °C (Supramaniyan and Jeeji 1992). It is beneficial in the treatment of diabetes, arthritis, anemia, cardiovascular disease and cancer (Mendiola et al. 2007). Though it is a beneficial for human beings, its toxic effect on insects reported by Aly and Abdou (2010) and Rania et al. (2020) found that water and ethanolic extract of *S. platensis* increased larval mortality and affected different biological parameters of *Spodoptera littoralis* (Boisduval). *N. muscorum* (cyanobacteria) is a free-living microorganism that inhabits both terrestrial and freshwater aquatic environments (Cameron 1960). *N. muscorum* cells are filamentous, gram-negative green–brown-colored algal cells that can form spores under desiccation conditions. *N. muscorum* is important for the nutrient cycling of carbon and nitrogen fixation within the soil ecosystems. The ideal environment for *N. muscorum* is one with a pH in the range of 7.0–8.5, with a lower pH limit of 5.7 and *N. muscorum* grows best when light intensity is less than that of direct sunlight, but can continue to grow and fix nitrogen in the presence of glucose and absence of sunlight (Allison et al. 1937). Due to the rapid rise in industrialization and urbanization, environmental pollution is a serious issue around the world; in particular, the widespread use of chemical pesticides has led to several environmental issues in many different nations (Aydinalp and Porca 2004). There is an urgent need for alternative management approaches since the overuse of conventional pesticides to eradicate insect pests disturbs ecosystem stability and breeds pests with greater pesticide resistance (Mohan and Gujar 2003).

The polyphagous pest fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), has been observed feeding on all parts of the plant causing economic damage to maize, rice, sorghum, millet, sugarcane, vegetable crops and cotton, as well as more than 80 other crop species (Chormule et al. 2019). It is invasive, wreaking havoc on cereal crops in general and maize crops in particular. This pest 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. 2018). The pest was thought to have migrated from Africa, where it was initially discovered in late 2016 (Nagoshi et al. 2019) and was found in 44 African countries (Rwomushana et al. 2018). The maize is a more favoured host, with infection rates ranging from 6 to 100% in Africa and India (Mallapur et al. 2018). In the absence of appropriate management techniques, the FAW is projected to cause maize yield losses in Africa ranging from 8.3 to 20.6 MT per year (Day et al. 2017).

The use of chemical pesticides has played a major role in increasing agricultural production and also in protecting crops from injury caused by insect pests. But the overuse of traditional pesticides to control insect pests leads to disturbance in ecosystem stability and increased resistance to pesticides in pests; therefore, there is an urgent need for alternative control methods (Mohan and Gujar 2003; Kariyanna 2019). The need for natural pesticides is one of the scientific research goals (Rahim and Mohamed 2013). Botanical pesticides are environmentally friendly alternative means to protect crops (Asaraja and Sahayaraj 2013). Examining natural alternative pesticides and extracts to identify new pesticides provides a method for researchers to uncover new chemical entities that have never been produced. Although plants hold considerable promise for the development of new pesticides, cyanobacteria (blue–green algae) make up the majority of the world's biomass and are a largely unexplored source. The insecticidal activity of *Spirulina* sp. and *Nostoc muscorum* extracts were not explored for their efficacy against *S. frugiperda*. Keeping this in view, an endeavor was made to study the toxic effect of cyanobacterial or blue–green algal extracts as natural pesticides for the management of fall armyworm, *S. frugiperda*.

Methods

Source of cyanobacteria and media suitable for their cultivation

The pure cultures of cyanobacterial species *Spirulina* sp. and *N. muscorum* were procured from the National Centre for Industrial Microorganisms (NCIM), Pune, Maharashtra. *Spirulina* media (NCIM, Pune) and Zarrouk media (Zarrouk, 1996) were prepared for mass production of *Spirulina* sp. Fogs media (NCIM, Pune) and BG-11 media (Allen and Stanier 1968) for mass production of *N. muscorum*.

Growth conditions and mass multiplication of cyanobacterial cultures (*Spirulina* sp. and *N. muscorum*)

A loopful of pure culture were inoculated into test tubes containing 10 ml liquid media for subculturing purposes in a laminar airflow, plugged with cotton wool and kept in a growth chamber which was set at a temperature of

25 ± 2 °C and 75 ± 2 percent relative humidity with a constant illumination of a light source. Cultures attained maximum growth after one month of inoculation, and further 100 ml liquid media was prepared and inoculated with 1 ml of culture and then kept in a growth chamber. Similarly, the same procedures were followed for 500, 1000, 2000 and 3000 ml liquid media to get a sufficient quantity of culture according to Rahim and Hamed (2013) with a slight modification. As the dry weight of the culture is very low, so it is required to maintain in different quantities of liquid media (Figs. 1 and 2).

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

After complete growth of cultures, it was filtered through a muslin cloth and shade dried at room temperature for one week. Cultures were powdered by using an electric blender. Dry powder forms of *Spirulina* sp. and *N. muscorum* were kept in the refrigerator (4 °C) until further use (Figs. 1 and 2).

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

Extraction was carried out by taking five grams of *Spirulina* sp. and *N. muscorum* powder. Each species were extracted using two different solvents like hexane and petroleum ether (PE) through the Soxhlet apparatus, whereas ethanol and methanol were used for extraction through the mechanical shaking method.

Soxhlet extraction

Extractions were carried out according to Rahim and Hamed (2013) with a slight modification. Five grams of the powdered material was extracted using 120 ml of hexane and petroleum ether in automated Soxhlet apparatus. The extract was concentrated under a desiccator to yield crude extracts. Standard stock solutions were prepared by dissolving crude material in dimethyl sulfoxide and stored at 4 °C.

Mechanical shaking method of extraction

Five grams of *Spirulina* sp. and *N. muscorum* powder was soaked in 50 ml of ethanol and methanol solvent and then kept in a mechanical shaker for three days continuously for the complete extraction of bioactive compounds, and then, it was filtered through Whatman no. 1 filter paper after that extract was kept in room temperature for about one day for the evaporation of solvents and to get a concentrated form of crude extract. Standard stock solutions were prepared by dissolving crude material in dimethyl sulfoxide and stored at 4 °C. Extractions were carried out according to Saber et al. (2018) with a slight modification.

Bioassay study of cyanobacterial extracts against fall armyworm, *S. frugiperda*

Insect rearing

The larvae of *S. frugiperda* were reared using an insect-breeding circular dish containing the maize cut leaf bits and closed with a lid and maintained at 25 ± 2 °C, 75 ± 2% RH and L12: D12 photoperiod. The emerged adults were allowed into oviposition cages. In these cages, paper towels were lined as an oviposition substrate. In each cage, pair of male and female adults were released. The adults were fed with 10% honey solution, soaked on cotton pads offer in small plastic caps inside the cages and replaced daily. Eggs were collected and kept in an insect-breeding circular dish for hatching. The eggs were examined at an interval of 12 h for hatching. The second instar larvae were used for the bioactivity test (Sharanabasappa et al. 2018b). Larvae were also reared on an artificial diet in the laboratory and the diet was prepared according to Omar and David (2019).

Bioassay test

The bioassay test was conducted by using the diet overlay method of bioassay. A series of six different concentrations, viz. 0, 100, 200, 400, 800 and 1600 ppm, were prepared from the stock solutions extracted through the Soxhlet apparatus and 0, 500, 1000, 2000, 4000 and 8000 ppm were prepared from the stock solutions extracted through mechanical shaking method, based on bracketing technique.

Diet overlay method

In the diet overlay method, the artificial diet of FAW was poured into a multi-well cavity tray. After drying to each well, 100 µl of the extract was gently smeared on the surface and allowed for the evaporation of solvents under room temperature until the formation of a thin layer of the extract on the diet. In each well, a one-second instar larva was placed for feeding. Thus, for each treatment, 40 larval individuals were used. After the exhaustion of the treated diet, a fresh diet without extract was provided until the larvae enter into pupation (Omar et al. 2019). LC_{50} values were calculated after 96 h. Similarly, a second set of experiments was conducted for the confirmation of the first set of bioassay results.

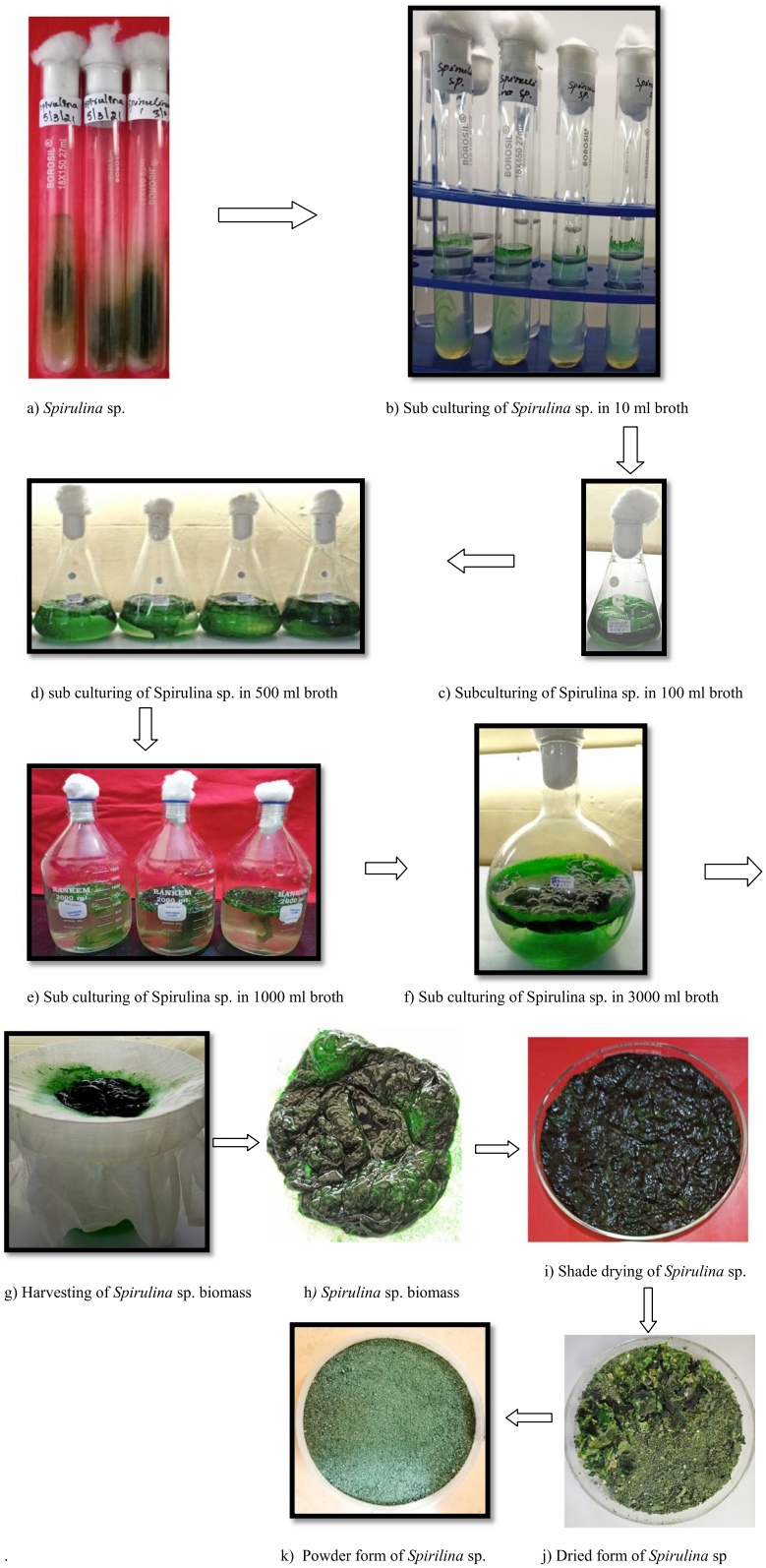


Fig. 1 Mass production of *Spirulina* sp. **a** *Spirulina* sp., **b** subculturing of *Spirulina* sp. in 10 ml broth, **c** subculturing of *Spirulina* sp. in 100 ml broth, **d** subculturing of *Spirulina* sp. in 500 ml broth, **e** subculturing of *Spirulina* sp. in 1000 ml broth, **f** subculturing of *Spirulina* sp. in 3000 ml broth, **g** harvesting of *Spirulina* sp. biomass, **h** *Spirulina* sp. biomass, **i** shade drying of *Spirulina* sp., **j** dried form of *Spirulina* sp, **k** powder form of *Spirilina* sp

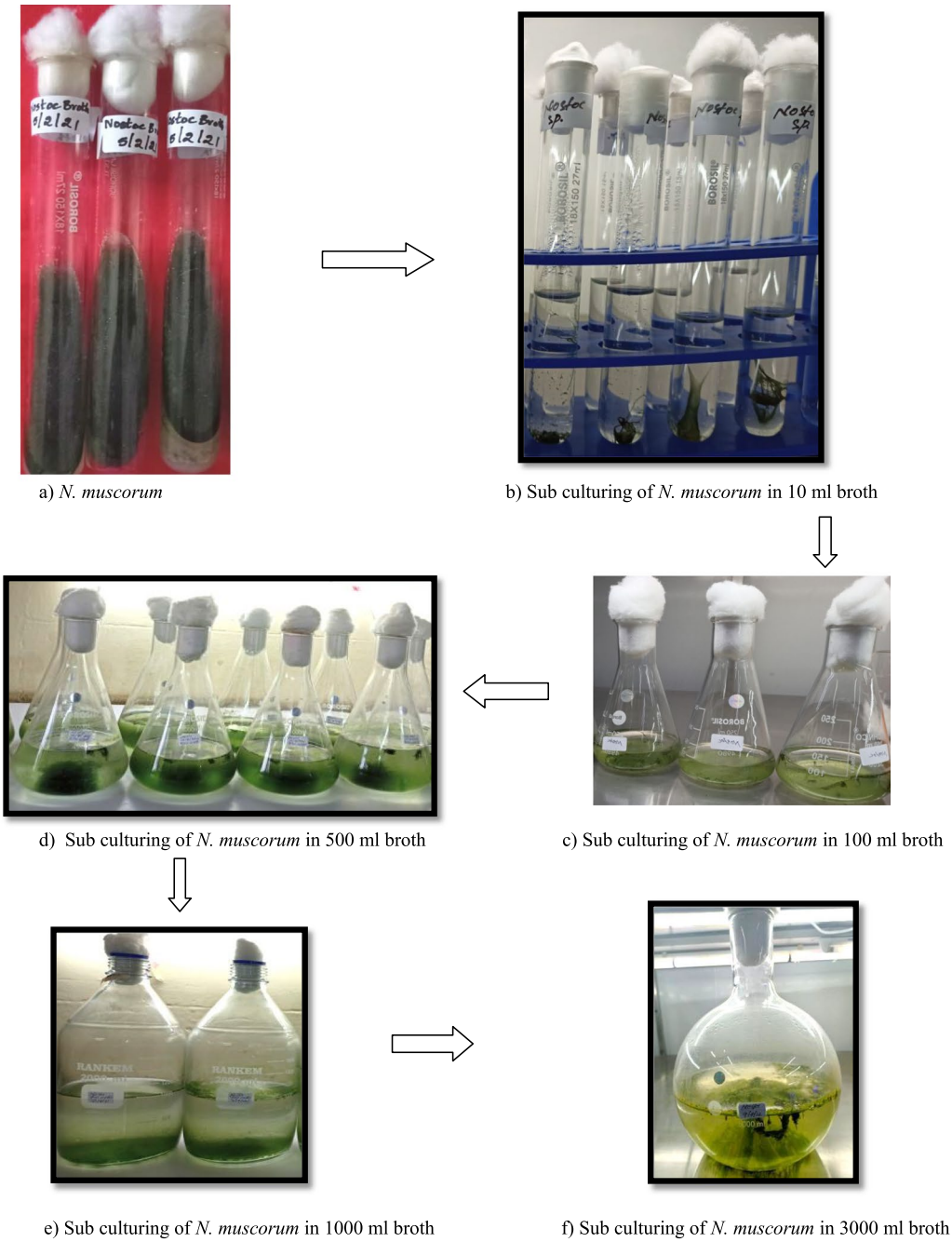


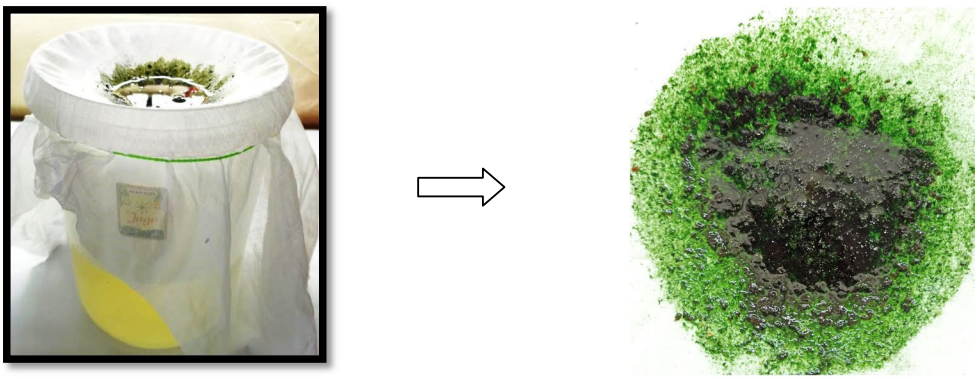
Fig. 2 Mass production of *N. muscorum*, **a** *N. muscorum*, **b** subculturing of *N. muscorum* in 10 ml broth, **c** subculturing of *N. muscorum* in 100 ml broth, **d** subculturing of *N. muscorum* in 500 ml broth, **e** subculturing of *N. muscorum* in 1000 ml broth, **f** subculturing of *N. muscorum* in 3000 ml broth, **g** harvesting of *N. muscorum* biomass, **h** *N. muscorum* biomass, **i** shade drying of *N. muscorum*, **j** dried form of *N. muscorum*, **h** powder form of *N. muscorum*

Effect of LC_{50} values on different biological parameters

The toxic effect of *Spirulina* sp. and *N. muscorum* extracts at their LC_{50} values on some biological parameters such as larval and pupal duration, pupation, adult emergence, pupal weight, pupal and adult malformation, male and female adult longevity and fecundity was recorded.

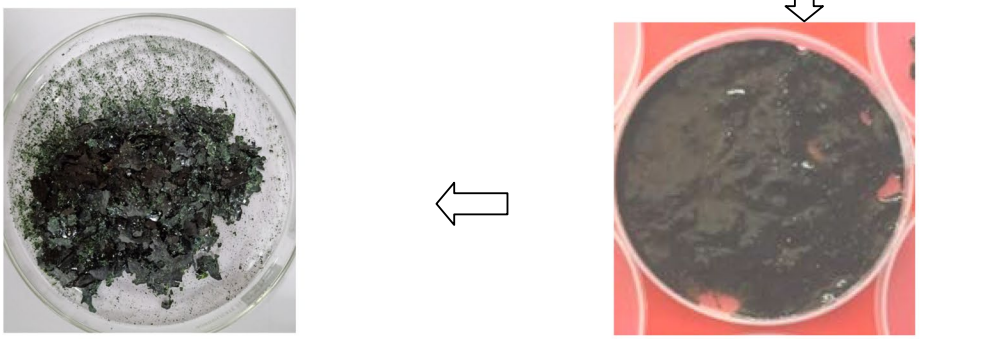
Statistical analysis

The LC_{50} values were calculated after 96 h of treatments and corrected according to the Abbott formula (Abbott 1925). The data were analyzed using the probit analysis (Finney 1971) for the determination of median lethal concentration (LC_{50}) and their 95% confidence



g) Harvesting of *N. muscorum* biomass

h) *N. muscorum* biomass



j) Dried form of *N. muscorum*

i) Shade drying of *N. muscorum*

h) Powder form of *N. muscorum*

Fig. 2 continued

limits (CLs) by using the POLO plus (Leora software 2002, Brkeliy, CA, USA). Bioassay data on different biological parameters of *S. frugiperda* were analyzed using one-way ANOVA (analysis of variance) with DMRT (Duncan multiple range test) using spss, wasp 2 (Ashok kumar, J. ICAR Research complex for Goa, Ela, old Goa, India) and opstat software (Sheoran, O. P., COBS & H CCS HAU, Hisar). The values were transformed to square root for the parameters, viz. larval duration, pupal duration, pupal weight, fecundity as well as male and female adult longevity. The values were transformed to arc sine for the parameters, viz. percent pupation, percent adult

emergence, pupal and adult malformation. The obtained data were also statically calculated through excel for windows computer program to determine P value, F value and degrees of freedom.

Results

The diet overlay method of bioassay revealed that among different treatments, *N. muscorum* hexane extract and *N. muscorum* petroleum ether extract were the most toxic and recorded the lowest LC_{50} values of 49.09 and 61.37 ppm, respectively. This was followed by *Spirulina* hexane extract (173.16 ppm), *Spirulina* petroleum ether extract (227.22 ppm), *N. muscorum* ethanol extract (530 ppm) and *N. muscorum* methanol extract (730 ppm). *Spirulina* methanol extract and *Spirulina* ethanol extract were recorded high LC_{50} values of 970 and 1000 ppm, respectively. Similar results were obtained during the second set of experiment wherein *N. muscorum* hexane and *N. muscorum* petroleum ether extract were recorded with the lowest LC_{50} values of 55.01 and 78.73 ppm, respectively, and *Spirulina* methanol extract recorded with a high LC_{50} value of 960 ppm (Table 1).

Toxic effects of *Spirulina* sp. and *N. muscorum* extracts at their LC_{50} values on different biological parameters of *S. frugiperda*

Larvae of *S. frugiperda* that were fed on a diet treated with different cyanobacterial extracts at their LC_{50} level had a significant effect on different biological parameters, as compared to their control.

Larval duration

Potential toxic activities of crude cyanobacterial extracts on the larval duration of *S. frugiperda* were observed. Second instar larvae of *S. frugiperda* fed on a diet treated with *N. muscorum* hexane extract at their LC_{50} level significantly increased the larval duration (18.33 days). This was followed by *N. muscorum* petroleum ether extract (17.73 days) and *Spirulina* hexane extract (17.33 days) and these treatments were at par with each other and significantly superior to the rest of the treatments. The remaining treatments, viz. *Spirulina* petroleum ether extract, *N. muscorum* ethanol extract, *N. muscorum* methanol extract, *Spirulina* ethanol extract and *Spirulina* methanol extract, were moderately effective in increasing the larval duration with a mean of 17, 16.83, 16.66, 16.50 and 16.16 days, respectively, as compared to their control which was recorded a low larval duration (15.33 days). Similarly, the second set of experiments was conducted for the confirmation of the first set results and the same trend was observed.

The pooled data on larval duration indicated that among different treatments *N. muscorum* hexane extract significantly increased the larval duration (18.42 days). This was followed by *N. muscorum* petroleum ether extract (17.73 days) and *Spirulina* hexane extract (17.42 days) and these treatments were at par with each other and significantly superior over the rest of the treatments. The remaining treatments, viz. *Spirulina* petroleum ether extract, *N. muscorum* ethanol extract, *N. muscorum* methanol extract, *Spirulina* ethanol extract and *Spirulina* methanol extract, were moderately effective in increasing the larval duration with means of 16.83, 16.67, 16.50, 16.33 and 16.17 days, respectively, as compared to their control (15.17 days) (Table 2).

Pupal duration

In the case of pupal duration, second instar larvae of *S. frugiperda* fed on a diet treated with *N. muscorum* hexane extract at their LC_{50} level significantly increased the pupal duration (13.83 days), followed by *N. muscorum* petroleum ether extract (12.83 days) and *Spirulina* hexane extract (12.33 days) and these treatments were at par with each other and significantly superior to rest of the treatments. The remaining treatments, viz. *Spirulina* petroleum ether extract, *N. muscorum* ethanol extract, *N. muscorum* methanol extract, *Spirulina* ethanol extract and *Spirulina* methanol extract, were moderately effective in increasing the pupal duration with means of 12, 11.83, 11.50, 11.33 and 10.66 days, respectively, as compared to their control (10.16 days). Similarly, an experiment was repeated and results, followed a similar trend wherein *N. muscorum* hexane extract significantly prolonged the pupal duration with a mean of 13.83 days. This was followed by *N. muscorum* petroleum ether extract (13 days), whereas the control population significantly recorded low pupal duration with a mean of 10 days.

The pooled data indicated that among different treatments, *N. muscorum* hexane extract significantly increased the pupal duration (13.83 days) followed by *N. muscorum* petroleum ether extract (12.92 days) and *Spirulina* hexane extract (12.42 days) and these treatments were at par with each other and significantly superior to rest of the treatments. The remaining treatments, viz. *Spirulina* petroleum ether extract, *N. muscorum* ethanol extract, *N. muscorum* methanol extract, *Spirulina* ethanol extract and *Spirulina* methanol extract, were moderately effective in increasing the pupal duration with means of 12.08, 11.92, 11.50, 11.25 and 10.75 days, respectively, as compared to their control which was recorded a low pupal duration (10.08 days) (Table 2).

Table 1 Insecticidal activity of *Spirulina* sp. and *Nostoc muscorum* extracts against *Spodoptera frugiperda*

Treatment details	Diet overlay 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	173.16	1.027 (±0.262)	0.068	282.696	65.447	165.22	0.965 (±0.261)	0.677	278.353	53.504
Spirulina PE ether extract	227.22	1.057 (±0.259)	0.195	359.319	107.163	223.18	1.030 (±0.259)	0.984	356.909	101.436
N. muscorum hexane extract	49.09	0.823 (±0.279)	0.042	122.517	0.965	55.01	0.790 (±0.274)	0.346	135.126	0.996
N. muscorum PE ether extract	61.37	0.753 (±0.267)	0.197	149.243	0.977	78.73	0.850 (±0.268)	0.012	165.614	6.029
Spirulina ethanol extract	970	1.090 (±0.264)	0.406	0.153	0.044	890	1.070 (±0.264)	0.642	0.142	0.036
Spirulina methanol extract	1000	0.898 (±0.255)	0.029	0.170	0.033	960	1.050 (±0.262)	0.260	0.153	0.040
N. muscorum ethanol extract	530	0.830 (±0.261)	0.116	0.105	0.006	520	0.886 (±0.265)	0.098	0.099	0.008
N. muscorum methanol extract	730	0.829 (±0.257)	0.361	0.135	0.014	710	0.906 (±0.260)	0.226	0.127	0.017

ppm Parts per million, *SD* Standard deviation, *Df* Degrees of freedom

Table 2 Toxic effect of *Spirulina* sp. and *Nostoc muscorum* extracts at their LC_{50} values on larval and pupal duration of *Spodoptera frugiperda*

Treatments	Larval duration Pooled data ± SE	Pupal duration Pooled data ± SE
Spirulina hexane extract	17.42 (4.23)^{abc} ± 0.18	12.42 (3.59)^{abc} ± 0.25
Spirulina PE extract	16.83 (4.16)^{bcd} ± 0.30	12.08 (3.54)^{bcd} ± 0.56
Nostoc muscorum hexane extract	18.42 (4.35)^{a} ± 0.41	13.83 (3.79)^{a} ± 0.14
N. muscorum PE extract	17.73 (4.27)^{ab} ± 0.44	12.92 (3.66)^{ab} ± 0.53
Spirulina ethanol extract	16.33 (4.10)^{cd} ± 0.25	11.25 (3.43)^{cde} ± 0.54
Spirulina methanol extract	16.17 (4.08)^{de} ± 0.14	10.75 (3.35)^{de} ± 0.31
N. muscorum ethanol extract	16.67 (4.14)^{cd} ± 0.18	11.92 (3.52)^{bcd} ± 0.30
N. muscorum methanol extract	16.50 (4.12)^{cd} ± 0.31	11.50 (3.46)^{bcd} ± 0.41
Control	15.17 (3.96)^{f} ± 0.07	10.08 (3.25)^{f} ± 0.56
P value	0.000124	0.003449
F value	8.184	4.597887
DF	26	26
CD (0.01)	0.163	0.307
CD (0.05)	0.119	0.224

Treatments found significant at 1% and 5% level of significance

* Figures in parentheses are square root transformed values

a,b,c,d,e,f: The values represented by same alphabet are statistically on par with each other by DMRT mean of three replications

Table 3 Toxic effect of *Spirulina* sp. and *Nostoc muscorum* extracts at their LC_{50} values on pupation and pupal weight of *Spodoptera frugiperda*

Treatments	Pupation (%) Pooled data	Pupal weight (g) Pooled data ± SE
Spirulina hexane extract	53.67 (47.10)^{abc}	0.18 (0.82)^{c} ± 0.00
Spirulina PE extract	54.50 (47.59)^{abcd}	0.19 (0.83)^{cd} ± 0.01
Nostoc muscorum hexane extract	50.83 (45.48)^{a}	0.12 (0.79)^{a} ± 0.01
N. muscorum PE extract	52.50 (46.43)^{ab}	0.15 (0.80)^{b} ± 0.01
Spirulina ethanol extract	58.67 (49.99)^{cd}	0.22 (0.85)^{e} ± 0.00
Spirulina methanol extract	60.00 (50.79)^{e}	0.23 (0.85)^{e} ± 0.00
N. muscorum ethanol extract	55.83 (48.35)^{abcd}	0.19 (0.83)^{cd} ± 0.00
N. muscorum methanol extract	57.50 (49.32)^{bcd}	0.20 (0.84)^{d} ± 0.00
Control	98.83 (83.83)^{f}	0.29 (0.89)^{f} ± 0.00
P value		0.000000000559
F value	127.8962	40.75302
DF	26	26
CD (0.01)	4.326	0.019
CD (0.05)	3.158	0.014

Treatments found significant at 1% and 5% level of significance

* Figures in parentheses are arcsine transformed values

a,b,c,d,e,f: The values represented by same alphabet are statistically on par with each other by DMRT mean of three replications

Percent pupation

The second instar larvae of *S. frugiperda* fed on a diet treated with *N. muscorum* hexane extract at their LC_{50} level showed a significant reduction in percent pupation (51.66%). This was followed by *N. muscorum* petroleum ether extract, *Spirulina* hexane extract, *Spirulina* petroleum ether extract and *N. muscorum* ethanol extract and these treatments were at par with each other and significantly superior to the rest of the treatments with means of 53.33, 54, 55 and 56.66%, respectively. The remaining treatments *N. muscorum* methanol extract, *Spirulina* ethanol extract and *Spirulina* methanol extract were minimum effect in reducing percent pupation with means of 58.33, 59 and 60%, respectively, but significantly superior over control (98.66%). A similar trend was followed during the second set of experiments wherein *N. muscorum* hexane extract and *N. muscorum* petroleum ether extract significantly decreased the percent pupation with a mean of 50 and 51.66%, respectively, as compared to 99% pupation in control.

In the case of pooled data, the results revealed that among different treatments, *N. muscorum* hexane extract at their LC_{50} level showed a significant reduction in percent pupation (50.83%), followed by *N. muscorum* petroleum ether extract, *Spirulina* hexane extract, *Spirulina* petroleum ether extract and *N. muscorum* ethanol extract and these treatments were at par with each other and significantly superior to rest of the treatments with means of 52.50, 53.67, 54.50 and 55.83%, respectively. The remaining treatments, viz. *N. muscorum* methanol extract, *Spirulina* ethanol extract and *Spirulina* methanol extract, were minimum effect in reducing % pupation with means of 57.50, 58.67 and 60%, respectively, and these treatments were significantly superior over control which was recorded higher pupation with a mean of 98.83% (Table 3).

Pupal weight

Similarly, in the case of pupal weight among different treatments *N. muscorum* hexane extract and *N. muscorum* petroleum extract significantly reduced the pupal weight with means of 0.12 and 0.15 g, respectively, followed by *Spirulina* hexane extract, *Spirulina* petroleum ether extract, *N. muscorum* ethanol extract and these treatments were at par with each other and recorded 0.17, 0.19 and 0.20 g, respectively. *N. muscorum* methanol extract (0.21 g), *Spirulina* ethanol extract (0.22 g) and *Spirulina* methanol extract (0.23 g) were moderately effective in reducing pupal weight but significantly

superior to the control which was recorded the highest pupal weight with a mean of 0.29 g. In the same manner, an experiment was repeated and the results followed the same trend as that of the first set of experiments.

The pooled data indicated that among different treatments, *N. muscorum* hexane extract significantly reduced the pupal weight with a mean of 0.12 g. This was followed by *N. muscorum* petroleum ether extract (0.15 g) and the treatments *Spirulina* hexane extract, *Spirulina* petroleum ether extract, *N. muscorum* ethanol extract were at par with each other and recorded 0.18, 0.19 and 0.19 g, respectively. *N. muscorum* methanol extract (0.20 g), *Spirulina* ethanol extract (0.22 g) and *Spirulina* methanol extract (0.23 g) were least effective in reducing pupal weight but significantly superior to the control which was recorded the highest pupal weight with a mean of 0.29 g (Table 3).

Pupal malformation

The second instar larvae of *S. frugiperda* fed on a diet treated with *N. muscorum* hexane and *N. muscorum* petroleum ether extract at their LC_{50} values showed a significant increase in pupal malformation with a mean of 56.67 and 53.33%, respectively, followed by *Spirulina* hexane extract (49%) and *Spirulina* petroleum ether extract (46.67%) and the treatments *N. muscorum* ethanol extract (42.33%), *N. muscorum* methanol extract (40%), *Spirulina* ethanol extract (38%) and *Spirulina* methanol extract (36.67%) were at par with each other but significantly superior over control, which had no effect in causing pupal malformation (0%). Likewise, the experiment was repeated for the confirmation of previous results and the same trend was followed during the second set of observations.

The pooled data showed that among different treatments *N. muscorum* hexane and *N. muscorum* petroleum ether extract showed a significant increase in pupal malformation with means of 57.50 and 54.17%, respectively, followed by *Spirulina* hexane extract (49.83%) and *Spirulina* petroleum ether extract (47.50%) and the treatments *N. muscorum* ethanol extract (43.50%), *N. muscorum* methanol extract (41.33%), *Spirulina* ethanol extract (39.33%) and *Spirulina* methanol extract (38%) were at par with each other but significantly superior over control, which was no effect in causing pupal malformation (0%) (Table 4) (Fig. 3A). Pupal and adult intermediates were also observed (Fig. 4).

Adult malformation

Similarly, in case of adult malformation, among different treatments *N. muscorum* hexane extract and *N. muscorum* petroleum ether extract at their LC_{50} values significantly increased the adult malformation with means of

Table 4 Toxic effect of *Spirulina* sp. and *Nostoc muscorum* extracts at their LC_{50} values on pupal and adult malformation of *Spodoptera frugiperda*

Treatments	Pupal malformation (%)	Adult malformation (%)
	Pooled data	Pooled data
Spirulina hexane extract	49.83 (44.90)^{bc}	44.50 (41.84)^{b}
Spirulina PE extract	47.50 (43.56)^{cd}	39.17 (38.74)^{c}
Nostoc muscorum hexane extract	57.50 (49.32)^{a}	58.17 (49.70)^{a}
N. muscorum PE extract	54.17 (47.39)^{ab}	53.17 (46.82)^{a}
Spirulina ethanol extract	39.33 (38.83)^{ef}	34.83 (36.14)^{cd}
Spirulina methanol extract	38.00 (38.05)^{g}	31.50 (34.13)^{e}
N. muscorum ethanol extract	43.50 (41.26)^{de}	38.17 (38.14)^{c}
N. muscorum methanol extract	41.33 (40.01)^{ef}	36.50 (37.16)^{cd}
Control	0.00 (0.00)^{h}	0.00 (0.00)^{f}
F value	247.4882	224.3165
DF	26	26
CD (0.01)	3.905	3.831
CD (0.05)	2.851	2.797

Treatments found significant at 1% and 5% level of significance

* Figures in parentheses are arcsine transformed values

a,b,c,d,e,f: The values represented by same alphabet are statistically on par with each other by DMRT mean of three replications

A)

B)

Fig. 3 **A** Pupal malformation of *S. frugiperda* resulted from the effect of *Spirulina* sp and *N. muscorum* extracts, **B** normal pupa

Fig. 4 Pupal–adult intermediates of *S. frugiperda* resulted from the effect of cyanobacterial extracts

58 and 53%, respectively, followed by *Spirulina* hexane extract (43.33%) and the remaining treatments like *Spirulina* petroleum ether extract (39%), *N. muscorum* ethanol extract (38%), *N. muscorum* methanol extract (36.33%), *Spirulina* ethanol extract (34.66%) and *Spirulina* methanol extract (31.33%) were at par with each other and significantly superior to control, which had no effect in causing adult malformation (0%). Similarly, the experiment was repeated in the second set for the confirmation of the first set of results.

The pooled data indicated that among different treatments *N. muscorum* hexane extract and *N. muscorum* petroleum ether extract at their LC_{50} values significantly increased the adult malformation with means of 58.17 and 53.17%, respectively, followed by *Spirulina* hexane extract (44.50%) and the remaining treatments like *Spirulina* petroleum ether extract (39.17%), *N. muscorum* ethanol extract (38.17%), *N. muscorum* methanol extract (36.50%), *Spirulina* ethanol extract (34.83%) and *Spirulina* methanol extract (31.50%) were at par with each other and significantly superior to control, which was no effect in causing adult malformation (0%) (Table 4) (Fig. 5A–C).

Percent adult emergence

The second instar larvae of *S. frugiperda* fed on a diet treated with *N. muscorum* hexane extract at their LC_{50} level significantly reduced the percent adult emergence with a mean of 50%. This was followed by *N. muscorum* petroleum ether extract, *Spirulina* hexane extract, *Spirulina* petroleum ether extract, *N. muscorum* ethanol extract, *N. muscorum* methanol extract and *Spirulina* ethanol extract which were at par with each other and recorded 51.66, 52.33, 53.33, 55, 56.66 and 56.66%, respectively. *Spirulina* methanol extract (58.33%) was the least effective in reducing percent adult emergence but significantly superior over control which was recorded a high percent adult emergence (98%). The study was repeated for the confirmation of previous results and the observations followed a similar trend.

The pooled data results revealed that among different treatments, *N. muscorum* hexane extract at their LC_{50} level significantly reduced the percent adult emergence with a mean of 50.83%. This was followed by *N. muscorum* petroleum ether extract, *Spirulina* hexane extract, *Spirulina* petroleum ether extract, *N. muscorum* ethanol extract, *N. muscorum* methanol extract and *Spirulina* ethanol which were at par with each other and recorded 52, 52.83, 54.17, 55.83, 57.17 and 57.50%, respectively. *Spirulina* methanol extract (59.17%) was least effective in reducing percent adult emergence but significantly superior over control (98.17%).

Fecundity

With respect to fecundity *S. frugiperda* at their LC_{50} value caused a significant reduction in the number of eggs laid by the female in all the treatments than their control. Among the different treatments, *N. muscorum* hexane extract significantly recorded the lowest number of eggs with a mean of 533 eggs. This was followed by *N. muscorum* petroleum ether extract (600.66 eggs) and *Spirulina* hexane extract (619 eggs), which were significantly superior to the rest of the treatments and the treatments *Spirulina* petroleum ether extract, *N. muscorum* ethanol extract, *N. muscorum* methanol extract, *Spirulina* ethanol extract and *Spirulina* methanol extract

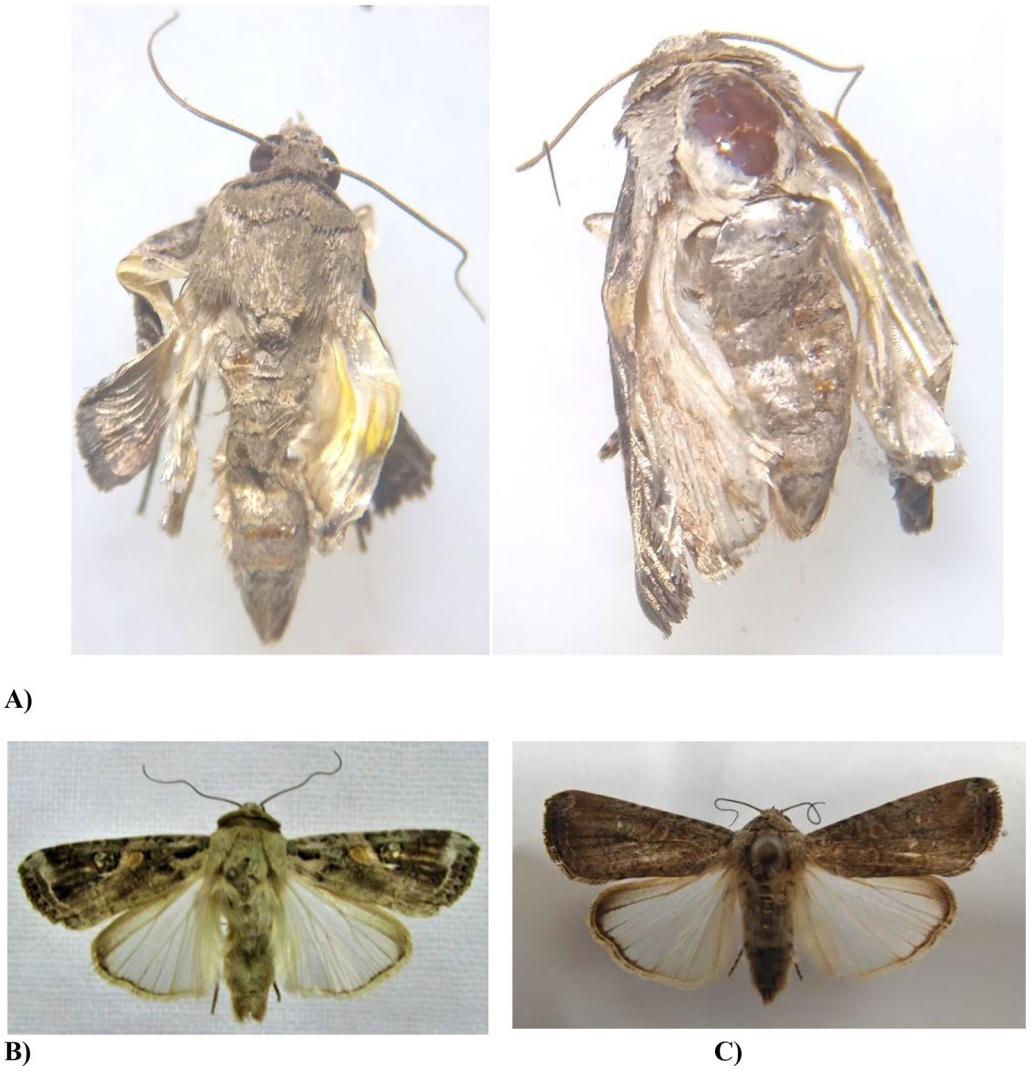


Fig. 5 **A** Adult malformation of *S. frugiperda* resulted from the effect of different cyanobacterial extracts, **B** normal male, **C** normal female

were moderately effective in recording a low number of eggs with means of 645.33, 720.33, 723.66, 733.66 and 749 eggs, respectively, and these treatments were at par with each other but significantly superior over control which has recorded the highest number of eggs (1064.66 eggs). The same trend was followed during the second set of experiments.

The pooled data indicated that among different treatments, *N. muscorum* hexane extract significantly recorded the lowest number of eggs with a mean of 532.17 eggs, followed by *N. muscorum* petroleum ether extract (599.83 eggs), *Spirulina* hexane extract (618.17 eggs) and *Spirulina* petroleum ether extract (644.50 eggs) which were significantly superior to the rest of the treatments and the treatments, *N. muscorum* ethanol extract, *N. muscorum* methanol extract, *Spirulina* ethanol extract and *Spirulina* methanol extract were moderately effective in recording the lowest number of eggs with a mean of 719.50, 722.83, 732.83 and 748.17 eggs, respectively, and these treatments were at par with each other but significantly superior over control which was recorded the highest number of eggs (1065.67 eggs) (Table 5).

Male adult longevity

The second instar larvae of *S. frugiperda* fed on a diet treated with *N. muscorum* hexane extract at their LC_{50} significantly reduced the male adult longevity (5.16 days). This was followed by *N. muscorum* petroleum ether, *Spirulina* hexane extract, *Spirulina* petroleum ether extract and *N. muscorum* ethanol extract which were at par with each other with means of 5.91, 6.16, 6.50 and 6.66 days, respectively, and the remaining treatments like *N.*

Table 5 Toxic effect of *Spirulina* sp. and *Nostoc muscorum* extracts at their LC_{50} values on adult emergence and fecundity of *Spodoptera frugiperda*

Treatments	Adult emergence (%) Pooled data	Fecundity (number) Pooled data ± SE
Spirulina hexane extract	52.83 (46.63)^{ab}	618.17 (24.86)^{ab} ± 25.79
Spirulina PE extract	54.17 (47.40)^{ab}	644.50 (25.34)^{bc} ± 48.44
Nostoc muscorum hexane extract	50.83 (45.48)^{a}	532.17 (23.06)^{a} ± 22.23
N. muscorum PE extract	52.00 (46.15)^{a}	599.83 (24.43)^{ab} ± 53.31
Spirulina ethanol extract	57.50 (49.32)^{ab}	732.83 (27.07)^{c} ± 23.82
Spirulina methanol extract	59.17 (50.29)^{c}	748.17 (27.36)^{c} ± 12.26
N. muscorum ethanol extract	55.83 (48.36)^{ab}	719.50 (26.83)^{c} ± 17.33
N. muscorum methanol extract	57.17 (49.12)^{ab}	722.83 (26.88)^{c} ± 23.35
Control	98.17 (82.23)^{d}	1065.67 (32.65)^{d} ± 18.31
P-value		0.00000288
F value	106.1688	13.92352
DF	26	26
CD (0.01)	4.572	2.968
CD (0.05)	3.337	2.167

Treatments found significant at 1% and 5% level of significance

* Figures in parentheses are arcsine transformed values

a,b,c,d: The values represented by same alphabet are statistically on par with each other by DMRT mean of three replications

muscorum methanol extract (6.83 days), *Spirulina* ethanol extract (6.83 days) and *Spirulina* methanol extract (7 days) were the least effective in reducing male adult longevity but significantly superior to control (8 days). Likewise, the experiment was repeated and the observations followed a similar trend.

The pooled data results indicated that among different treatments *N. muscorum* hexane extract (5.08 days) and *N. muscorum* petroleum ether (5.83 days) at their LC_{50} significantly reduced the male adult longevity. These were followed by *Spirulina* hexane extract, *Spirulina* petroleum ether extract and *N. muscorum* ethanol extract which were at par with each other with means of 6.08, 6.33 and 6.50 days, respectively, and the remaining treatments like *N. muscorum* methanol extract, *Spirulina* ethanol extract and *Spirulina* methanol extract were the least effective in reducing male adult longevity but significantly superior to control and recorded 6.67, 6.75, 6.92 days, respectively, than their control which was recorded higher male adult longevity (8 days) (Table 6).

Female adult longevity

Similarly, female adult longevity was significantly reduced by *N. muscorum* hexane extract (7.16 days). This was followed by *N. muscorum* petroleum ether extract and *Spirulina* hexane extract with a mean of 7.83 and 8 days, respectively, and the remaining treatments like *Spirulina* petroleum ether extract, *N. muscorum* ethanol extract, *N. muscorum* methanol extract, *Spirulina* ethanol extract and *Spirulina* methanol extract were at par with each other and recorded 8.5, 8.66, 8.83, 8.83 and 9 days,

Table 6 Toxic effect of *Spirulina* sp. and *Nostoc muscorum* extracts at their LC_{50} values on male and female adult longevity of *Spodoptera frugiperda*

Treatments	Male adult longevity (days) Pooled data ± SE	Female adult longevity (days) Pooled data ± SE
Spirulina hexane extract	6.08 (2.56)^{bc} ± 0.44	7.92 (2.90)^{abc} ± 0.25
Spirulina PE extract	6.33 (2.61)^{bc} ± 0.11	8.33 (2.97)^{bc} ± 0.18
Nostoc muscorum hexane extract	5.08 (2.36)^{a} ± 0.23	7.08 (2.75)^{a} ± 0.18
N. muscorum PE extract	5.83 (2.51)^{ab} ± 0.49	7.75 (2.87)^{ab} ± 0.51
Spirulina ethanol extract	6.75 (2.69)^{c} ± 0.20	8.75 (3.04)^{bc} ± 0.20
Spirulina methanol extract	6.92 (2.72)^{c} ± 0.11	9.00 (3.08)^{c} ± 0.24
N. muscorum ethanol extract	6.50 (2.64)^{bc} ± 0.24	8.50 (3.00)^{bc} ± 0.12
N. muscorum methanol extract	6.67 (2.68)^{c} ± 0.18	8.67 (3.03)^{bc} ± 0.14
Control	8.00 (2.91)^{d} ± 0.24	10.75 (3.35)^{d} ± 0.42
P value	0.001827	0.0000877
F value	5.177086	8.544791
DF	26	26
CD (0.01)	0.274	0.232
CD (0.05)	0.200	0.169

Treatments found significant at 1% and 5% level of significance

* Figures in parentheses are square root transformed values

a,b,c,d: The values represented by same alphabet are statistically on par with each other by DMRT mean of three replications

respectively, and showed significant difference over control (10.83 days) and similar trends were observed in second set of the experiment.

In the case of pooled data, the results revealed that *N. muscorum* hexane extract at their LC_{50} values significantly decreased the female adult longevity with a mean of 7.08 days. This was followed by *N. muscorum* petroleum ether extract (7.75 days) extract and *Spirulina* hexane extract (7.92 days) and the remaining treatments like *Spirulina* petroleum ether extract, *N. muscorum* ethanol extract, *N. muscorum* methanol extract, *Spirulina* ethanol extract and *Spirulina* methanol extract were at par with each other and recorded 8.33, 8.50, 8.67, 8.75 and 9 days, respectively, and showed significant difference over control which was recorded high female adult longevity (10.75 days) (Table 6).

The overall results revealed that crude cyanobacterial extracts of *Spirulina* sp. and *N. muscorum*, in general, had a potential effect against larvae and other developmental stages of *S. frugiperda*. However, the degree of toxicity varied among different extracts wherein *N. muscorum* hexane extract and *N. muscorum* petroleum ether extract were proved to be the most effective against different biological parameters of *S. frugiperda,* followed by *Spirulina* hexane extract and *Spirulina* petroleum ether extract. Since it is of the first kind of study, similar kinds of studies were not reported against *S. frugiperda* but some other algae and cyanobacteria having insecticidal properties against other crop pests were reported. However, from a larger perspective, cyanobacterial extracts were also a type of biopesticides so, extracts of plant-originated biopesticides were utilized for discussion.

Discussion

The increase in the larval and pupal periods may be due to the toxicant may bind to the midgut epithelium and affecting the digestive system this results in decreasing the efficiency of conversion of ingested food to the efficiency of conversion of digested food, this may also affect the percent pupation and pupal weight (Mandeep et al. 2019). An increase in pupal and adult malformation may be due to the effect of the toxicant during metamorphosis and a decrease in adult longevity may be due to a decrease in sugar, carbohydrate and fats, etc. in the body of the insect which are required for survival of adults and this may results in lower production of eggs (Mohan et al. 2013).

The insecticidal activity of *N. muscorum* and *Spirulina* sp. extracts on different biological parameters of *S. frugiperda* in the present study are in line with the insecticidal activity of other cyanobacterial, algal and plant extracts reported by the many authors, viz. hexane extract of *Inula racemosa* Hook, when mixed with diet against *Spodoptera litura* (Fab) lead to a significant increase in larval duration than the control. Later significantly affected pupal malformation, decreased adult emergence, male and female adult longevity (Mandeep et al. 2019). Similarly, hexane extract of *Eurybia divaricate* (L.) (Asterace) significantly increased larval and pupal duration of *S. litura* (Amala et al. 2021), hexane extract of *Cascabela peruviana* (L.) (Apocynaceae) significantly increased the pupal and adult malformation of *S. litura* than the control (Ramanan and Muthukumaran 2020), hexane extract of castor seed resulted in growing inhibition of *S. frugiperda* and in prolonged larval and pupal durations than the control (López et al. 2010) and petroleum ether extracts of *Ulva lactuca* caused the highest percentages of inhibition of adult emergence in *S. littoralis* than the control (Moustafa et al. 2014).

The results are contradictory with some of the reports such as ethanolic extract of *N. muscorum* and *A. fertilissima* significantly increased the larval and pupal durations than the control. Later noticed significantly decreased percent pupation, pupal weight and percent moth emergence of *Agrotis ipsilon* (Hufnagel) (Rahim and Mohamed 2013), ethanolic extract of the *N. carneum* significantly prolonged the larval and pupal duration of *S. littoralis*, whereas *P. kessleri* and *N. carneum* distinctly reduced the formation of pupae and significantly decreased the pupal weights, adult emergence, fecundity, and male and female longevity and increased pupal and adult malformation (Saber et al. 2018), ethanol extract of *S. platensis* significantly increased larval, pupal durations, pupal, adult malformation and significantly decreased fecundity of *S. littoralis* (Rania et al. 2020), and hydroalcoholic extracts of three seaweeds *Sargassum asperifolium*, *S. dentifolium* and *S. linifolium* affected different biological parameters of *S. littoralis* and *S. frugiperda* (Matloub et al. 2012). These contradictory results may be because each solvent has the capability of dissolving different bioactive compounds; moreover, hexane and petroleum ether extracts of *N. muscorum* and *Spirulina* sp. were not reported against *S. frugiperda* and results also varied with a different strain of cyanobacterial species, method of extraction and varied with each species of insects.

Conclusions

The novel study of the insecticidal activity of *Spirulina* sp. and *N. muscorum* extracts had a potential impact on larval mortality and different biological parameters of *S. frugiperda*. From the various cyanobacterial extracts, *N. muscorum* hexane and petroleum ether extracts recorded low LC_{50} values and significantly affected different biological parameters, viz. larval duration, pupal duration, pupation, pupal weight, pupal malformation, adult

malformation, adult emergence, fecundity, and male and female adult longevity. These treatments were followed by *Spirulina* hexane and petroleum ether extract. The cyanobacterial extracts of *Spirulina* sp. *N. muscorum* were also a type of biopesticides which are eco-friendly in nature and serve as a natural and alternative source for the management of fall armyworm and other crop pests in various crop ecosystems.

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
DMRT	Duncan multiple range test

Author contributions

The individual contributions of authors to the manuscript should be specified in this section. SCH methodology, experimentation, analysis and writing—original draft preparation, reviewing. MB, AP and HRN were responsible for methodology and writing—reviewing and editing, resource providing. NMN and SNR contributed to methodology, statistical analysis and editing, and BK participated in design, conceptualization and writing—reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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