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Evaluating differential effect of deltamethrin and carbofuran on growth characteristics of *Westiellopsis prolifica* Janet, a dominant nitrogen fixing cyanobacterium of tropical rice field ecosystem

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ABSTRACT

Recent trends of indiscriminate use of synthetic pesticides in different tropical rice fields has been affecting the soil flora and fauna including the most beneficial nitrogen fixing cyanobacteria. As nitrogen fixing cyanobacteria play an important role in soil fertility and acts as a natural bio fertilizer, the present study was carried out to investigate the differential effects of deltamethrin and carbofuran on *Westiellopsis prolifica*, an abundantly grown nitrogen fixing cyanobacterium in the rice fields of tropics. The study was conducted considering its dry biomass, protein, carbohydrate along with chlorophyll-a, and carotenoid content in a time- and dose-dependent exposure for 16 days. The resultant effect of both the pesticides was found to be pleiotropic for different concentrations of deltamethrin (25–75ppm) and carbofuran (20–60ppm). Results revealed that the growth parameters were more affected with carbofuran treatment than that of deltamethrin at their highest treated concentrations. Carbohydrate showed a gradual increase with carbofuran in a time and dose dependent manner whereas deltamethrin enhanced carbohydrate content up to 16th day from the day of inoculation only at 25ppm. Carotenoid showed a little but insignificant increase at the initial concentrations of both the pesticides whereas protein showed positive enhancement up to 4th day of inoculation when treated with deltamethrin. Though low concentrations stimulated some of the growth characteristics of the test organism, higher concentrations of both the pesticides were observed to be detrimental.

1. Introduction

Pesticides which have been deliberately applied in the agricultural fields in the recent years to protect the crops from unwanted pests (Venkatarman, 1975; Mellanby, 1978) are basically synthetic organic compounds (Aktar et al., 2009). Though they are used with an aim to get rid of harmful pests and subsequently, to enhance the crop yield, the ultimate fate of those non-degradable organic compounds is now becoming a matter of concern (Coupe et al., 2000). It is estimated that only around 0.1% of the pesticides reaches the target organisms, and the remaining 99.9% is found to be dispersed through air, soil and water to the nearby ecosystems somehow (Pimentel, 1995). The pesticide residues are hence, frequently reported from the surface water in agricultural fields in any tropical countries (T. Hoysater, 1994; Larson et al., 1999; Ulén et al., 2002; Maruthanayagam and Sharmila, 2004) and the concentration of which has been increasing day by day particularly in the south Asian and African countries (Zhang et al., 2011). It has been a matter of concern that the magnified amount of compounds affect the beneficial soil micro flora including the soil borne nitrogen fixing cyanobacteria (Roger et al., 1995; Ma et al., 2002). Depending on the chemical properties of the pesticides and their concentration along with the species on which it is applied, the magnitude of their effect varies (Maly and Ruber, 1983).

Cyanobacteria are a group of prokaryotes containing unicellular to multicellular microorganisms with a great diversity and carrying out oxygenic photosynthesis (Carr and Whitton, 1982; Vermaas, 2001). A few of them have the dual capacity of fixing carbon and nitrogen simultaneously in the substratum, which makes them as one of the important sources of natural N_2 supplement to any crop fields (Roger, 1995; Fernández-Valiente et al., 2000; Irisarri et al., 2001) and thus, contribute to a greater extent in maintaining soil fertility therein. N_2 fixing cyanobacteria has already been recorded as one of the ideal and potential natural biofertilizer by its ability to tolerate or resist the effects of chemical fertilizers and pesticides in rice agro ecosystem (Singh et al.,

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2014).

Rice is the second largest staple cereal crop in the world (Koesukwiwat et al., 2014). In tropical countries, cultivation of rice extends from 8 to 35 N latitudes across different ecosystems such as irrigated, rain fed low land, upland, semi deep and deep water including coastal saline regions (Krishnaiah and Varma, 2013). Hence, to feed or to cater the need of large number of peoples residing there through higher rate of rice productions, rice fields are being deliberately sprayed with deltamethrin and carbofuran to get rid of majority of insects and nematodes (Shafer et al., 2008). According to records, 2.5–15g ai/ha of deltamethrin and 600–2000 g ai/ha of carbofuran are being used in tropical rice fields (Mullie et al., 1991; Tomlin, 2004) per annum.

As the non-targeted organisms are being affected by the toxic effects of carbofuran (Anton et al., 1993; Dobsikova, 2003) and deltamethrin (Shrivastave et al.,2011) particularly in rice fields, study of these pesticides on soil micro flora becomes essential and need of the hour for better management of rice field soils. Keeping these in mind, *Westiellopsis prolifica*, an important member of filamentous nitrogen fixing cyanobacteria which is found to be growing profusely in the rice field agro ecosystems in entire tropics (Tiwari et al., 2005; Sethi et al., 2012) was selected as test organism to understand the systemic effects of pesticides on the organism. The study was conducted under controlled laboratory conditions for the two commonly used pesticides deltamethrin and carbofuran in different concentrations as prepared based on the LC_{50} value in response to its growth. Accordingly, the study was conducted to ascertain the impact of both the pesticides on the test organism considering its dry biomass, chlorophyll-a, carotenoid, protein and carbohydrate content for 16 days of time period at an interval of 4 days.

2. Materials and methods

2.1. Test organism

The test organism *W. Prolifica* Janet was isolated from an organic rice field (Kakojan, Jorhat: 26°48′22.13″N; 94°21′43.60″E) of Assam (India) following Strainer et al. (1971). The axenic culture of the organism was maintained following Pabbi and Dhar (2010) at a temperature of 25 ± 2 °C and pH 7.3 using BG-11 media in the Plant Ecology Laboratory of Department of Botany (Gauhati University).

2.2. Pesticide used

The commercial grade carbofuran and deltamethrin (Manufactured by Biostadt India ltd.) was obtained from an authorized dealer of Guwahati city of Assam (India).

2.2.1. Carbofuran

It is a broad-spectrum systemic carbamate pesticide (Table 1) which is widely used throughout the world in the crop fields to get rid of insects, mites and nematodes (Dobsikova, 2003). Due to its very low absorption into soil and high solubility to water, it is frequently reported in rain, surface and ground water (Dobsikova, 2003; Trotter et al., 1991) in the vicinity of the crop fields.

2.2.2. Deltamethrin

It is a synthetic pyrethroid pesticide (Table 1) that kills insects through dermal contact and digestion. Deltamethrin is considered as one of the most powerful toxic pyrethroid (Shrivastava et al., 2011). High stability and its recalcitrant nature makes them highly stable in nature, which is a matter of serious concern.

2.3. Pesticide treatment

The test organism from the exponential growth phase was inoculated to estimate the lethal concentration. LC_{50} of the pesticides was determined in terms of chlorophyll-a following Kumar et al. (2016). On the basis of that, treatments were set up for both the pesticides (Table 2). Experiments were conducted in batch culture. The test organism was transferred to different test tubes containing 10 ml of BG-11 media with different pesticide concentrations along with the control (without pesticides) and incubated at the same culture condition as set for the axenic culture. At the end of each incubation period (4,8,12and 16 days) as set up for the test, the cultures were washed thrice with double distilled water to remove the excess pesticides adhering to the samples and used for measuring the growth parameters. All the tests were conducted in triplicates and average calculated values were considered for further analysis.

2.4. Biomass estimation

For determination of dry biomass, the test organism was centrifuged and filtered through pre-dried what man No. 1 filter paper using sintered glass apparatus. The pellets obtained after centrifugation were washed thrice with double distilled water to remove the remaining pesticides and then dried in an oven until constant weight was observed at 70 °C. Weights were measured using an analytical balance readable to 0.01 mg.

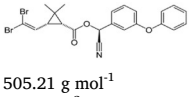
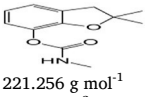
2.5. Chlorophyll-a estimation

Chlorophyll-a estimation was done following the protocol of Mackinney (1941). The pesticide treated test organism was centrifuged to obtain the pellet and each pellet was washed with double distilled water,

Table 2
LC_{50} value of the two pesticides and treatments based on it.

Test Organism	Sl. No	Pesticide used	LC_{50} value (ppm)	Treatment decided based uponLC_{50} value (ppm)
Westiellopsis prolifica	1	Deltamethrin	55	25 55 75
	2	Carbofuran	40	20 40 60

Table 1
Properties of the two pesticides used in the present study.

Properties	Deltamethrin	Carbofuran
Class	Pyrethroid ester insecticide	Carbamate pesticide
IUPAC name	(S)-α-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate	2,2Dimethyl2,3dihydro1benzofuran7yl Methylcarbamate
Chemical structure		
Molar mass	505.21 g mol^{-1}	221.256 g mol^{-1}
Density	1.5 gcm^{-3}	1.18 gcm^{-3}
Trade names	Decamethrin, Decis and K- othrin	Furadan, Curater, Furacarb

homogenised with 95% methanol and kept in a water bath at 65 °C for 30 min. The resultant suspension formed was centrifuged at 3000 rpm for 10 min and the supernatant was read at 650nm and 665nm against 95% methanol as blank in uv–visible spectrophotometer 119 (SYSTRONICS).

2.6. Carotenoid estimation

For estimation of carotenoid, the treated cyanobacterial pellet was extracted, washed with double distilled water, homogenised, suspended in 80% acetone and kept overnight at 4 °C. Next day, the suspension was centrifuged at 3000 rpm for 10 min and supernatant was read at 480nm against 80% acetone as blank in uv–visible spectrophotometer. The value obtained was calculated following Myers and Kratz (1955).

2.7. Protein estimation

Protein estimation was done following the method of Lowry et al. (1951). The test samples were centrifuged at 4000 rpm for 5 min. The pellet obtained after centrifugation was suspended in 1N NaOH and kept in a boiling water bath for 10 min. This was followed by addition of reagent A (prepared by adding 1 ml freshly prepared 1% Na–K tartarate solution containing 0.5% CuSO4 into 50 ml 2% Na_2CO_3 solution) and incubated at room temperature for 10 min. To it, 0.5 ml of reagent B (Folinciocalteu's phenol reagent) was added, mixed thoroughly and incubated at room temperature for another 30 min. The absorbance of the supernatant was read at 650 nm against Folin reagent as blank. The protein concentration was determined from the calibration curve prepared using bovine serum albumin (BSA) as a standard.

2.8. Carbohydrate estimation

The carbohydrate content was estimated according to Spiro (1966). 0.5ml of homogenised algal suspension was taken in a test tube and the volume was made to 1ml with double distilled water. It was followed by addition of 4ml anthrone reagent (prepared by addition of 100mg of anthrone and 1 gm thiourea to 100ml of 75% sulphuric acid). The tubes were then kept at boiling water bath for 10 min and then brought to the room temperature. The absorbance was read at 620 nm against anthrone reagent as blank. Carbohydrate content was determined from the curve calibrated with glucose as standard and expressed in μg/ml.

2.9. Statistical analysis

All experimental values were presented as mean ± standard deviation of three replicates (N = 3). The significant difference between the control and pesticide treated groups were analysed by doing One-way analysis of variance with post hoc (Dunnett) test at significance level 0.05.

3. Results and discussions

3.1. Effect of pesticides on biomass

Biomass is an important parameter of growth of any microorganisms. Biomass content of the test organism treated with deltamethrin and carbofuran are depicted in Fig. 1a and b. The overall biomass content of the test organism was observed to be decreasing in a time and dose dependent manner up to 16th day whereas a steady growth of biomass was observed up to 16th day from the date of inoculation in the control sets. The biomass generation of the test organism were recorded to be decreased by 11% ($p > 0.05$), 52% ($p<0.01$) and 63% ($p<0.001$) at 25ppm, 55ppm, 75 ppm deltamethrin solutions respectively. When treated with carbofuran, the dry pellet biomass were decreased by 25% ($p<0.01$), 46% ($p<0.001$), and 59% ($p<0.001$) at 20ppm, 40ppm, and 60ppm concentrations just over the control on the 16th day from the date of inoculation. A little but insignificant increase in biomass was recorded when treated with 20ppm (2%) and 40ppm (24%) of carbofuran on 4th day and increase up to 7% at 20 ppm on 8th day.

A little but insignificant enhancement in biomass with lower dose of carbofuran could be due to the adaptability of the test organism in the stress environment (Gupta and Baruah, 2015). A reverse effect i.e. decrease in biomass was observed with increase in both the pesticides, which could be attributed to the inhibition of photosynthesis in the test organism with the application of pesticides (Galhano et al., 2009). The inhibition of photosynthesis may be due to the reduction in synthesis of chlorophyll-a, one of the most important pigment required for photosynthesis (Okmen and Ugur, 2011). Similar results were reported by Battah et al. (2001) while working on *Anabaena variabilis* treated with thiobencarb herbicide and by Zeeshan and Prasad (2007)on *Nostoc muscorum* treated with monocrotophos.

3.2. Effect of pesticides on chlorophyll-a

The chlorophyll-a content of the test organism was seen to be

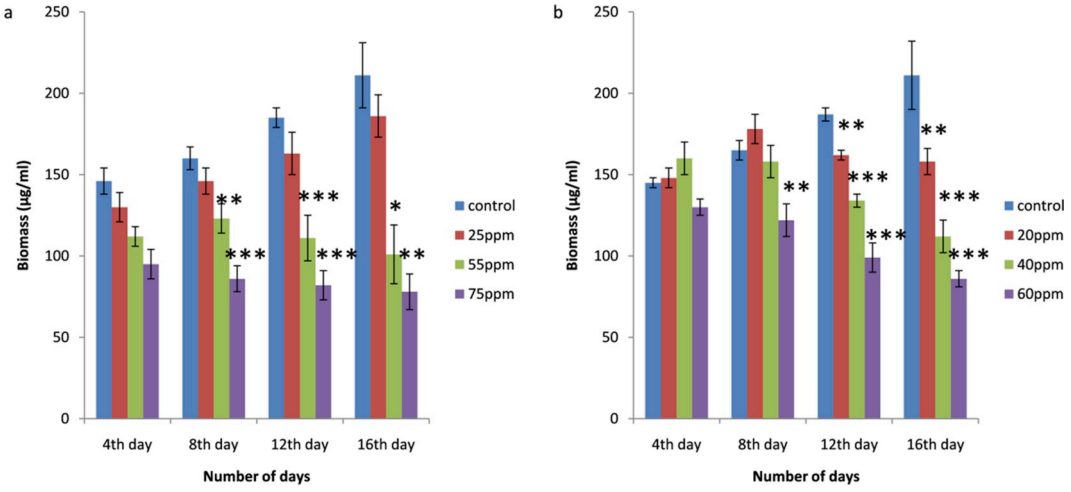


Fig. 1. (a & b). Effect of different concentrations of deltamethrin (25, 55 &75ppm) and carbofuran (20, 40, & 60ppm) on biomass contents of *Westiellopsis prolifica*. Experimental values were presented as mean ± SD, N = 3. Asterisks (*$p<0.05$, **$p<0.01$, ***$p<0.001$) above the histogram bars signifies significant increase and decrease in the pesticides treated samples over the control.

decreasing in a time and dose dependent manner by the application of both deltamethrin and carbofuran (Fig. 2a and b). There was a significant reduction in chlorophyll-a content up to 76% ($p<0.001$) on the 16th day at 75 ppm of deltamethrin and up to 77% ($p<0.001$) when treated with 60ppm of carbofuran over the control.

Chlorophyll-a is considered as a major light harvesting pigment in cyanobacteria through which it absorbs sunlight for carrying out photosynthesis. In the present study the decrease in chlorophyll-a with increase in pesticide concentration with time could be attributed to the hindrance put forth by the pesticide in porphyrin ring formation, a part of a chlorophyll molecule that absorbs light energy(Lal and Saxena, 1980). The present finding is in agreement with the findings of Porsbring et al. (2009) who worked on the effect of fungicides on marine microalgal communities and observed that fungicides had deleterious effects on Chl-a, carotenoid and phycobiliproteins of the marine algae. Similar findings were reported in *Anabaena variabilis* and *Protosiphon botryoides* treated with simazine, atrazine herbicide (Kobbia et al., 2001) and in *Anabaena cylindrica* treated with molinate. (Galhano et al., 2009).

3.3. Effect of pesticides on carotenoid

The carotenoid content of the test organism after the application of deltamethrin was seen to be decreasing in time and dose dependent manner from the 8th to the 16th day with a little but insignificant increase on the 4th day by 8% at 25 ppm over the control (Fig. 3a and b). Similarly, with carbofuran application, carotenoid showed insignificant enhancement at 20 ppm on 4th, 8th and 12th day by 10%, 5% and 5% respectively against the control sets.

Carotenoid plays an important role in photosynthetic process of green plants including cyanobacteria. The pigment is responsible for transferring energy to chlorophyll-a, light harvesting, photo protection by quenching triplet chlorophyll-a molecule and scavenging singlet oxygen (Shen et al., 2009). The present study showed that with low pesticide concentration, there was an insignificant stimulation of carotenoid, which could be due to carotenoid acting as an antioxidant under stress (Phukan et al., 2019) or slower rate of impact of the applied pesticides on the photosynthetic activity of the cyanobacterium (Azizullah et al., 2011). In contrast, with higher dose of both the pesticides, carotenoid production was inhibited in a dose and time dependent manner. The present study supports the findings of Galhano et al. (2009) and Chen et al. (2013) who recorded time and dose dependent decrease of carotenoid contents in *Anabaena cylindrica*, on application of Bentazon and Molinate and in Nostoc sp. when treated with butachlor. There might have several reasons for such inhibition in higher dose of pesticides. For example, solubilisation of membrane associated proteins due to pesticides effect (Hirschberg and Chamovitz, 1994)could be one of the reasons and further, as carotenoid is synthesized by the membrane bound enzymes, interaction of these enzymes with the pesticides might cause reduction in carotenoid biosynthesis (Mohapatra et al., 2003).

3.4. Effect of pesticides on protein

Protein content of the test organism was decreased from the 8th to the 16th day from the date of inoculation in a time and dose dependent manner with deltamethrin and carbofuran. On the 4th day of incubation it was seen to increase in protein contents of about 11% ($p<0.05$), 28% ($p<0.001$) and 34% ($p<0.001$) at 25ppm, 55ppm and 75ppm of deltamethrin respectively. In contrast, the protein contents of the test organism were gradually decreased with time and dose of carbofuran (Fig. 4a and b).

A little but significant enhancement in protein content was observed with lower dose of deltamethrin which could be attributed to the synthesis of stress retarding protein by the test organism due to the impact of the applied pesticide (Fatma et al., 2008). It is known that stress induce synthesis of heat shock proteins(Hsps) and stress proteins in cells undergoing stress (De Maio, 1999 and Feige et al., 1996). The result is in accordance with Fatma et al.(2008) who reported increase in protein content due to the formation of stress protein at low pesticide concentration. In contrast, at higher concentration of deltamethrin and carbofuran, the protein content was found to be around 55% ($p<0.001$) less

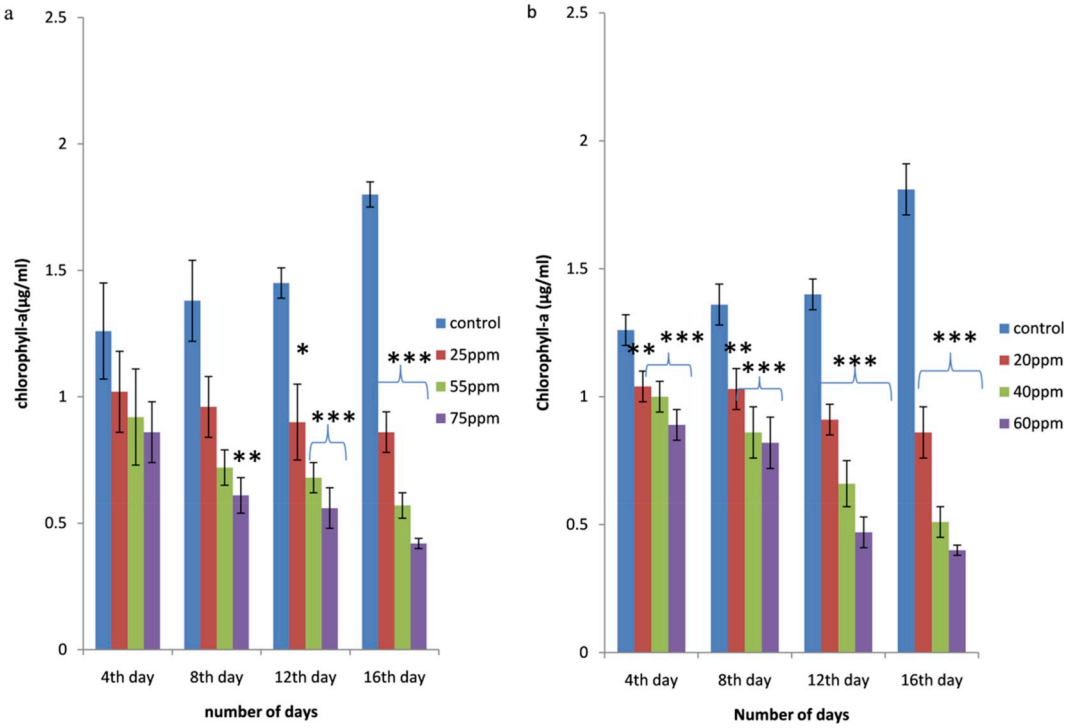


Fig. 2. (a & b). Effect of different concentrations of deltamethrin (25, 55 & 75ppm) and carbofuran (20, 40, & 60ppm) on chlorophyll-a contents of *Westiellopsis prolifica*. Experimental values were presented as mean ± SD, N = 3. Asterisks (*$p<0.05$, **$p<0.01$, ***$p<0.001$) above the histogram bars signifies significant increase and decrease in the pesticides treated samples over the control.

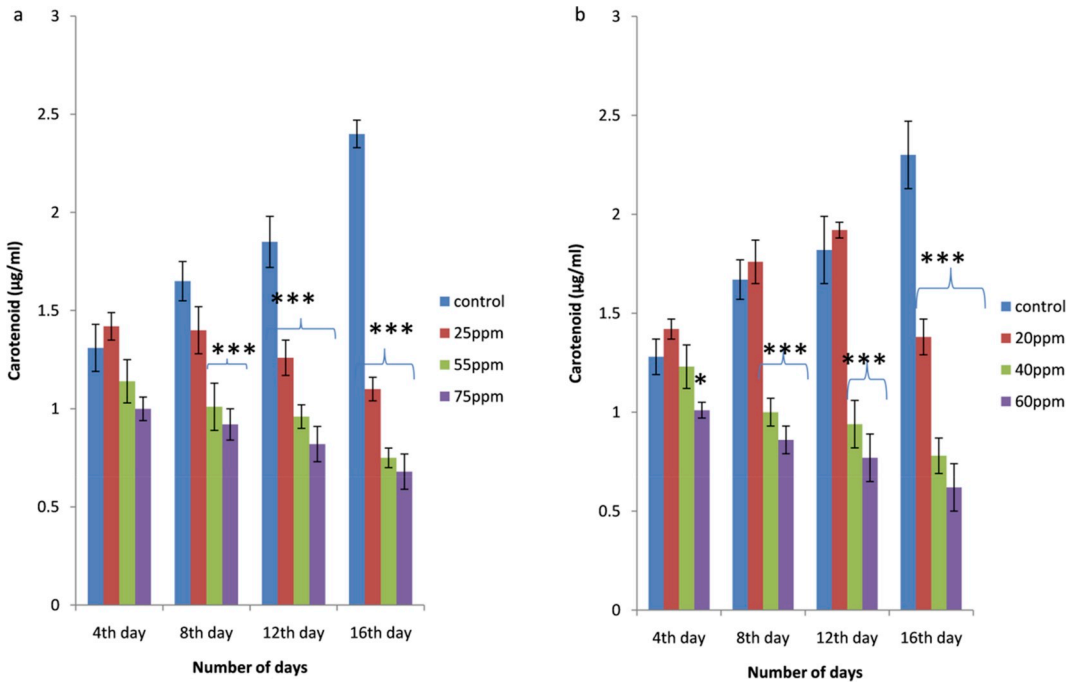


Fig. 3. (a & b). Effect of different concentrations of deltamethrin (25, 55 & 75ppm) and carbofuran (20, 40, & 60ppm) on carotenoid contents of *Westiellopsis prolifica.* Experimental values were presented as mean ± SD, N = 3. Asterisks (*p<0.05, **p<0.01, ***p<0.001) above the histogram bars signifies significant increase and decrease in the pesticides treated samples over the control.

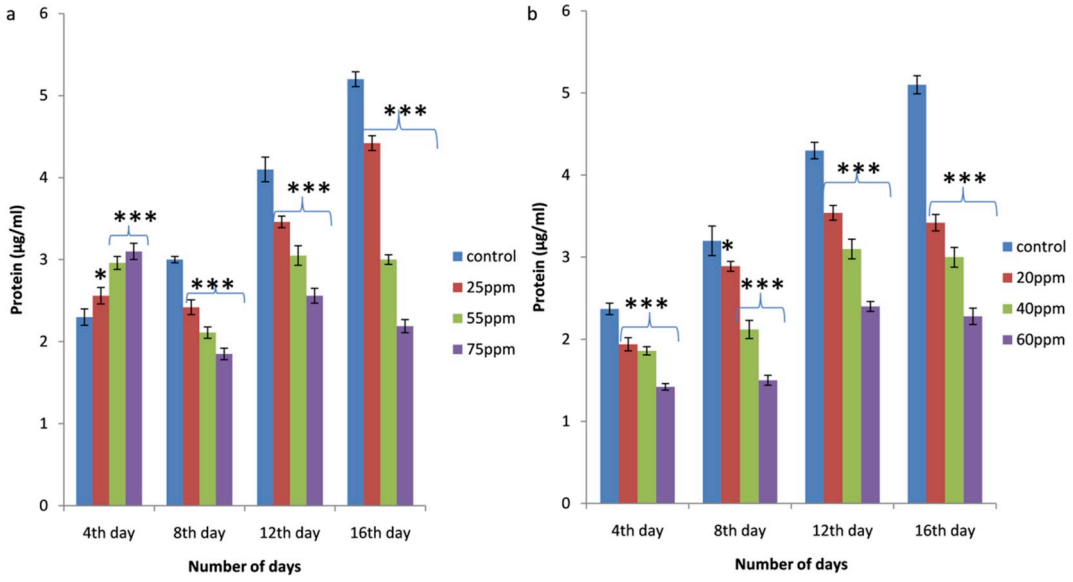


Fig. 4. (a & b). Effect of different concentrations of deltamethrin (25, 55 & 75ppm) and carbofuran (20, 40, & 60ppm) on protein contents of *Westiellopsis prolifica.* Experimental values were presented as mean ± SD, N = 3. Asterisks (*p<0.05, **p<0.01, ***p<0.001) above the histogram bars signifies significant increase and decrease in the pesticides treated samples over the control.

at 60 ppm of carbofuran on 16th day from the date of inoculation as compared to the control set for the same time period. The result is in conformity with the findings of Battah et al. (2001), who observed reduction of protein content in *Anabaena variabilis* treated with thiobencarb and in *Westiellopsis prolifica treated* with tebuconazole (Kumar et al., 2010). The reduction in protein content might be due to the inhibition of structural proteins and enzymes essential for the survival of the cyanobacterium (Kapoor et al., 1996) or might be due to the reduced growth and increase in protease activity under the pesticide stress condition (Suresh Babu et al., 2001).

3.5. Effect of pesticides on carbohydrate

The carbohydrate content of the test organism was seen to be decreasing with the gradual increase of deltamethrin concentration, except at 25 ppm where a little but insignificant increase in carbohydrate content was recorded from the date of inoculation over the control by 12.5%, 9.5%, 1.8% and 13% on 4th,8th,12th and 16th day (Fig. 5a). On the other hand, from the Fig. 5b it was observed that in the carbofuran treated samples, carbohydrate content gradually increases in a time and dose dependent manner. At 60 ppm the carbohydrate content increases by48.5%(p<0.01), 56.8%(p<0.01), 57.1%(p<0.001), 30.7%

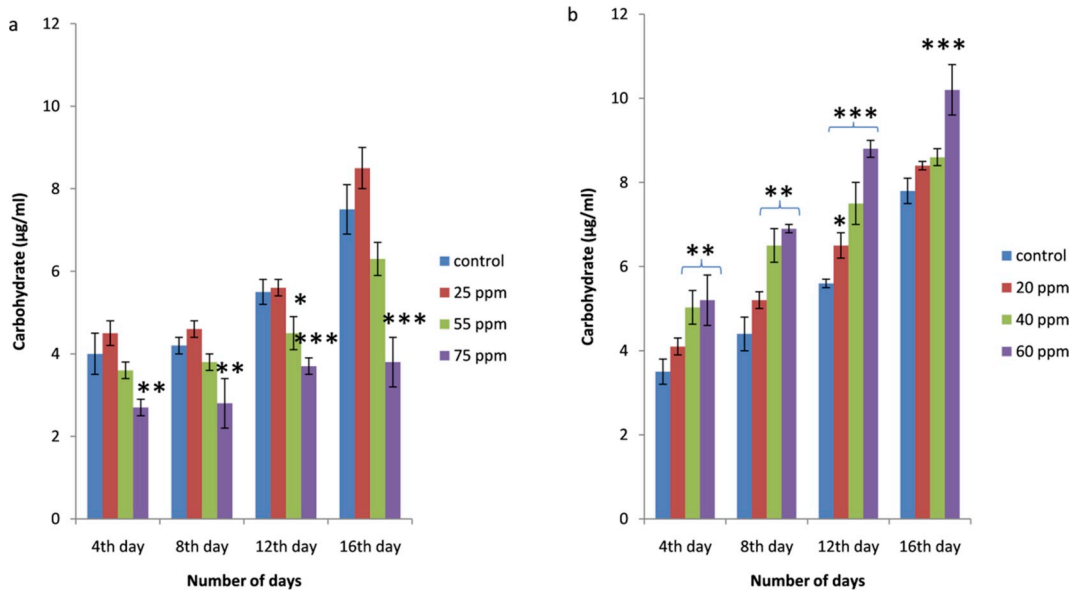


Fig. 5. (a & b). Effect of different concentrations of deltamethrin (25, 55 & 75ppm) and carbofuran (20, 40, & 60ppm) on carbohydrate contents of *Westiellopsis prolifica*. Experimental values were presented as mean ± SD, N = 3. Asterisks (*p<0.05, **p<0.01, ***p<0.001) above the histogram bars signifies significant increase and decrease in the pesticides treated samples over the control.

(p<0.001) on 4th, 8th, 12th and 16th day with carbofuran application respectively.

The impact of pesticides on the carbohydrate content of an organism depends on the type of the test organism, duration and dose of the pesticides used (Moreland, 1980). The reduction in carbohydrate with deltamethrin application could be attributed to the inhibition of the photosynthetic process by interfering with the chemical used (Kumar, 1991) or could be due to decrease in the rate of CO_2 photo assimilation (Mansour et al., 1994). This result is in agreement with the findings of Galhano et al. (2009). The result is also in consonance with Fatma et al. (2008) who reported low production of carbohydrate in *Aulosira fertilissima, Anabaena variabilis, Nostoc muscorum* treated with higher concentration of endosulfan. On the other hand, the data obtained further revealed that carbofuran stimulates the production of carbohydrate in relation to time and dose. The increase in carbohydrate content may be an extracellular adaptive measure of *W. Prolifica* by synthesising extracellular polysaccharides (EPS), an adaptive strategy utilised by cyanobacteria for its survival under pesticide stress condition (Ehling Schulz and Scherer 1999; Nicolaus et al. 1999). Probably, due to the stress put forth by carbofuran on the cell membrane integrity, it triggeres the synthesis of EPS in the test organism, which might have subsequently binded with carbofuran and tried to reduce its toxicity (Galhano et al., 2009). Further, the role of carbohydrate in the ROS scavenging mechanism (Van Den Ende and Valluru, 2009; Matros et al., 2015) might also be the reason for time and dose dependent increase of carbohydrate under carbofuran stress. Similar results were reported by Battah et al. (2001) and Habib et al. (2013) while working on *Anabaena Variabilis* treated with carbamate pesticide thiobencarb and *Calothrix brevissima* treated with Carbaryl.

4. Conclusion

Increasing human population has been creating a demand for higher productivity of rice crops. For which higher amount of pesticides are constantly being used in the tropical countries. As around 99.9% of these chemicals leach out into the nearby ecosystem creating pollution (Pimentel, 1995), the present study was carried out to observe the effect of two very commonly used commercial pesticides on *Westiellopsis prolifica,* a nitrogen fixing cyanobacterium of rice field ecosystem. Results showed inhibitory effect of both the pesticides on its growth, pigments and photosynthesis with an exception in carbohydrate content which gradually increases with concomitant increase of carbofuran concentration which might be an adaptive measure adopted by the cyanobacterium to overcome the stress condition. Between the pesticides considered for the present study, carbofuran is observed to be more toxic than deltamethrin and hence, its invites restrictions. As sustainable agriculture aims to enhance soil fertility with renewable resources, use of biopesticides could be suggested in the crop field as a whole. Further, an in-depth study is needed on the pesticide degrading ability of the test cyanobacterium with a futuristic approach to recommend it as a better candidate for rice biofertilization programmes in the tropics.

Author contributions

The study was carried out by Jyotishmita Dutta under the supervision of Prof. Partha Pratim Baruah for her Ph.D. programme.

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Declaration of competing interest

The authors declare that they have no conflict of interests.

CRediT authorship contribution statement

Jyotishmita Dutta: Conceptualization, Methodology, Writing - original draft. **P.P. Baruah:** Investigation, Supervision, Validation, Writing - review & editing.

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