The Pharma Innovation Journal 2019; 8(6): 877-883

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(6): 877-883

www.thepharmajournal.com
Received: 21-04-2019
Accepted: 25-05-2019

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Effect of acetamiprid on *Anabaena aequalis* Borge and *Oscillatoria salina* Biswas: A biochemical study

DS Yasodha, KM Umarajan and S Malathy

Abstract
Acetamiprid is a new generation, highly active neonicotinoid insecticide which has been used to control insect pests and mites in our study the effect of this insecticide on blue green algae namely, *Anabaena aequalis* Borge and *Oscillatoria salina* Biswas was been investigated. Cyanobacteria plays an important role in enhancing the soil fertility by fixing the atmospheric nitrogen in addition to this they also help to degrade harmful pollutants from the soil thereby reducing the toxicity from the environment. The insecticide acetamiprid which are applied to eradicate the insect pest have impact on the cyanobacteria. Hence in our study the estimation of chl-a, carotenoids, phycobilins, protein and nitrogen reductase activity showed that the insecticide drastically decreases the above said components at the selected concentration during the first week. All the response were found to be dose dependant and satistically significant. Hence the insectidcide acetamiprid was found to have microbial ecotoxicity effect.

Keywords: Insecticide, acetamiprid, BGA, *Anabaena aequalis* Borge, *Oscillatoria salina* Biswas

1. Introduction
The most prominent group of insecticides in the world is the neonicotinoid type of insecticides. They are used to control the agricultural pests as they posses broad spectrum activity and they have proven versatile application. These insecticides were commercialized in more than 100 countries. The risk of acetamiprid to the various soil zones was found to be significant and undesirable [1]. It has the potential to alter behavioural and physiological characters on micro crustaceans [2]. Rodents exposed to acetamiprid showed a notable suppression on immune system with unique humoral response [3]. The term Blue Green Algae (BGA) was coined by sachs in 1874. They are diverse, widespread and large group of prokaryotes, comprising unicellular to multicellular microorganisms. The cyanobacteria dwell in diverse environments [4]. Apart from improving the soil fertility the BGA can degrade environmental pollutants including pesticides [5, 6]. Introduction of BGA in the rice fields is an age –old technique and these BGA fixes the nitrogen thereby improves the soil fertility and crop productivity [7]. New age agricultural practices depend extensively on synthetic fertilizers, fungicides, herbicides and insecticides. They cause acute or chronic toxic effect upon microorganisms [8] pesticides was found to have three specific impacts on the soil algal and BGA. (a) Its selective toxicity to green algae and it indirectly encourages the BGA growth (b) it temporarily decreases the invertebrates that feeds on algae (c)an unique effect of insecticides on BGA by recruiting algal grazers, which promotes the growth of mucilaginous macrocolonies resistance to grazers [9, 10]. Organophosphorous insecticide was found to affect the release of extracellular products from the cyanobacteria [11] green algae was more sensitive to pesticides than the cyanobacteria [12].
Indiscriminate pesticide use can result in one or more of the following (a) health impairment due to direct or indirect exposure to hazardous chemicals (b) contamination of ground and surface water through runoff and seepage (c)the transmittal of pesticide residues through the food chain to the farm family and urban consumers (d) an increase in the resistance of pest populations to pesticides, thereby reducing their efficacy and consequently causing pest outbreaks; (e) the reduction of beneficial insects like parasites and predators, thereby reducing the effectiveness of pest control strategies that attempt to minimize pesticide use and (f) the reduction in the populations of microorganism in the paddy soil and water that help sustain soil fertility while lowering chemical fertilizer use. The incidence and magnitude of each of these effect depend on the types of chemical, frequently and quantities applied and their persistence [13]. Pesticides may directly or indirectly affect the vital biochemical reactions such as mineralization of organic matter, nitrogen fixation, nitrification,

denitrification and ammonification by activating /deactivating specific soil microorganisms [14]. Hence the effect of acetamiprid on two specific BGAs especially *Anabaena aequalis* Borge and *Oscillatoria salina* Biswas was been investigated by analyzing the chlorophyll–a, carotenoid, phycobilin, protein and nitrate reductase activities using standard protocols. The results were tabulated and statistically analyzed.

2. Materials and Methods

The insecticide acetamiprid was suspended in the BGA culture in specified concentration. *A. aequalis* and *O. salina* were used in this experiment was grown in 250mL Erlenmeyer flask using 100mL of BG-11 medium [15] before use the medium was autoclaved at 15 psi for 15 minutes. Exponentially grown algal cultures were used throughout the experiment. The known concentration of acetamiprid stock solutions was prepared by diluting the commercial formulation with sterile BG 11 medium in the laminar incubation chamber. All the test concentrations (7.5ppm to 60ppm) were prepared based on the active ingredients in the formulations then the flasks were incubated at 25 ± 1^0 c for 21 days. The cultivation was performed in a thermostatically controlled room under continous illumination using fluroscent lamps at an intensity of 33µmol photons $m^{-2}s^{-1}$ with a 12: 12 h light: dark cycle regime. The growth, pigment composition and protein content were measured at 7days intervals. All the experiments were carried out in triplicate and their values are represented as mean ±SD of three replication

2.1 Estimation of chlorophyll –a

The *A. aequalis* and *O. salina* treated with acetamiprid was harvested and estimation of chlorophyll –a was done using Jeffrey and Humphrey (1975) [16] and expressed as μgmL^{-1}

Chl a (μgmL^{-1}) = 11.43E663 – 0.64E630.

2.2 Estimation of carotenoid content

Estimation of carotenoids was done using the method of Strickland and Pausons (1972) [17] and expressed as μgmL^{-1}.

Carotenoid = E480 X10

2.3 Estimation of phycobilin pigments

It was estimated using the method of Bennett and bogorad, (1973) [18] and was expressed in μgmL^{-1}.

$$\text{Phycobilin (PC)} = \frac{\text{A615} - 0.474(\text{A652})}{5.34}$$

$$\text{Allophycocyanin (APC)} (\mu gmL^{-1}) = \frac{\text{A652} - 0.208(\text{A615})}{5.09}$$

$$\text{Phycoerythrin (PE)} (\mu gmL^{-1}) = \frac{\text{A562} - 2.41(\text{pc}) - 0.849(\text{APC})}{9.62}$$

2.4 Estimation of protein

Estimation of protein was done using Lowry *et al*. 1951 [19]

2.5 Nitrate reductase assay

Nitrate reductase, are the enzymes which reduces nitrate to ammonia, it is very sensitive to a number of environmental changes. Samples for NR activity were collected by centrifugation at 800rpm for 10 minutes and measured. Samples were always analyzed immediately after collection. NR was extracted in 200mM phosphate buffer, pH 7.9 containing 5mM EDTA .0.10/0(v/v) triton x-100, 0.03% (W/v) dithiothritol 0.3% (W/V) polyvinyl pyrrolidone, and 3% (W/V) bovine serum albumin (BSA). Assays was conducted in 200mM phosphate buffer, pH 7.9 with 0.2mM NADH, 20% assay volume as algal extract and 10m M KNO_3 added to initiate the reaction at growth temperature and incubated for 45 minutes. At the end of the incubation period, the reaction was terminated with 1.4mM zinc sulfate. Precipitates were removed by centrifugation at 8000g for 10 minutes at 20^o c.

Nitrite concentration were determined by measuring absorption at 543nm by uv-visible spectrophotometer after the addition of 9.6mM sulphanilamide and 0.7mMn – (1- naphtyl) ethylenediamine dihydrochloride. One unit of NR(u) is the enzymatic activity producing 1µmol of nitrite per minute (Chapman & Harrison 1988) [20]. The nitrate reductase activity expressed as µM/min/g.

3. Results

Effect of acetamiprid on chl –a, biliproteins, carotenoids, total protein and nitrate reductase activity were analysed. The effect of acetamiprid on *A. aequalis* and *O. salina* was presented in table 1 and 2; fig 1 and 2. Treatment with various concentrations of acetamiprid produced a dose- dependent inhibition of growth. Treatment with all concentrations and durations of acetamiprid significantly (P <0.05) affected the chlorophyll content in dose –dependent manner. A drastic reduction was observed in the 1st week but there was increased chl-a content in 2nd and 3rd week at all concentrations significantly (P <0.05). Significant differences were also observed within the groups at higher concentrations for both the blue green algae.

Phycocyanins was found to decrease in the 1st week for *anabaena* sp but there was increased phycocyanins in the second week again they were found to show decreased phycocyanins in the third week. But for the *O. salina* the phycocyanins was found to decrease in the 1st week and there was significant (P <0.05) increase in the phycocyanins in the 2nd and 3rd week. The acetamiprid was found to increase the APC content from 1st week to third week significantly from 94.32 to 108.55 µg/mL at 7.5 ppm for *A. aequalis* but there was significant decrease in the APC content with increased acetamiprid concentration (P <0.05) within the week duration.

Similar results were also observed for *O. salina* for the APC parameters. Carotenoid concentration were found to decrease significantly (P <0.05) with increased concentrations of acetamiprid for both.

A. aequalis and *O. salina*. however carotenoids was found to increase in the 2nd and 3rd week for both the blue green algae. phycoerythrin was also found to decrease significantly (P <0.05)with increased concentrations of acetamiprid for both *A. aequalis* and *O. salina*. But phycoerythrin was found to increase significantly (P <0.05) with respect to duration for both the BGA.

Total protein content was significantly (P <0.05) decreased during the 1st week. But it was found to increase in subsequent weeks. Treatments at 60ppm significantly increases the protein content than the other dosages in longer

durations (2nd and 3rd week) (Table 3 and 4; Fig. 3 and 4) for both the BGA.

Acetamiprid treatments significantly (P <0.05) affected the enzyme nitrate reductase activity in all concentrations and durations. Dose dependant decrease in the activity was observed in all duration and significant differences were found in each concentrations within the groups (Table 3 and 4; Fig. 3 and 4) for both the *A. aequalis* and *O. salina*.

Table 1: Effect of Acetamiprid on pigment composition in *A. aequalis*

Cultivation Time (Days)	Blue colour chemical Concentration (ppm)	Chl a (µg/mL) (Mean ±SD)	PC (µg/mL) (Mean ±SD)	APC (µg/mL) (Mean ±SD)	PE (µg/mL) (Mean ±SD)	Carotenoids (µg/mL) (Mean ±SD)
0-7	Control	163.12±0.34	346.12±0.21	96.21±0.21	82.81±0.21	58.31±0.12
	7.5	160.18±0.12	329.21±0.32	94.32±0.31	80.92±0.31	56.32±0.32
	15	109.31±0.31	93.12±0.41	84.21±0.23	70.81±0.21	43.12±0.33
	30	56.44±0.33	59.41±0.22	43.12±0.21	29.72±0.22	32.11±0.14
	45	42.41±0.15	37.22±0.12	34.22±0.22	20.82±0.41	19.31±0.42
	60	19.12±0.24	21.14±0.21	24.21±0.24	10.81±0.33	14.33±0.11
8-14	Control	188.24±0.11	364.33±0.23	104.68±0.14	87.89±0.14	67.63±0.23
	7.5	173.12±0.05	347.42±0.33	102.79±0.23	86.01±0.21	65.64±0.12
	15	126.32±0.31	111.33±0.41	92.68±0.21	75.89±0.22	52.44±0.32
	30	69.31±0.22	77.62±0.11	51.59±0.24	34.81±0.31	41.43±0.33
	45	52.22±0.13	55.43±0.13	42.69±0.31	25.91±0.32	28.63±0.21
	60	26.41±0.41	39.35±0.23	32.68±0.32	15.89±0.32	23.65±0.23
15-21	Control	206.45±0.12	357.59±0.22	110.44±0.21	89.67±0.22	72.52±0.41
	7.5	191.33±0.13	340.68±0.14	108.55±0.11	89.78±0.14	70.53±0.42
	15	144.13±0.24	104.59±0.32	98.44±0.23	77.67±0.14	57.33±0.12
	30	87.22±0.21	70.88±0.31	57.35±0.41	36.58±0.32	46.32±0.43
	45	70.43±0.13	48.69±0.14	48.45±0.31	26.68±0.31	33.52±0.14
	60	44.32±0.14	32.61±0.12	38.44±0.23	18.67±0.21	28.54±0.24

Table 2: Effect of Acetamiprid chemical on pigment composition in *O. salina*

Cultivation Time (Days)	Blue colour chemical Concentration (ppm)	Chl a (µg/mL) (Mean ±SD)	PC (µg/mL) (Mean ±SD)	APC (µg/mL) (Mean ±SD)	PE (µg/mL) (Mean ±SD)	Carotenoids (µg/mL) (Mean ±SD)
0-7	Control	221.21±0.21	387.21±0.21	102.21±0.32	80.47±0.21	60.62±0.21
	7.5	193.21±0.31	374.33±0.42	93.21±0.12	78.58±0.32	58.63±0.34
	15	63.42±0.22	102.32±0.31	90.21±0.24	68.47±0.33	45.43±0.12
	30	46.32±0.32	93.21±0.43	49.12±0.32	27.38±0.21	34.42±0.42
	45	21.41±0.22	52.21±0.31	41.23±0.24	18.48±0.31	21.62±0.21
	60	12.41±0.14	19.21±0.24	30.32±0.42	8.47±0.42	16.64±0.23
8-14	Control	230.24±0.41	395.01±0.41	106.42±0.12	87.46±0.41	61.79±0.21
	7.5	202.14±0.32	382.13±0.21	98.32±0.43	85.57±0.21	59.80±0.24
	15	72.25±0.23	110.12±0.23	94.42±0.31	75.46±0.32	46.61±0.31
	30	55.23±0.21	101.01±0.22	53.33±0.23	34.37±0.21	35.59±0.12
	45	30.44±0.24	60.24±0.32	44.43±0.23	25.47±0.32	22.79±0.23
	60	21.34±0.13	27.32±0.21	34.42±0.31	15.46±0.24	17.81±0.21
15-21	Control	234.42±0.24	396.23±0.31	112.21±0.22	88.13±0.11	63.91±0.14
	7.5	206.12±0.34	383.14±0.23	106.54±0.31	86.24±0.32	61.92±0.23
	15	76.23±0.32	111.42±0.24	96.43±0.24	76.13±0.42	48.72±0.32
	30	63.21±0.24	102.32±0.21	55.34±0.21	35.04±0.21	37.71±0.21
	45	34.12±0.12	61.14±0.22	46.44±0.31	26.14±0.31	24.91±0.22
	60	27.21±0.32	28.23±0.34	36.43±0.34	16.13±0.21	19.93±0.14

Table 3: Effect of Acetamiprid on Protein and Nitrate reductase activity in *A. aequalis*

Cultivation Time (Days)	Blue colour chemical Concentration (ppm)	Protein (%) (Mean ± SD)	Nitrate Reductase (µmoles/g/m) (Mean ± SD)
0-7	Control	0.165±0.24	0.153±0.22
	7.5	0.164±0.31	0.148±0.31
	15	0.123±0.13	0.101±0.41
	30	0.102±0.21	0.096±0.42
	45	0.098±0.41	0.061±0.43
	60	0.086±0.32	0.021±0.24
8-14	Control	0.285±0.41	0.183±0.31
	7.5	0.284±0.22	0.176±0.24
	15	0.243±0.43	0.132±0.43
	30	0.222±0.31	0.126±0.31
	45	0.218±0.34	0.091±0.42
	60	0.206±0.22	0.051±0.31

15-21	Control	0.341±0.12	0.193±0.42
	7.5	0.335±0.42	0.181±0.13
	15	0.298±0.32	0.143±0.43
	30	0.272±0.24	0.130±0.33
	45	0.268±0.33	0.100±0.32
	60	0.256±0.12	0.061±0.41

Table 4: Effect of Acetamiprid on Protein and Nitrate reductase activity in *O. salina*

Cultivation Time (Days)	Blue colour chemical Concentration (ppm)	Protein (%) (Mean ± SD)	Nitrate Reductase (µmoles/g/m) (Mean ± SD)
0-7	Control	0.145±0.21	0.162±0.22
	7.5	0.143±0.42	0.154±0.41
	15	0.132±0.31	0.091±0.33
	30	0.961±0.42	0.079±0.22
	45	0.082±0.32	0.066±0.32
	60	0.073±0.32	0.046±0.42
8-14	Control	0.205±0.41	0.182±0.11
	7.5	0.203±0.21	0.174±0.42
	15	0.192±0.22	0.098±0.41
	30	0.151±0.31	0.089±0.32
	45	0.142±0.22	0.075±0.22
	60	0.133±0.31	0.056±0.21
15-21	Control	0.225±0.41	0.192±0.11
	7.5	0.223±0.14	0.179±0.12
	15	0.201±0.21	0.101±0.22
	30	0.189±0.32	0.100±0.32
	45	0.162±0.12	0.098±0.41
	60	0.153±0.32	0.081±0.21

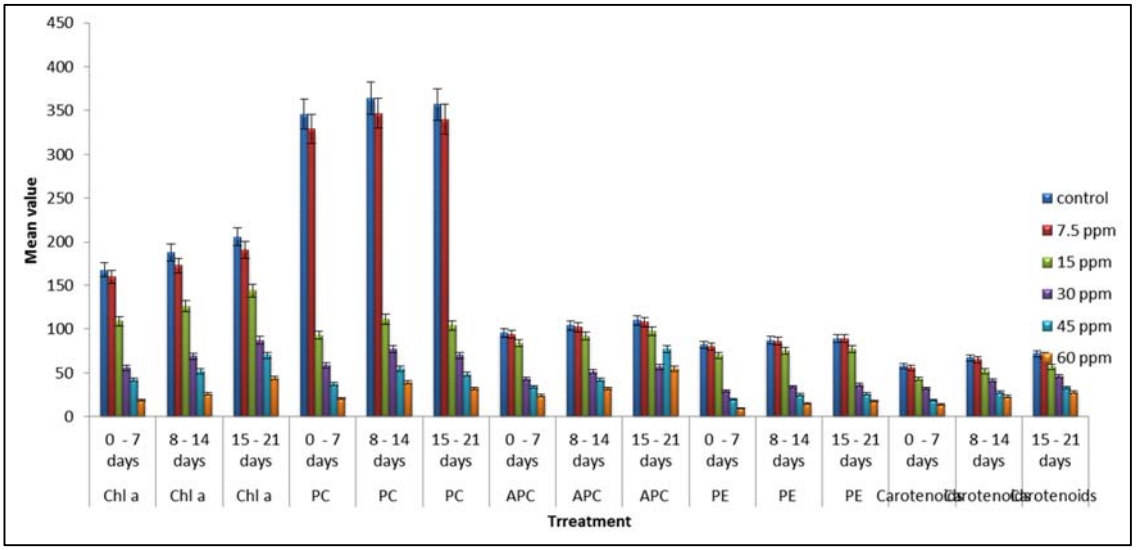

Fig 1: Effect of Acetamiprid on pigment composition in *A. aequalis*

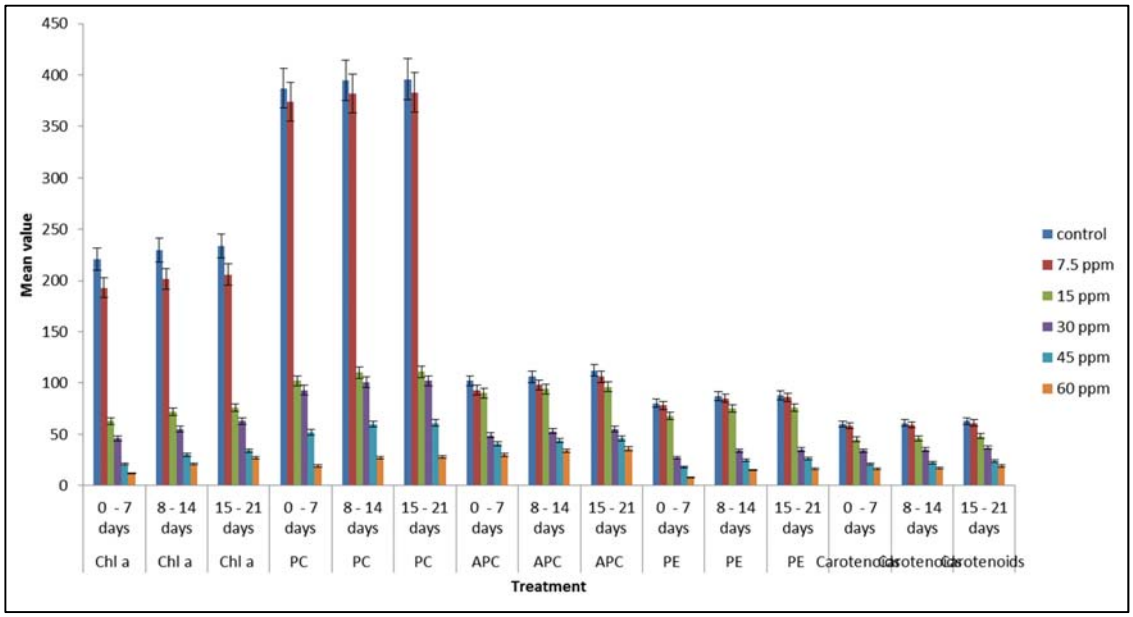

Fig 2: Effect of Acetamiprid on pigment composition in *O. salina*

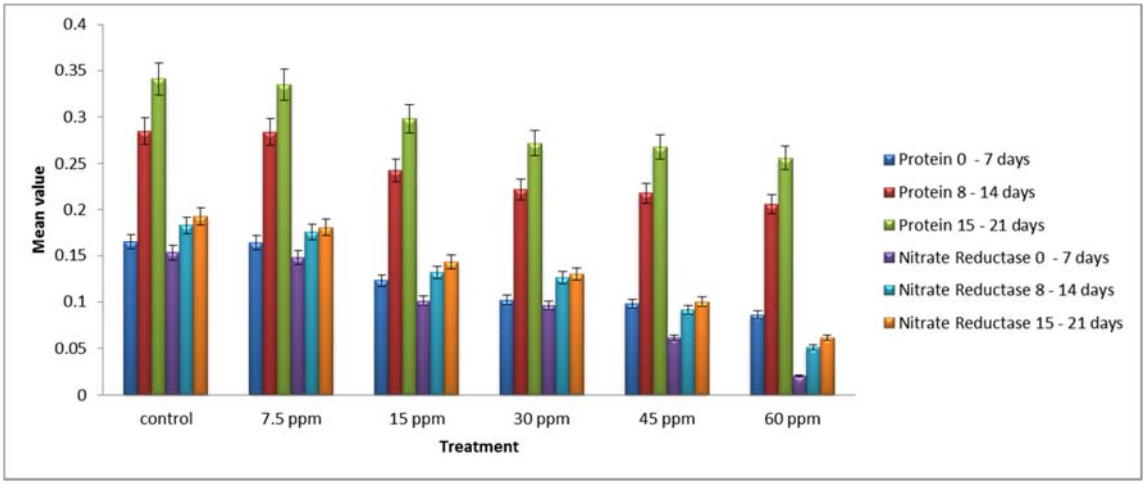

Fig 3: Effect of Acetamiprid on Protein and Nitrate reductase activity in *A. aequalis*

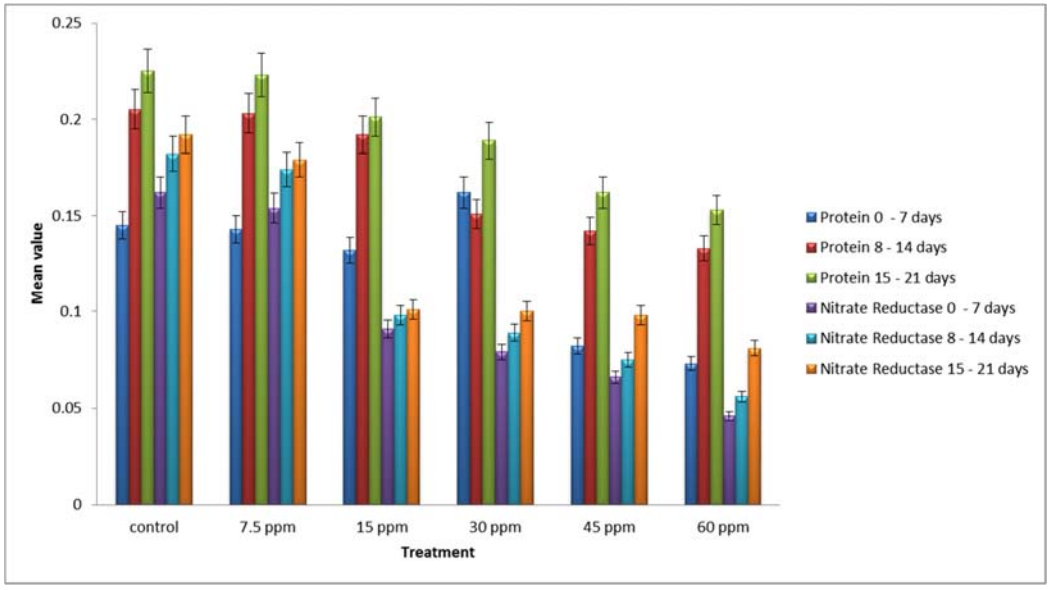

Fig 4: Effect of Acetamiprid on Protein and Nitrate reductase activity in *Oscillatoria* sp

4. Discussion

The effect of neonicotinoid insecticide acetamiprid on cyanobacteria, *A. aequalis* and *O. salina* species was investigated using standard biochemical assays. The results proved that the insecticide acetamiprid drastically decreased the chl-a, phycocyanins, carotenoids and the enzyme nitrate reductase activity in the first phase of exposure. Insecticides were found to inhibit the nitrogenase activity of cyanobacteria at 50µg/g. This is in akin to our results where 60ppm of acetamiprid drastically decreases the nitrate reductase enzyme [21]. Insecticide Divap 1000 deleteriously affects the release of protein, ammonia, carbohydrates, aminoacids and phenols in *A. aequalis* and *O. salina*. This is in consensus with our results wherein both BGA were found to show significant decrease in their biochemical products [22]. *O. salina* was found to show adverse cellular activity when exposed to pesticide glyphosate [23], Similarly in our study also *O. salina* was affected more than *A. aequalis* in the various biochemical assay performed. Decreased photosynthetic pigments, carbohydrates, lipids, proteins and aminoacids in *Anabena* and *oscillatoria* species was observed when treated with carbaryl at 3ppm [24]. This is in akin to our results where a neonicotinoid insecticide, acetamiprid also affects the above said biochemical product synthesis in our selected blue green algae. The effects of acetamiprid on the parasitoid were evaluated [25]. Many of the literatures prove that neonicotinoids are harmful to moderately harmful (IOBC Class) [26]. The degradation of these photosynthetic pigments could be also attributing to the pesticide –thylakoid membrane interaction. Significant reduction in chl-a, biliproteins, carotenoid and total protein content was observed in acetamiprid treatments in 1st week of treatments. This was increased in later duration of treatments. This clearly indicates the resistant potential of the organism to pesticide and this supported the earlier reports [27].

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