CHAPTER IV

RESULTS

In order to meet the challenges that have been confronted by the agricultural scientist, farmers and policy makers, synthetic pesticides and herbicides etc. are being deliberately applied in the crop fields throughout the globe which again have some positive and negative effects on the natural biota in the agricultural systems. To investigate the differential effects of malathion, deltamethrin and carbofuran on the autochthonous strains of *Westiellopsis,* a nitrogen fixing cyanobacterial taxa commonly occurring in rice field soils, a survey was made throughout Assam (Table –7) to isolate different strains under the taxa from the different rice field soils where pesticides are usually not applied.

4.1 Isolation and identification of the test organisms

A total of three strains of *Westiellopsis* were isolated from the organic rice field soil samples (Table-7) and raised to a axenic culture in BG-11 medium (Ripkka *et al*., 1979) without nitrogen separately and identified following standard manuals of Desikachary (1959), Komarek and Anagnostidis (1989), Komarek (2013), Komarek *et al*. (2014) and authenticated following Algaebase (www.algaebase.org).

4.1.1Systematic position of the genus *Westiellopsis*

Empire- Prokaryota

Kingdom- Eubacteria

Phylum- Cyanobacteria

Class- Cyanophyceae

Order- Nostocales

Family- Hapalosiphonaceae

Genus- ***Westiellopsis***

4.1.2 Taxonomic description of the isolated species

***1. Westiellopsis prolifica* Janet 1941: 170** (Plate 1A)

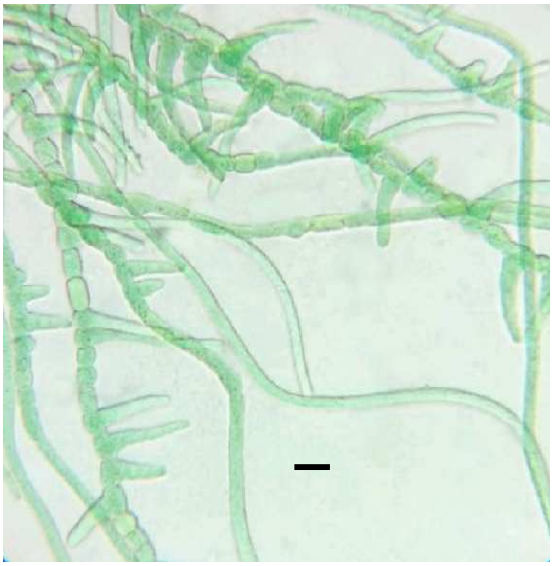
Filaments torulose shaped,T- shaped branching, cells of main filaments are barrel shaped, the branched cells are thinner and elongated in shape; cells 10-12µm long and 6-8 µm broad; heterocyst 10.5-22 µm long and 5-5.6 µm broad.

***2. Westiellopsis indica* Bourrelly 1970: 360**(Plate 1B)

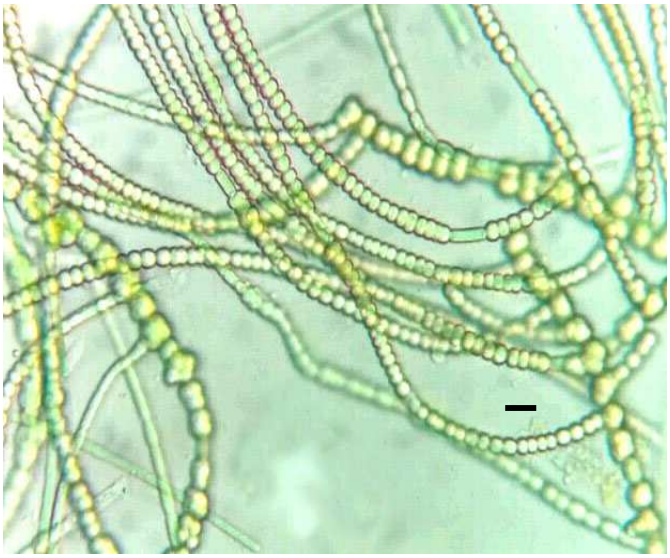
Entangled filaments, sometimes fasciculated, T- shaped branching, cells are barrel shaped, elongated and almost spherical in shape, elongated and narrow branch cell; heterocyst various shaped, cells 5-15 µm long and 3.6-14 µm broad; heterocyst 13-16 µm long and 4-6 µm broad.

***3. Westiellopsis ramosa* Bagchi in Singh & al. 2017: 1247, fig. 1**(Plate 1C)

Monoseriate filaments, constricted cross walls, T- shaped and unidirectional branching, barrel shaped irregular cells in main filament. Irregularly round to round branched cells; cylindrical to barrel shaped heterocyst; cells 6-9 µm long and 4-5 µm broad; heterocyst 5-11 µm long and 3-7 µm broad.



A



B

C

Plate 1. Microphotographs of isolated *Westiellopsis* strains

A= *Westiellopsis prolifica*, **B** = *W. indica* **C**= *W. ramosa*,

Table 7: Organically rice cultivated locations of Assam selected for screening of *Westiellopsis* species

("+" indicates presence of the species and "-" indicates absence of the species)

Sl No.	Collection sites	*Geo-cordinates*	*Westiellopsis prolifica*	*Westiellopsis indica*	*Westiellopsis ramosa*
1.	Tinsukia	27°29′22″N & 95°21′36″E	+	-	-
2.	Chabua	27°29'00"N & 95°11'00"E	+	+	-
3.	Dibrugarh	27°42'30"N & 95°29'08"E	+	-	+
4.	Lakhimpur	27°13′48″N & 94°06′00″E	+	-	-
5.	Tezpur	26°39'04"N & 92°47'01"E	+	-	-
6.	Sivasagar	26°59′04″N & 94°37′53″E	+	-	+
7.	Demow	26°07'38"N & 94°44'23"E	+	+	-
8.	Kohora	26°34'33"N & 93°10'01"E	-	+	-
9.	Nagaon	26°21′00″N & 92°40′00″E	+	-	-
10.	Karbi anglong	26°11′00″N & 93°34′00″E	+	-	-
11.	Sonapur	26°07'12"N & 91°58'48"E	-	-	+
12.	Panikhaity	26°12'12"N & 91°51'29"E	+	-	-
13.	Basistha chariali	26°06'00"N & 91°47'00"E	-	+	-
14.	Lankeshwar	26°08'43"N & 91°38'45"E	+	-	-
15.	Rani	26°05'00"N & 91°35'00"E	+	-	-
16.	North Guwahati	26°10'48"N & 91°43'12"E	+	-	-
17.	Nalbari	26°27′00″N & 91°26′24″E	+	-	+
18.	Pathsala	26°30'42"N & 91°10'51"E	+	+	-
19.	Bongaigaon	26°28′00″N & 90°34′00″E	-	+	-
20.	Goalpara	26°26′00″N & 90°22′00″E	+	-	+

4.3 LC_{50} values

The LC_{50} value of all the three test organisms for Malathion, Deltamethrin and Carbofuran were determined in terms of chlorophyll-a following Kumar *et al*. (2016). The treatment concentrations were deduced based on the LC_{50} values (Table 8, 9 & 10) and the experimental setups were designed accordingly.

Table 8:LC_{50} value of Malthion for three test organisms

Sl No.	Pesticide used	Test specimen	LC_{50} value determined	Treatment based on the LC_{50} value
1	Malathion	*Westiellopsis prolifica*	60	30 60 90
		W. indica	60	30 60 90
		W. ramosa	56	26 56 86

Table 9: LC_{50} value of deltamethrin for three test organisms

Sl no.	Pesticide used	Test specimen	LC_{50} value determined	Treatment based on LC_{50}value
2	Deltamethrin	*Westiellopsis prolifica*	55	25 55 75
		W. indica	35	15 35 55
		W. ramosa	40	20 40 60

Table 10: LC_{50} value of carbofuran for three test organisms

Sl no.	Pesticide used	Test specimen	LC_{50} value determined	Treatment based on LC_{50}value
3	Carbofuran	*Westiellopsis prolifica*	40	20 40 60
		W. indica	42	22 42 62
		W. ramosa	30	10 30 50

EFFECT OF MALTHION, DELTAMETHRIN AND CARBOFURAN ON *Westiellopsis prolifica*

4.4 Effect of Malathion on *Westiellopsis prolifica*

4.4.1 Biomass

Biomass is an important parameter of growth of any microorganisms. The biomass content of *W. prolifica* with Malathion treatment is shown in the Fig. 1. It was observed that the biomass contents of the test species decreased from lower to higher concentrations up to 16th day from the day of inoculation over the control, but on the 4th day, at 30ppm concentration there was a little but insignificant increase in the biomass content as compared to the control. The growth was found to be highest in the control set on the 16th day (210±12) µg/ml and the lowest growth was observed at 90ppm on the 16th day of treatment (72±16) µg/ml ($p < 0.001$).

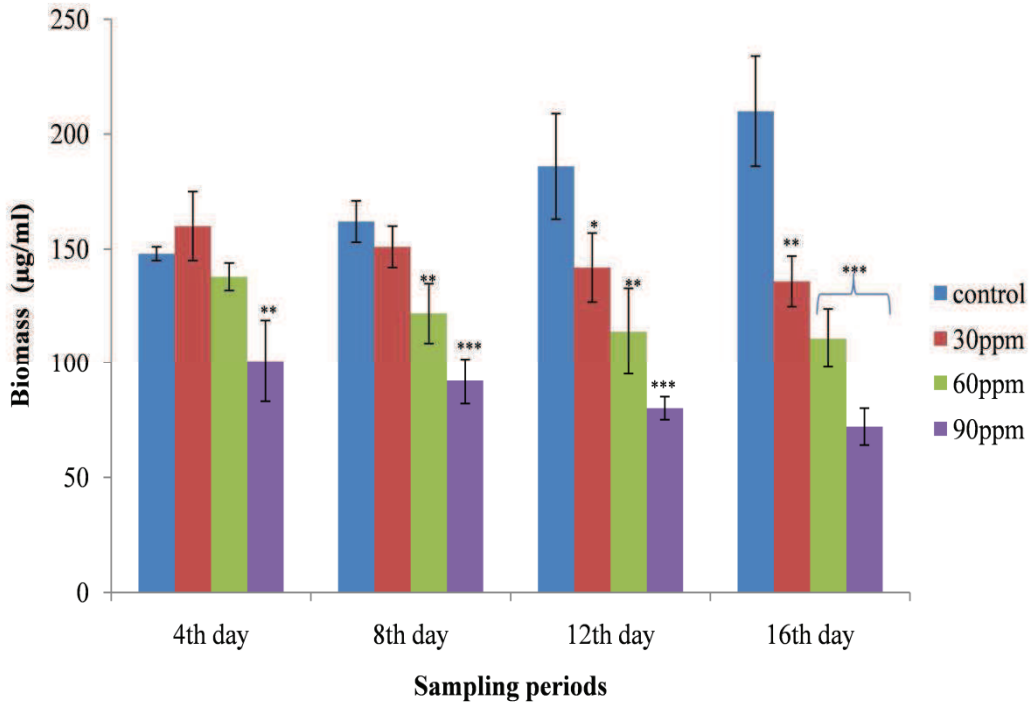


Fig.1. Effect of different concentrations of Malathion on the biomass content of *W. prolifica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p< 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.4.2 Chlorophyll-a

The growth of the test organism in terms of Chlorophyll-a is depicted in Fig. 2. From the figure, chlorophyll-a production was found to be slightly higher than the control at 30ppm on the 4thday ($p<0.05$) and on 8thday from the day of inoculation. There was a gradual reduction in chlorophyll-a content from lower dose to higher dose of pesticide on the 12th and 16th day. The highest chlorophyll-a content was found in the untreated set that was in the control on the 16th day (1.82 ± 0.1 µg/ml) and the highest inhibitory concentration was found to be 90ppm with lowest reduction of chlorophyll-a on the 16th day (0.42 ± 0.15 µg/ml) ($p <0.001$).

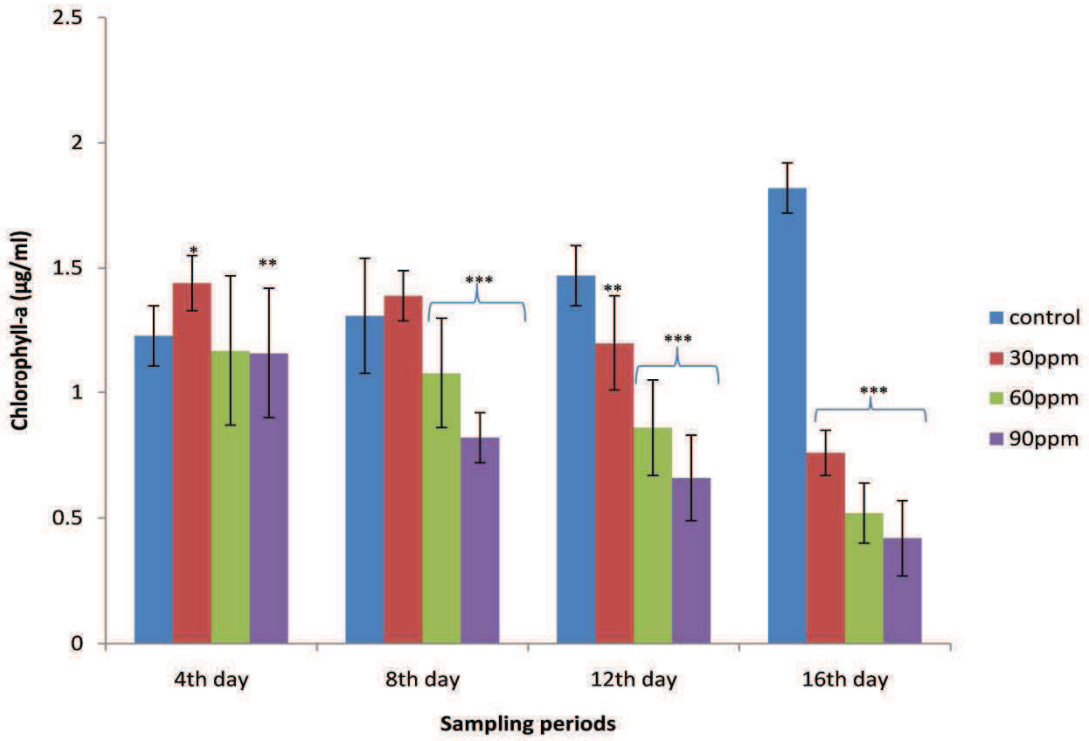


Fig. 2. Effect of different concentrations of Malathion on the chlorophyll-a content of *W. prolifica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control

4.4.3 Carotenoid

Results obtained after exposing the test organism to different concentrations of Malathion is presented in Fig.3. A remarkable degree of carotenoid inhibition was observed in *W. prolifica* after exposure to Malathion treatment for a period of 16 days. Carotenoid contents of the test species was observed to be affected by the pesticide in a time dose dependent manner. In the control sets, an exponential growth was observed up to the 16th day from the day of inoculation throughout the experiment period. The highest carotenoid content was observed in the control during the 16th day (2.5 ±0.06 µg/ml) and lowest reduction was observed on the 16th day (0.12 ± 0.02 µg/ml) at 90ppm ($p<0.001$).

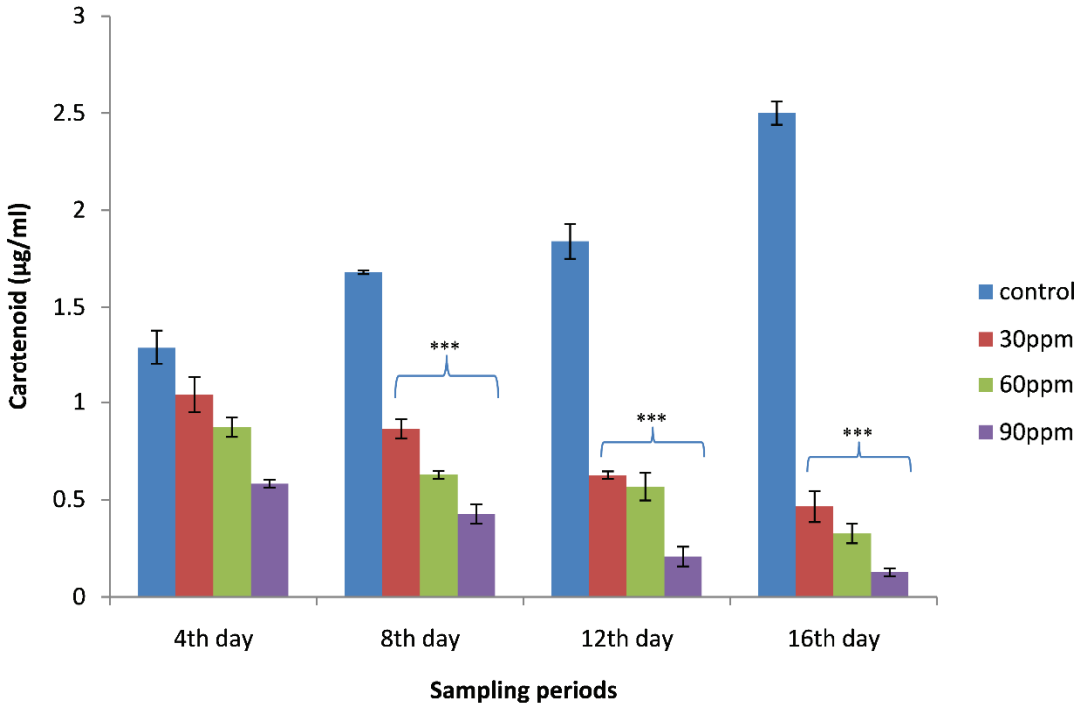


Fig.3. Effect of different concentrations of Malathion on the carotenoid content of *W. prolifica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.4.4 Protein

The effect on the protein content of the test species with Malathion treatment is shown in Fig.4. There was a gradual reduction in the protein content with increase in pesticide concentration in a time and dose dependent manner. The highest protein content was found in the control sets (5.3±0.18) µg/ ml and lowest at 90ppm (0.66±0.1) µg/ml on the 16th day ($p<0.001$). On 12th and 16th day, the reduction in protein content was found to be significant at 30, 60 and 90 ppm over the control ($p<0.001$). At the highest treated concentration i.e. at 90 ppm, the reduction in protein content was significant ($p<0.001$) up to 16th day from the day of inoculation.

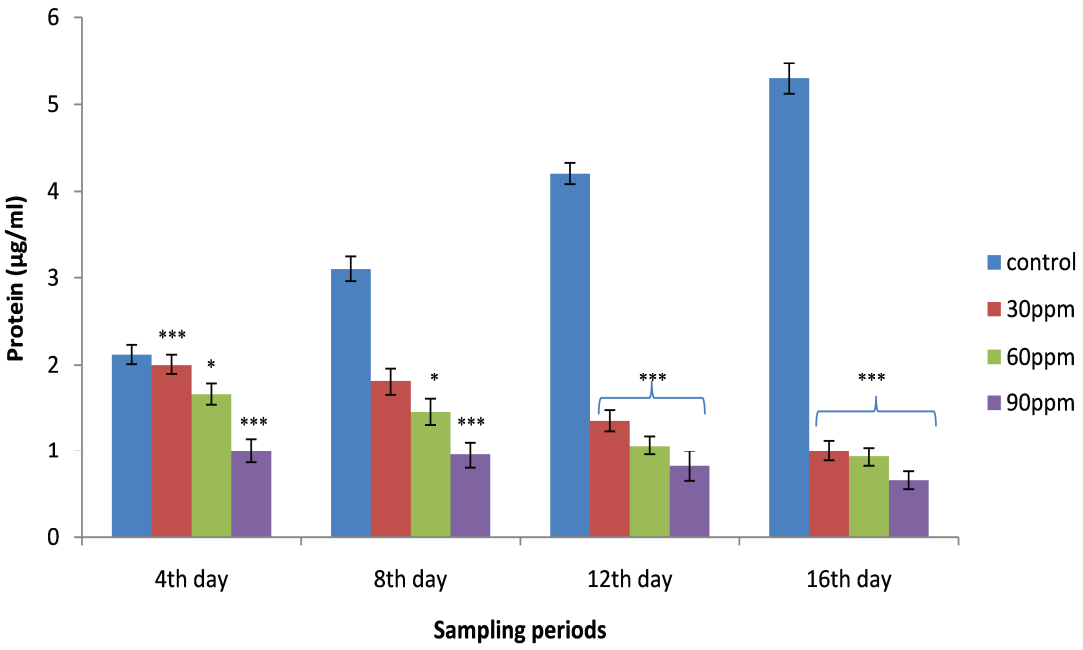


Fig.4. Effect of different concentrations of Malathion on the protein content of *W. prolifica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.4.5 Carbohydrate

Results of carbohydrate content obtained after exposing the test organism to varying Malathion concentrations is depicted in the Fig.5. The carbohydrate content was found to be decreasing gradually from lower to higher pesticide concentration with increase in time period of the treatment. There was a significant reduction in carbohydrate content at 60 and 90ppm on 12th day ($p<0.001$) and at 90 ppm of concentration on 16th day from the day of inoculation .On 4th day, though the protein content was found to be decreasing in a time and dose dependent manner but the decrease was not significant over the control.

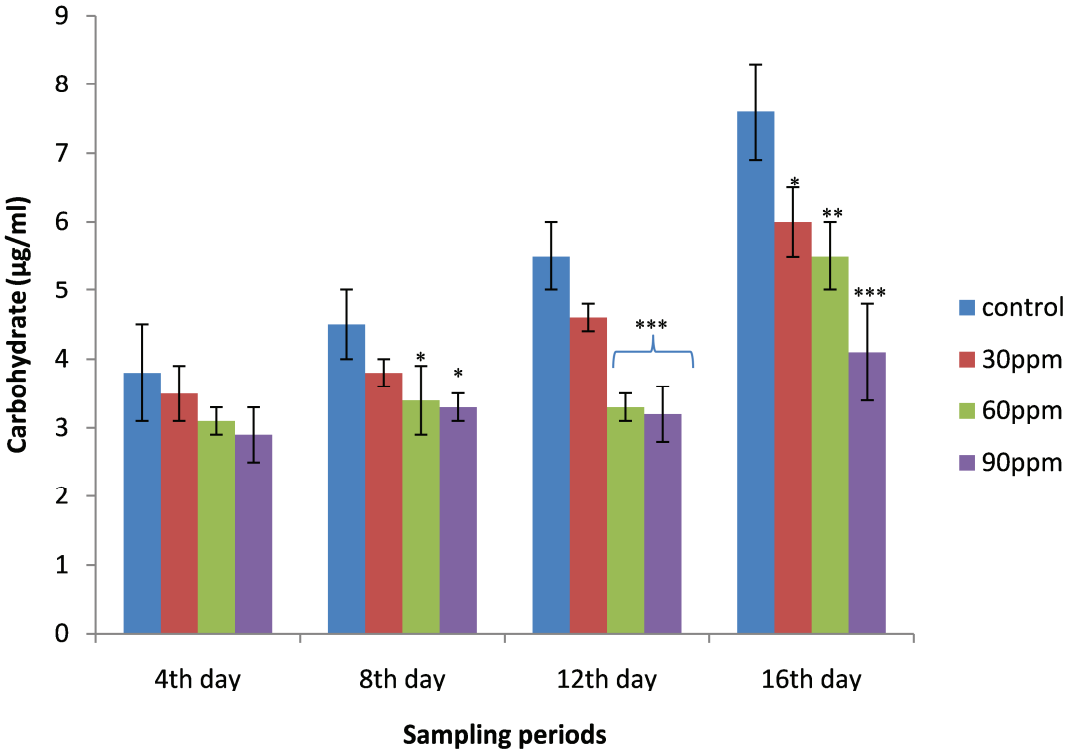


Fig. 5. Effect of different concentrations of Malathion on the carbohydrate content of *W. prolifica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.5.6 Rate of Nitrogen fixation

Results of rate of nitrogen fixation obtained after exposing the test organism to varying Malathion concentrations is depicted in the Fig.6. The nitrogen fixing capacity was found to be decreasing gradually from lower to higher pesticide concentrations in relation to time of the treatment. There was a significant reduction in the rate of nitrogen fixation on 8^{th} and 16^{th} day at 30 ($p<0.001$), 60 ($p<0.001$) and 90 ppm ($p<0.001$) over the control. On 4^{th} day, though the nitrogen fixed by the test organism was found to be decreasing in a time and dose dependent manner but the decrease was not significant over the control.

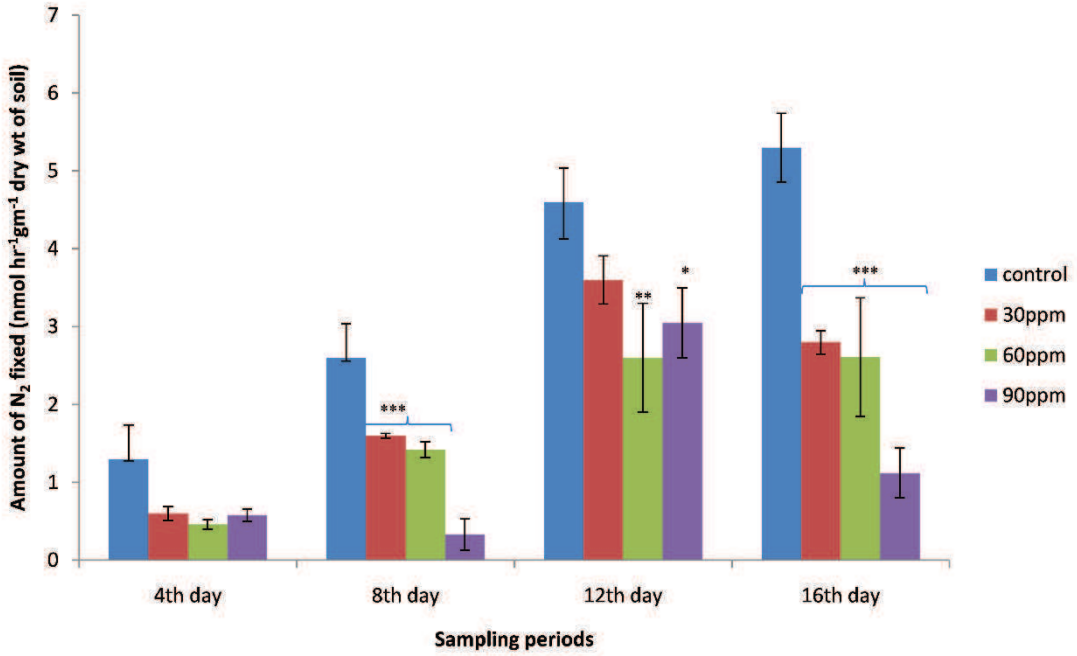


Fig.6. Effect of different concentrations of Malathion on the nitrogen fixing rate of *W. prolifica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.5 Effect of Deltamethrin on *W.prolifica*

4.5.1 Biomass

Biomass content of the *W.prolifica* treated with Deltamethrin is depicted in Fig.7. The overall biomass content of the test organism was observed to be decreasing in a time and dose dependent manner up to 16th day from the date of inoculation. A steady increase in biomass was observed in the control sets up to 16th day from the day of inoculation. The highest biomass was recorded in the control sets on 16th day (211 ± 20) µg/ml and lowest was recorded at 75ppm on the same day (78 ± 11) µg/ml.

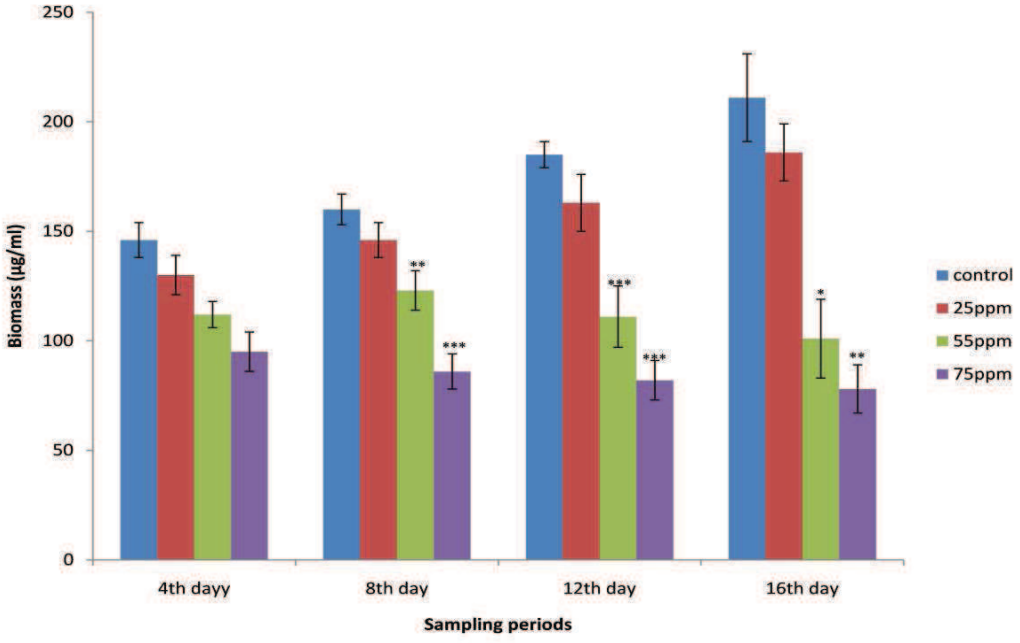


Fig.7. Effect of different concentrations of Deltamethrin on the biomass content of *W. prolifica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.5.2 Chlorophyll-a

The chlorophyll-a content of the test organism was seen to be decreasing in a time and dose dependent manner by the application of Deltamethrin (Fig. 8). There was a significant reduction in chlorophyll-a content up to 76% ($p<0.001$) on the 16th day at 75ppm of Deltamethrin over the control. At 75ppm, the chlorophyll-a content reduces by 55% ($p<0.01$) on 8th day and by 61% ($p<0.001$) on 12th day over the control. On 4th day, the reduction in the pigment content was not found to be insignificant at all the tested concentrations of Deltamethrin over the control.

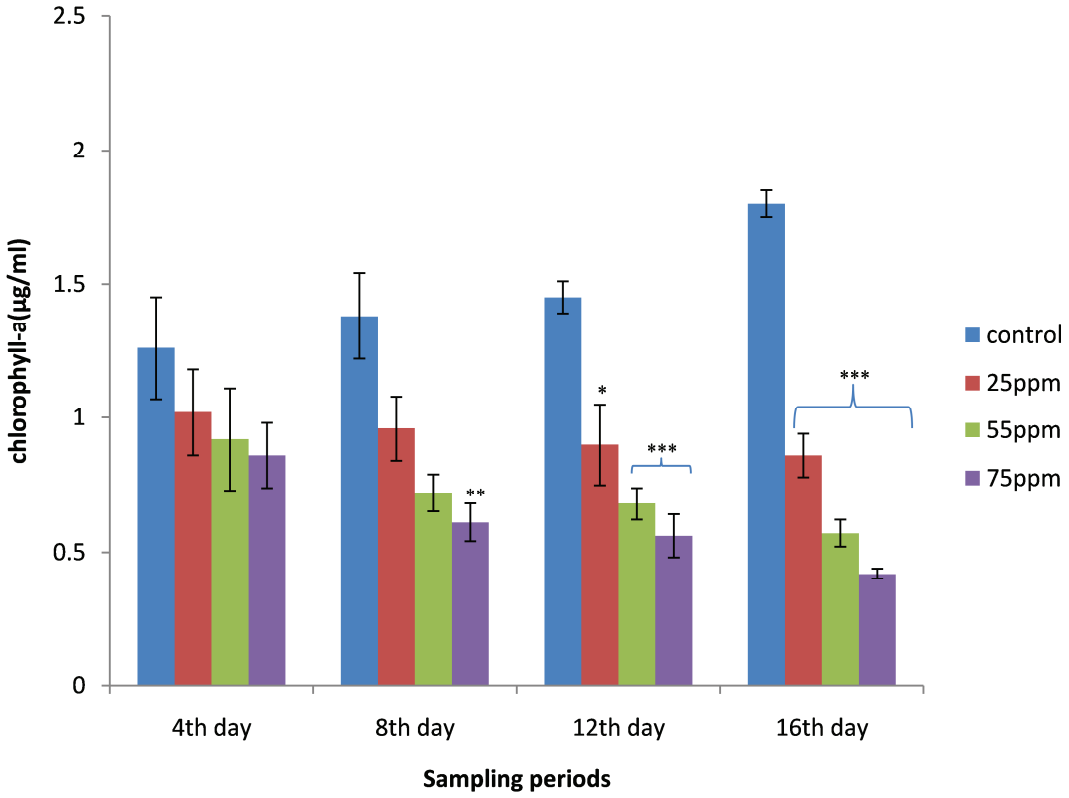


Fig. .8. Effect of different concentrations of deltamethrin on the chlorophyll-a content of *W. prolifica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.5.3 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 4th to the 16th day with a little but insignificant increase on the 4th day by 8% at 25ppm over the control (Fig. 9). On 8th day, a significant reduction in carotenoids content was observed at 55 and 75ppm of pesticide concentration over the control. On the 12th day, it was reduced by 31% ($p<0.001$), 48% ($p<0.001$) and 55% ($p<0.001$) at 25, 55 and 75 ppm compared to the control. Similarly, on 16th day, it was reduced by 54% ($p<0.001$), 68% ($p<0.001$) and 71% ($p<0.001$) at 25, 55 and 75 ppm over the control.

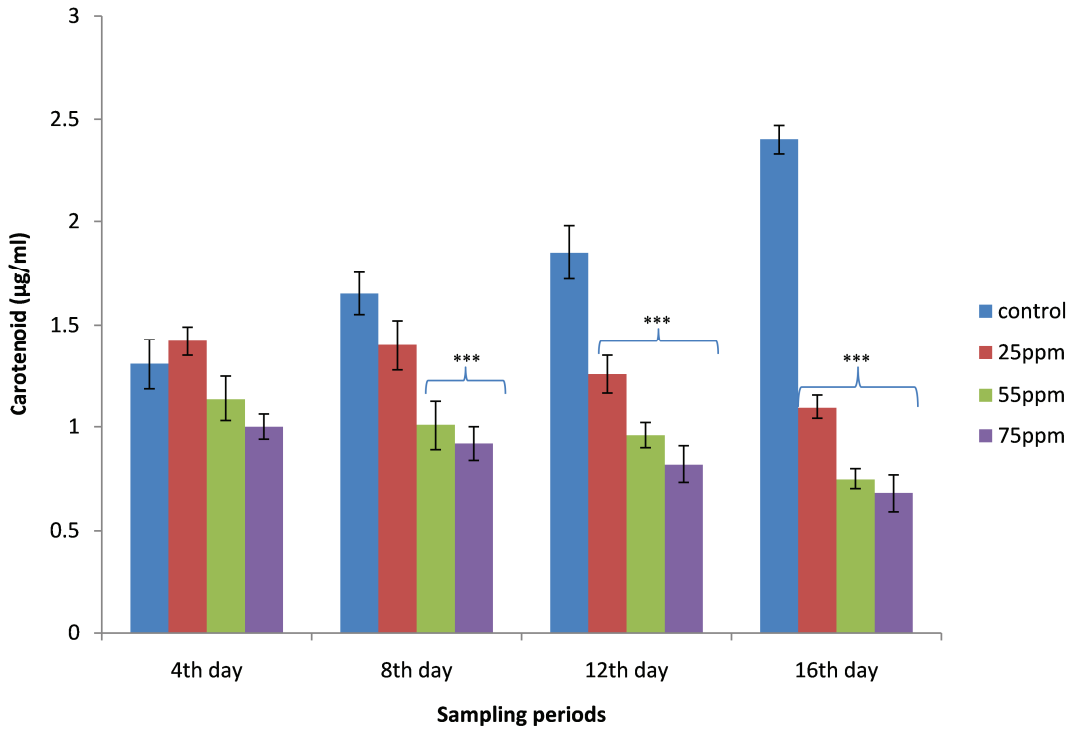


Fig. 9. Effect of different concentrations of Deltamethrin on the carotenoid content of *W. prolifica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.5.4 Protein

Protein content of the test organism was found to be decreased from the 8th to the 16th day from the date of inoculation in a time and dose dependent manner with Deltamethrin application. 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 (Fig. 10). In the rest of the days i.e. 8th, 12th and 16th, the protein content was found to be significantly decreased in a time and dose dependent manner.

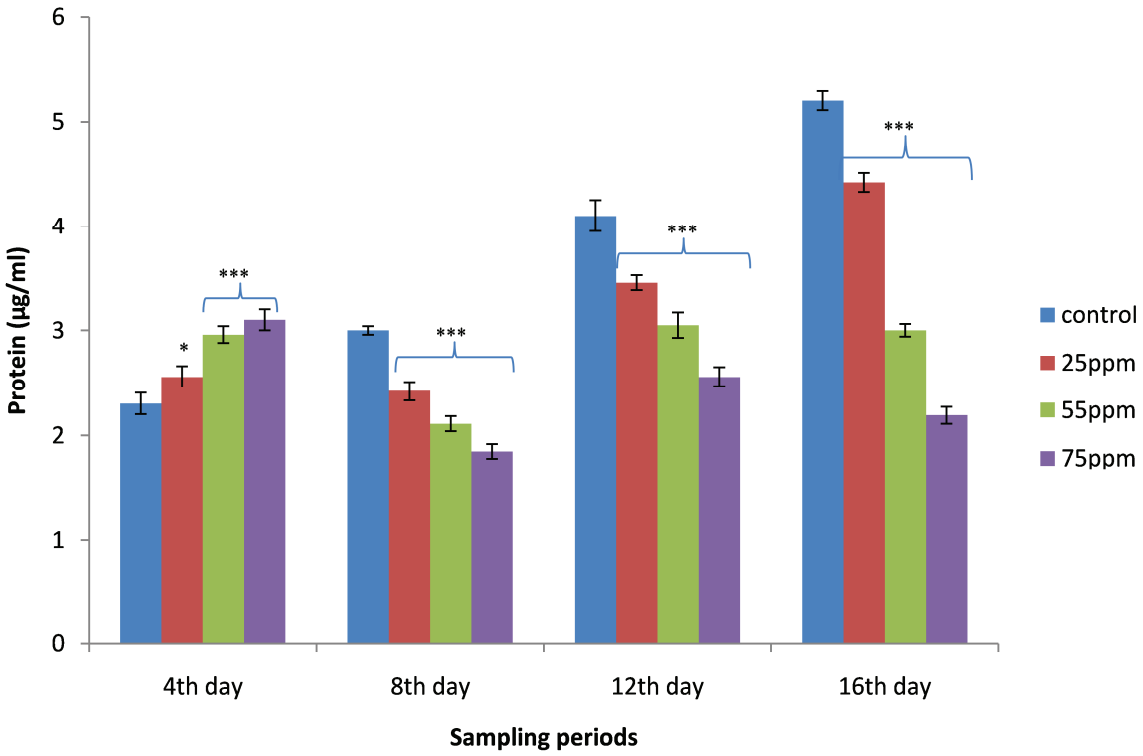


Fig.10. Effect of different concentrations of Deltamethrin on the protein content of *W. prolifica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.5.5 Carbohydrate

The carbohydrate content of the test organism was seen to be decreasing with the gradual increase of Deltamethrin concentration, except at 25ppm where an insignificant increase in carbohydrate content was recorded from the date of inoculation up to 16th day over the control by 12.5%, 9.5%, 1.8% and 13% on 4th, 8th, 12th and 16th day (Fig. 11). At the highest tested concentration i.e. at 75ppm, the reduction in carbohydrate content was significant by 32% ($p<0.01$) on 4th day, 33% ($p<0.01$) on 8th day, 32.7% ($p<0.001$) on 12th day and 49% ($p<0.001$) on 16th day respectively over the control.

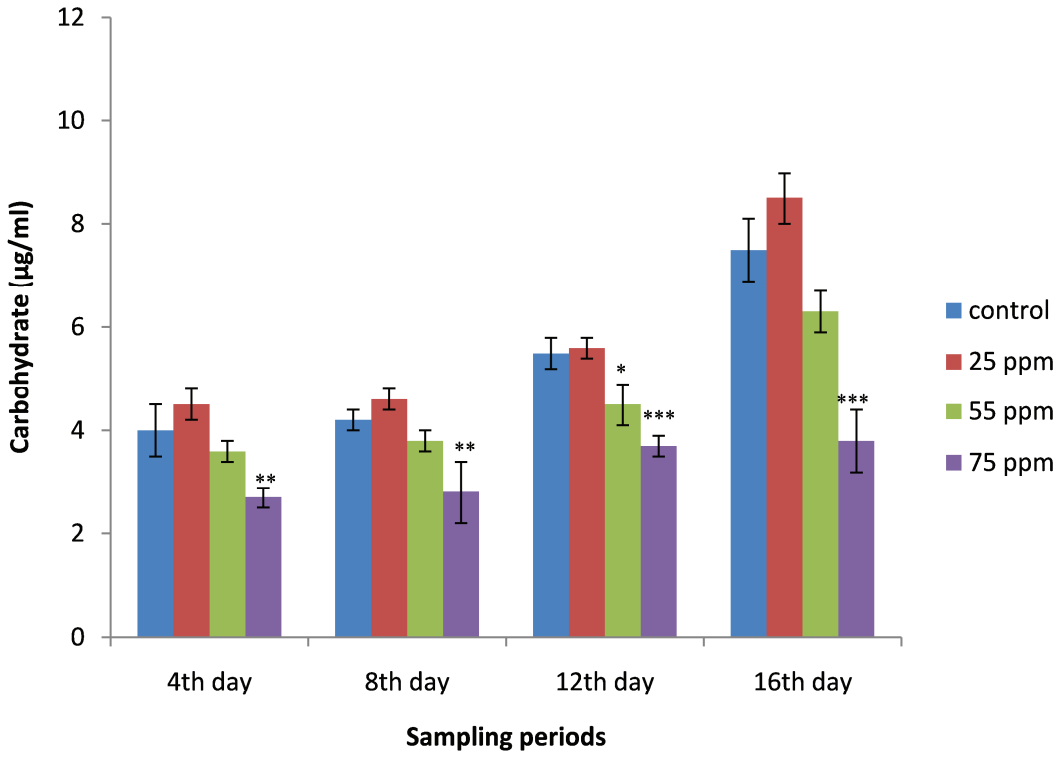


Fig.11. Effect of different concentrations of Deltamethrin on the carbohydrate content of *W. prolifica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.5.6 Rate of Nitrogen fixation

The nitrogen fixation rate of the test organism was seen to be decreasing with the gradual increase of Deltamethrin concentration, except on 8th day where a significant increase in rate of nitrogen fixation was observed at 25ppm by 88% ($p<0.05$) and at 55 ppm by 64% as compared to the control (Fig. 12). A substantial as well as significant reduction in the rates of nitrogen fixation were observed at 25, 55 and 75ppm of treatments of Deltamethrin by 51% ($p<0.001$), 67% ($p<0.001$) and 68% ($p<0.01$) respectively on 16th day over the control.

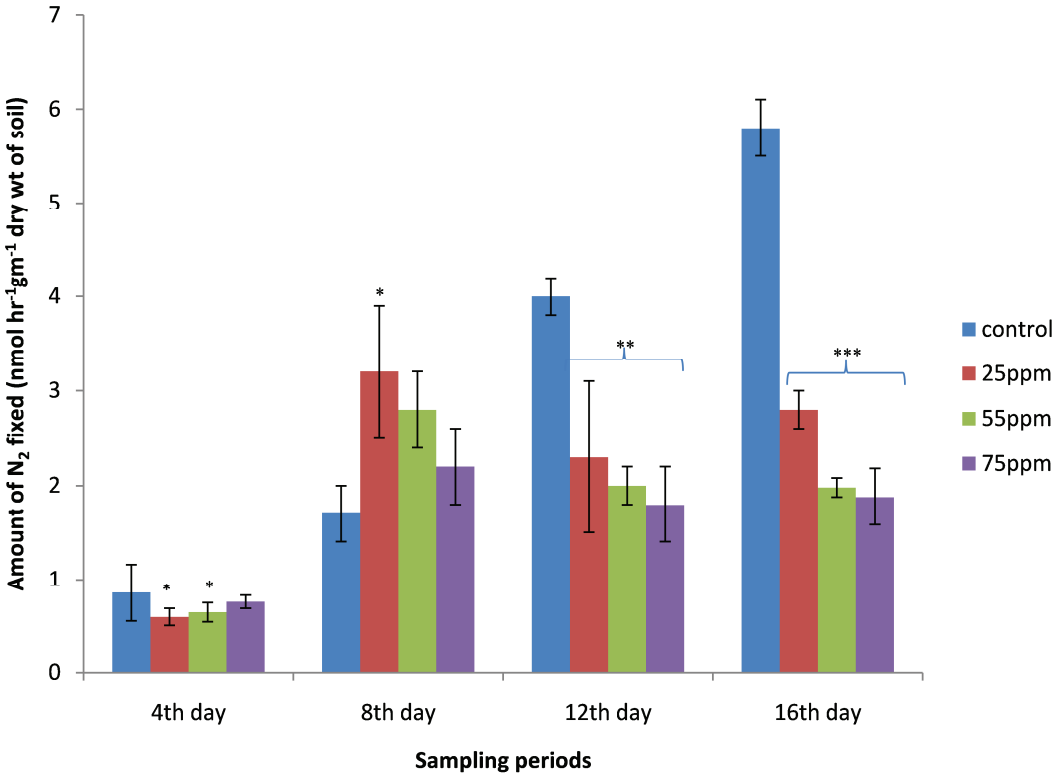


Fig.12. Effect of different concentrations of Deltamethrin on the nitrogen fixing rate of *W. prolifica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.6 Effect of Carbofuran on *W. prolifica*

4.6.1 Biomass

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. An 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. On 12th day, at 20 ppm the biomass was found to be decreased by 13% ($p<0.01$), at 40 ppm by 28% ($p<0.001$) and at 60 ppm by 59% ($p<0.001$).

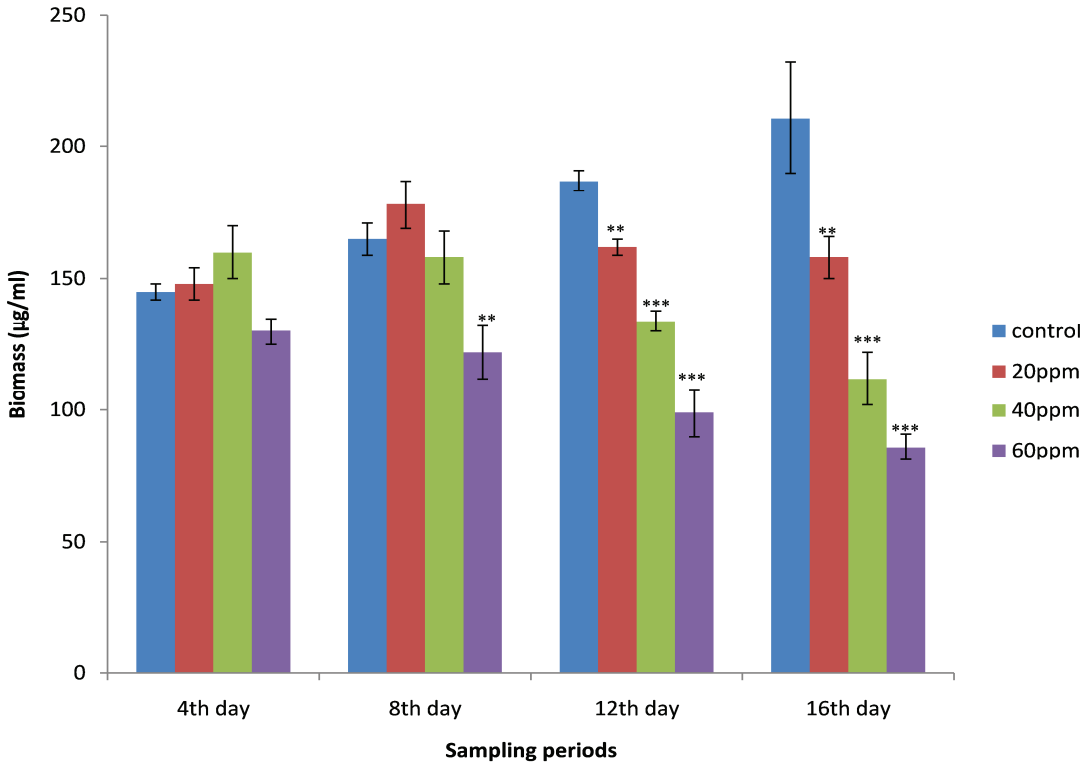


Fig.13. Effect of different concentrations of Carbofuran on the biomass content of *W. prolifica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.6.2 Chlorophyll-a

The chlorophyll-a content of the test organism was found to be decreasing in a time and dose dependent manner by the application of Carbofuran. There was a significant reduction in chlorophyll-a content up to 77% ($p<0.001$) when treated with 60ppm of Carbofuran over the control on 16th day. The overall reduction of chlorophyll-a was found to be significant at all the tested concentrations (20ppm, 40ppm, 60ppm) over the control up to 16th day from the day of inoculation. The highest chlorophyll-a content was found in the control sets on 16th day (1.81±0.1µg/ml) and lowest chlorophyll-a content was found at 60ppm on the same day (0.4±0.02 µg/ml).

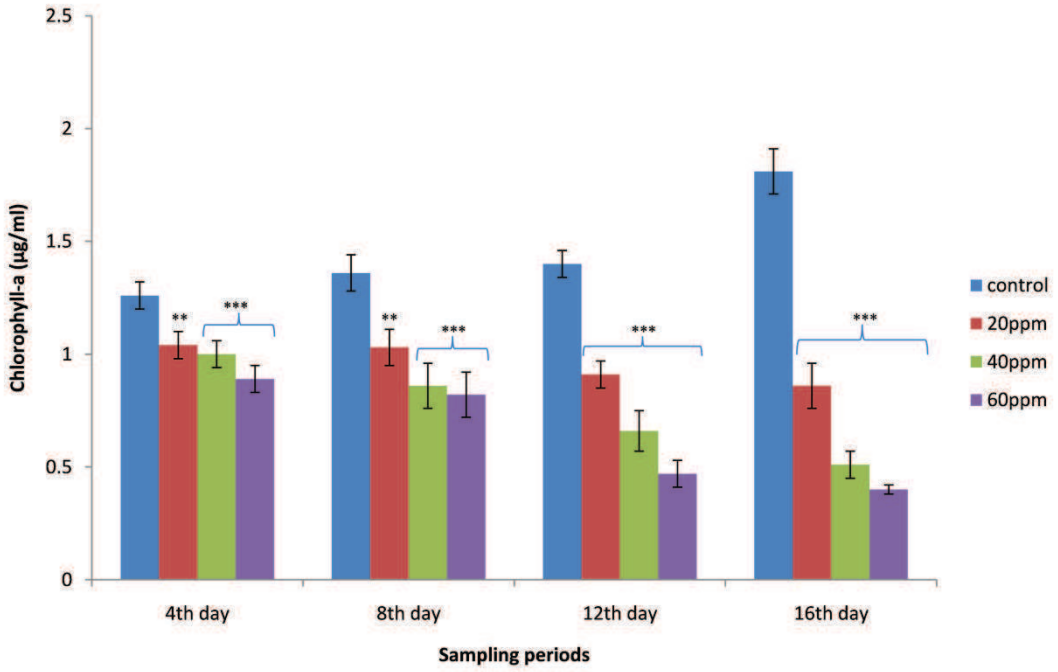


Fig.14. Effect of different concentrations of Carbofuran on the chlorophyll-a content of *W. prolifica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.6.3 Carotenoid

From the Fig.15, it was observed that with Carbofuran application, carotenoid showed insignificant enhancement at 20ppm on 4th, 8th and 12th day by 10%, 5% and 5% respectively against the control sets. A significant reduction in carotenoid content was observed at 60ppm up to 16th day from the day of inoculation. It was reduced by 21% ($p < 0.05$) on 4th day, by 48% ($p < 0.001$) on 8th day, by 57% ($p < 0.001$) on 12th day and by 73% ($p < 0.001$) on 16th day at 60ppm respectively over the control. The highest carotenoid content was observed in the control sets (2.3± 0.17 µg/ml) and lowest was observed at 60ppm on the same day (0.62±0.12 µg/ml).

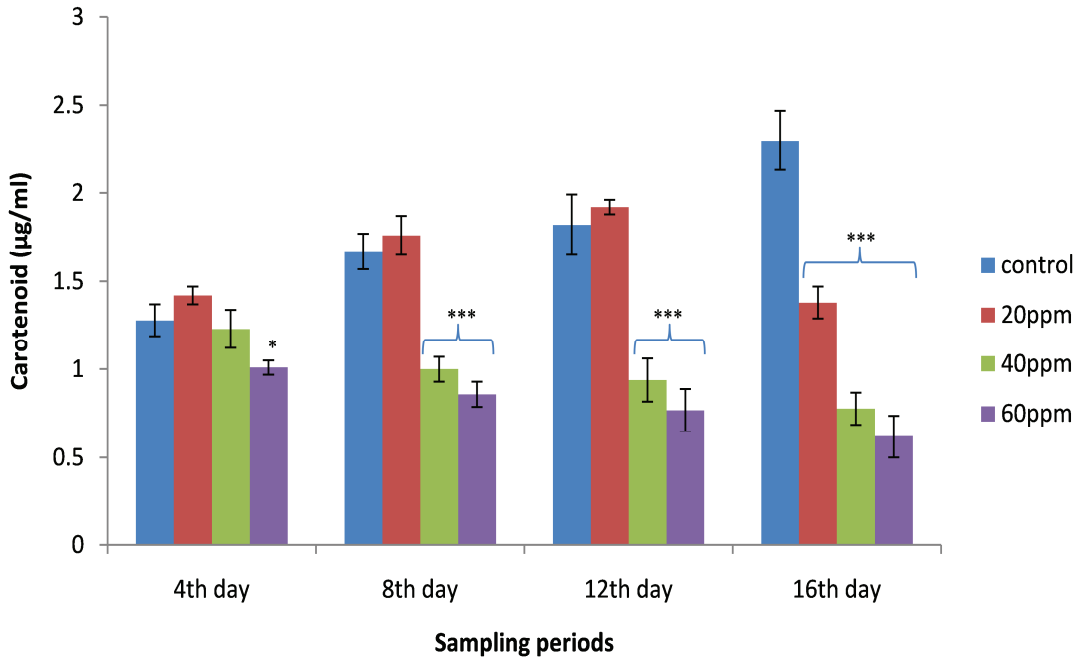


Fig. 15. Effect of different concentrations of Carbofuran on the carotenoid content of *W. prolifica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.6.4 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 Carbofuran application. The protein contents of the test organism were gradually decreased with increase in time and dose of Carbofuran. The reduction in protein content was observed to be significant at all the tested concentrations from 4th-16th day of inoculation. At 60ppm, the protein content was decreased by 40% ($p<0.001$), 53% ($p<0.001$), 44% ($p<0.001$) and 55% ($p<0.001$) on 4th, 8th, 12th and 16th day respectively.

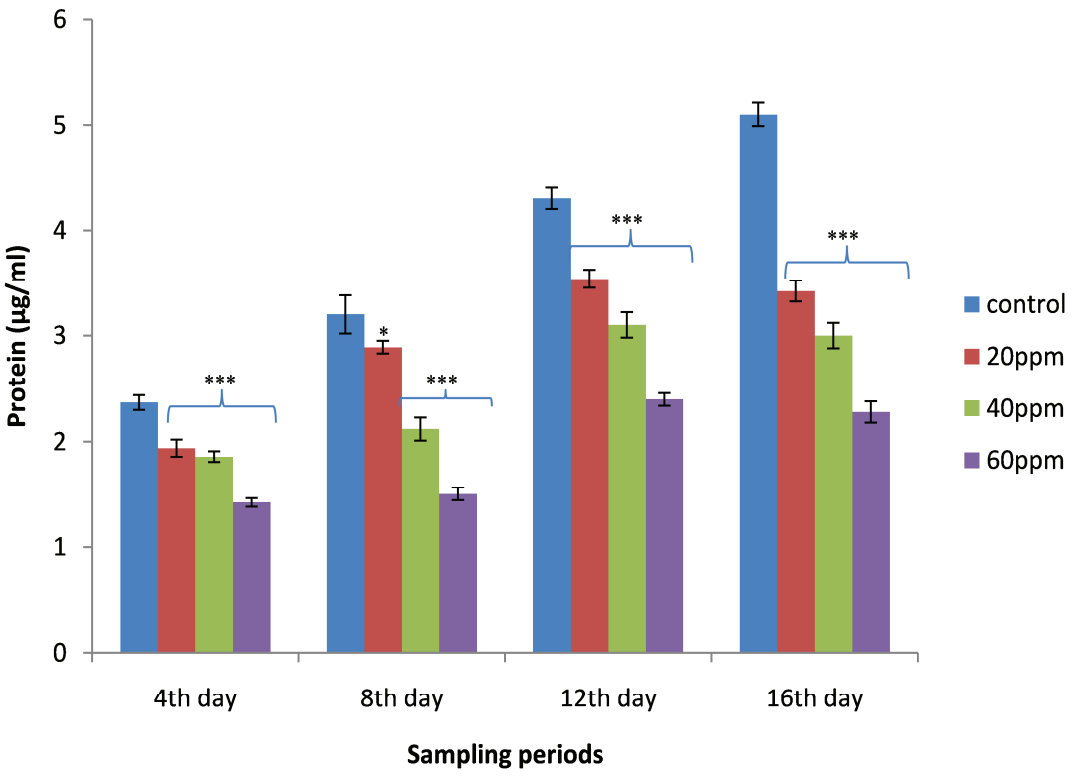


Fig.16. Effect of different concentrations of Carbofuran on the protein content of *W. prolifica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.6.5 Carbohydrate

The effect of carbofuran on the carbohydrate content of *W. prolifica* is depicted in Fig.17. From the figure, it was observed that in the Carbofuran treated samples, carbohydrate content was gradually increased in a time and dose dependent manner. At 60ppm, the carbohydrate content was increased by 48.5% ($p<0.01$), 56.8% ($p<0.01$), 57.1% ($p<0.001$), 30.7% ($p<0.001$) on 4^{th}, 8^{th}, 12^{th} and 16^{th} day with Carbofuran application respectively. The highest carbohydrate content was found at 60ppm on 16^{th} day (10.2±0.6 µg/ml) and the lowest was recorded in the control sets on 4^{th} day (3.5±0.3 µg/ml).

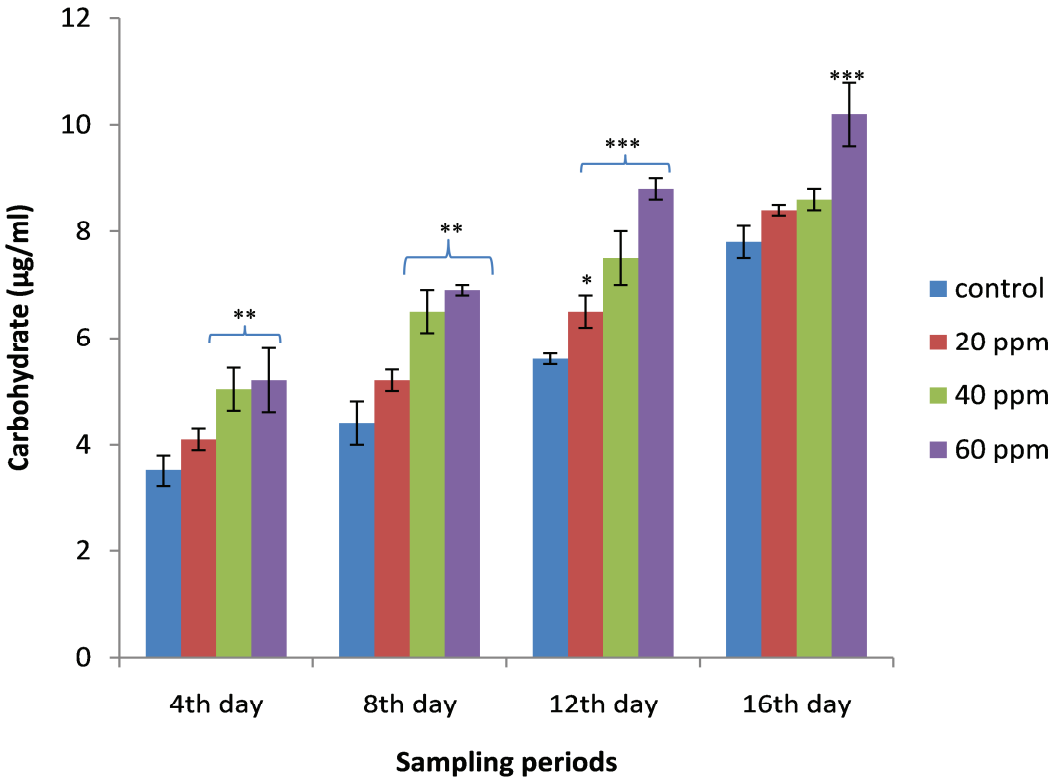


Fig.17. Effect of different concentrations of Carbofuran on the carbohydrate content of *W. prolifica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.6.6 Rate of Nitrogen fixation

The rate of nitrogen fixation by the test organism was decreased in a time and dose dependent manner with the Carbofuran application with an exception at 20ppm concentration on 4th and 8th day where the nitrogen fixing capacity of the test organism increases by 42% (not significant) and 60% ($p<0.01$) over the control. The reduction in nitrogen fixation was observed to be significant at all the tested concentrations on 12th and 16th day of inoculation. At 40ppm, the nitrogen fixation by the organism was decreased by 57% ($p<0.01$) on 12th day and 67% ($p<0.001$) on 16th day. At 60ppm, the nitrogen fixation by the organism was decreased by 68% ($p<0.01$) on 12thday and 91% ($p<0.001$) on 16th day respectively.

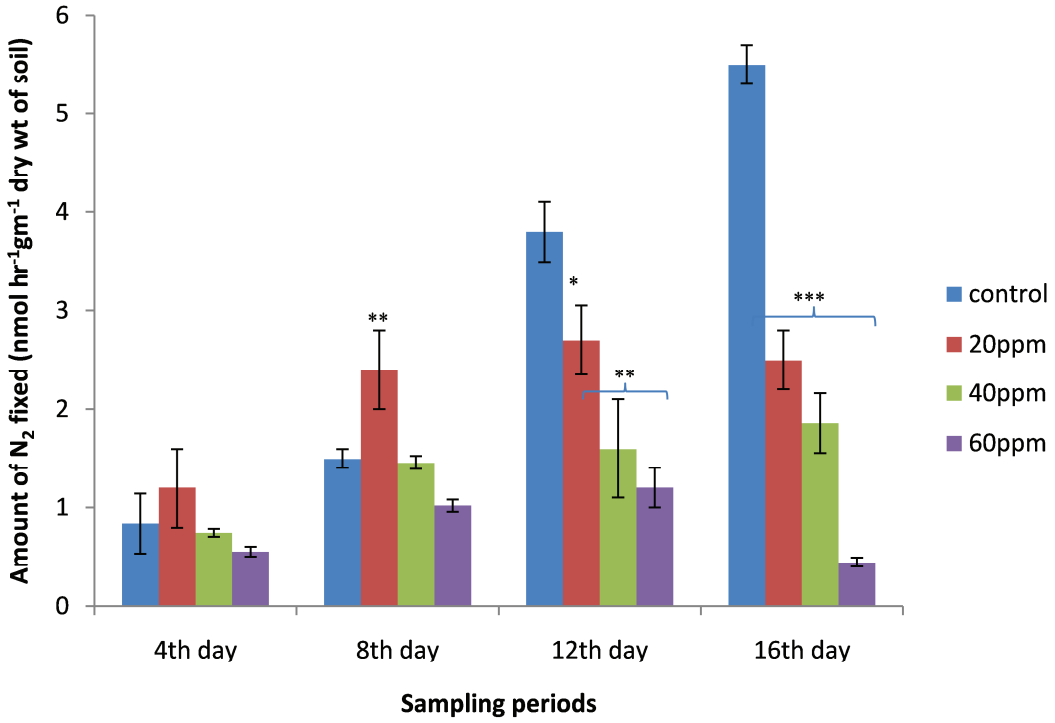


Fig. 18. Effect of different concentrations of Carbofuran on the nitrogen fixing rate of *W. prolifica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

EFFECT OF MALTHION, DELTAMETHRIN AND CARBOFURAN ON *W. indica*

4.7 Effect of Malathion on *W. indica*

4.7.1 Biomass

The effect of Malathion on the biomass content of *W. indica* is depicted in the Fig.19. It was seen that the biomass content decreased with the concomitant increase of the pesticide concentration with time. An insignificant increase in biomass at 30ppm over the control was observed on 4th day. At the highest treated concentration i.e. at 90ppm, a significant reduction in biomass was observed on 12th day (47%) ($p<0.001$) and 16th day (36%) ($p<0.001$). The highest biomass was observed in the control sets on 16th day (123±12 µg/ml) and lowest biomass was observed at 90ppm on 8th day (50±4 µg/ml).

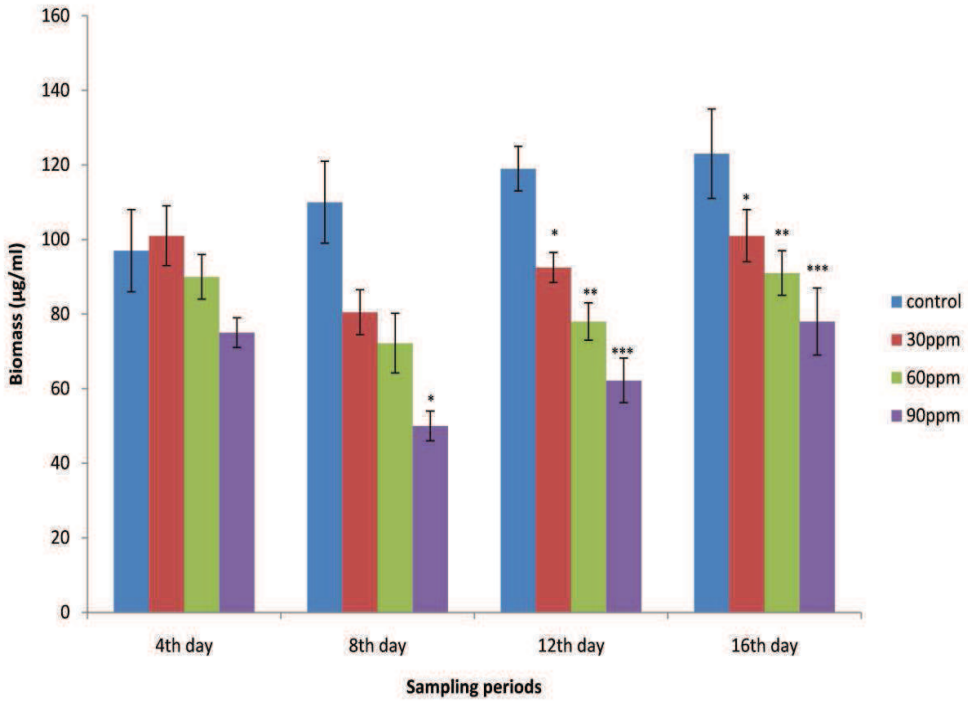


Fig. 19. Effect of different concentrations of Malathion on the biomass content of *W. indica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.7.2 Chlorophyll-a

The effect of Malathion on the chlorophyll-a content of the test organism is summarized in Fig.20. From the Figure, it was observed that the chlorophyll-a contents of the test organism with Malathion application varied considerably at different concentrations and time. On 4th day at 30ppm, the pigment was found to be increased by 24% ($p<0.01$) over the control. On 8th day, it was increased by 10% ($p<0.01$) at 30ppm and by `4% ($p<0.001$) at 60ppm over the control. On 12th day, an insignificant increase in chlorophyll-a was observed at 30ppm over the control. While on the other hand, a significant decrease in biomass was observed at 60 and 90ppm on 12th and 16th day respectively.

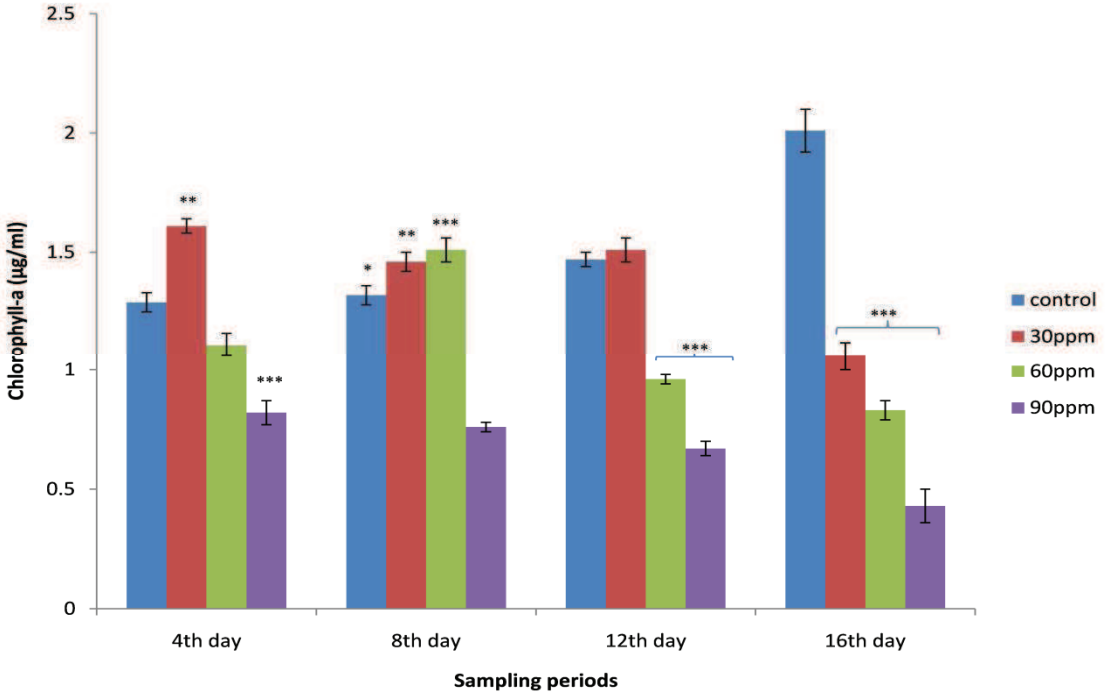


Fig. 20. Effect of different concentrations of Malathion on the chlorophyll-a content of *W. indica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.7.3 Carotenoid

The effect of Malathion on the carotenoid content of *W.indica* is shown in Fig.21. The carotenoid content was seen to be decreasing in a time and dose dependent manner with an exception at 30 and 60ppm on 4th day which was increased by 37% ($p<0.01$) and 63% ($p<0.001$) compared to the control sets. At 90ppm, the reduction was observed to be significant over the control on 8th, 12th and 16th day i.e. it reduces by 34% ($p<0.01$), 72% ($p<0.001$) and 78% ($p<0.001$). The highest carotenoid content was recorded in the control sets on 16thday (1.8 ± 0.08 µg/ml) and lowest was observed at 90ppm on 16th day (0.38 ± 0.05 µg/ml).

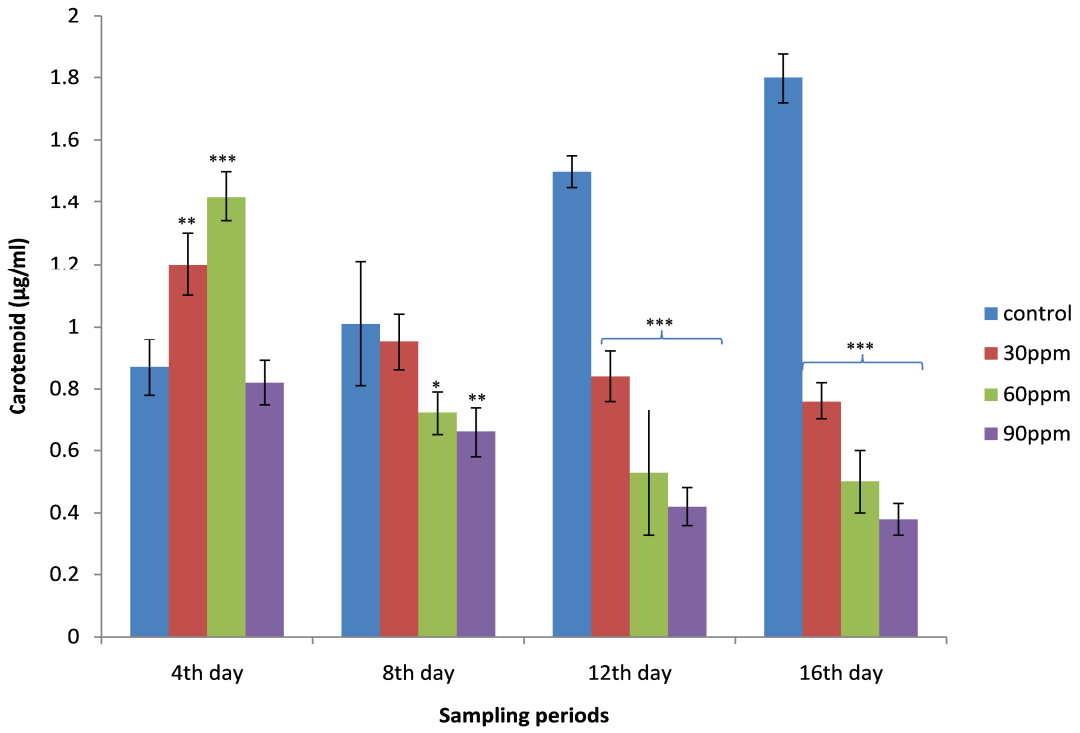


Fig. 21. Effect of different concentrations of Malathion on the carotenoid content of *W. indica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.7.4 Protein

The effect of Malathion on the protein content of the test organism can be observed from the Fig.22. The results showed that the protein content of the test organism was decreased with the increase in pesticide concentration with time. At 90ppm, the protein content was reduced by 40% ($p<0.001$), 28% ($p<0.001$), 31% ($p<0.001$) and 36% ($p<0.001$) over the control on 4th, 8th, 12th and 16th day respectively. The highest protein content was recorded in the control sets on 16th day (5.1 ± 0.15 µg/ml) and lowest was observed at 90 ppm on 4th day (2.1 ± 0.12 µg/ml). At 90ppm of pesticide concentration, the decrease in pesticide concentration was observed to be significant from 4th -16th days of inoculation.

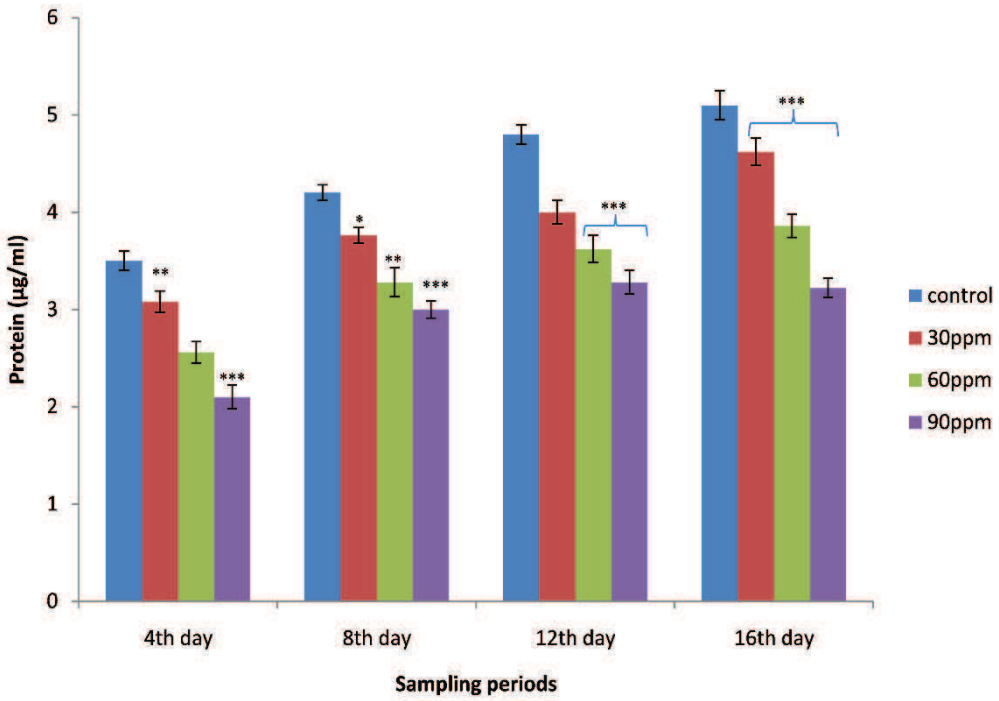


Fig. 22. Effect of different concentrations of Malathion on the protein content of *W. indica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.7.5 Carbohydrate

The carbohydrate content of the test organism was seen to be decreasing with the concomitant increase in the pesticide concentrations with time. On 4th day, an insignificant increase in carbohydrate was observed at 30ppm, 60ppm and 90ppm over the control. Similarly, on 8th day it showed insignificant decrease at 30ppm and 60ppm concentrations of Malathion, with a significant decrease at 90ppm over the control. On 12th day, the carbohydrate content decreases by 28% ($p < 0.001$) at 30ppm, 45% ($p<0.001$) at 50ppm and 56% ($p<0.001$) at 90ppm over the control. On 16th day, the carbohydrate content decreases by 25% ($p<0.01$) at 30ppm, 60% ($p<0.001$) and 60.86% ($p<0.001$) respectively over the control.

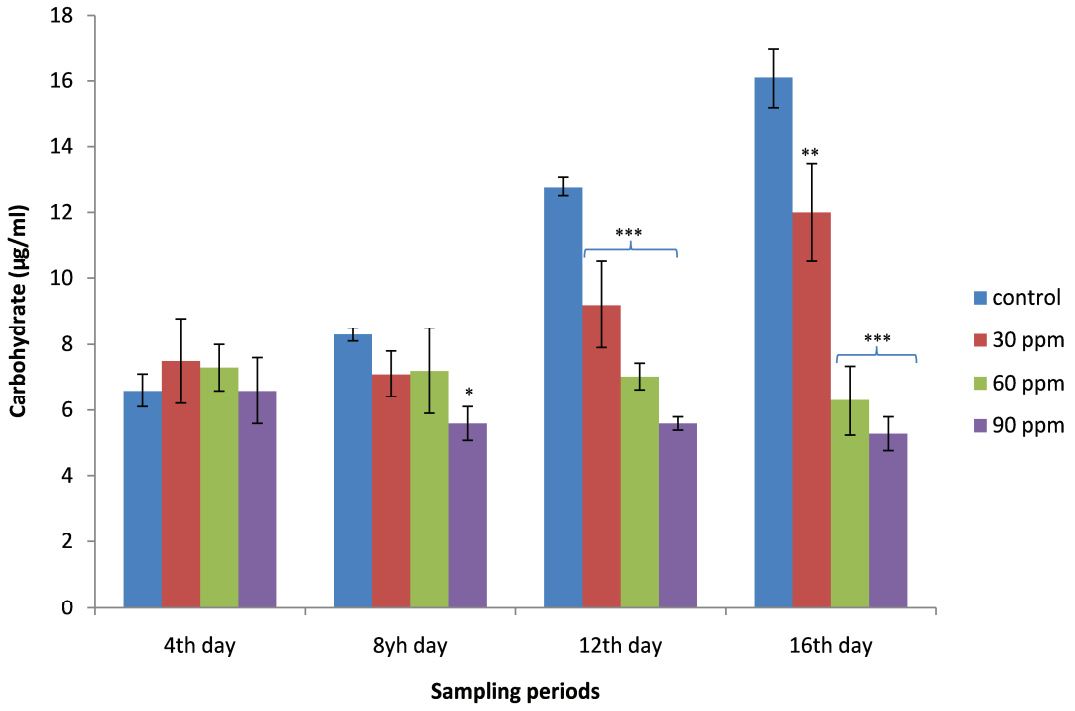


Fig. 23. Effect of different concentrations of Malathion on the carbohydrate content of *W. indica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.7.6 Rate of Nitrogen fixation

The rate of nitrogen fixation by the test organism under Malathion stress is depicted in Fig.24. The rate of nitrogen fixed by the test organism was seen to be decreasing with the concomitant increase in the pesticide concentrations with time. On 4th day, a significant increase in nitrogen fixation was observed at 30ppm, 60ppm and 90ppm over the control by 71% ($p<0.05$), 80% ($p<0.05$) and 90% ($p<0.01$). On 12th and 16th day, the nitrogen fixation by the test organism decreases significantly over the control. At 90ppm, the nitrogen fixation reduces by 39% ($p<0.01$), 75% ($p<0.001$) and 74% ($p<0.01$) on 8th, 12th and 16th day respectively.

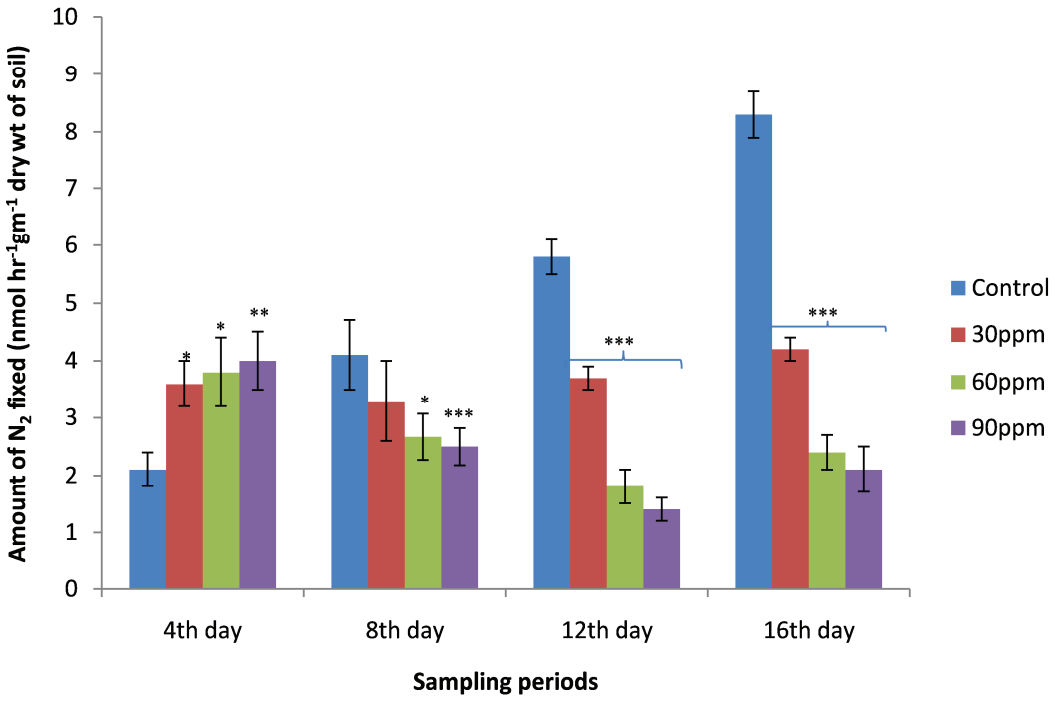


Fig. 24. Effect of different concentrations of Malathion on the nitrogen fixing rate of *W. indica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.8 Effect of Deltamethrin on *W .indica*

4.8.1 Biomass

Deltamethrin had an inhibitory effect on the biomass content of *W. indica* with an exception at 15ppm concentration where the biomass was observed to be higher than the control sets. An insignificant decrease in biomass was observed at 35ppm concentration from 4th day to 16th day from the day of inoculation. A significant decrease in biomass was observed at 55ppm concentration on 4th day by 24%, on 12th day by 42% and on 16th day by 36%. Highest biomass was observed in the 15ppm treated cultures on 16th day (148.2 ± 20 µg/ml) and lowest was observed at 55ppm on 12th day (68 ± 15 µg/ml).

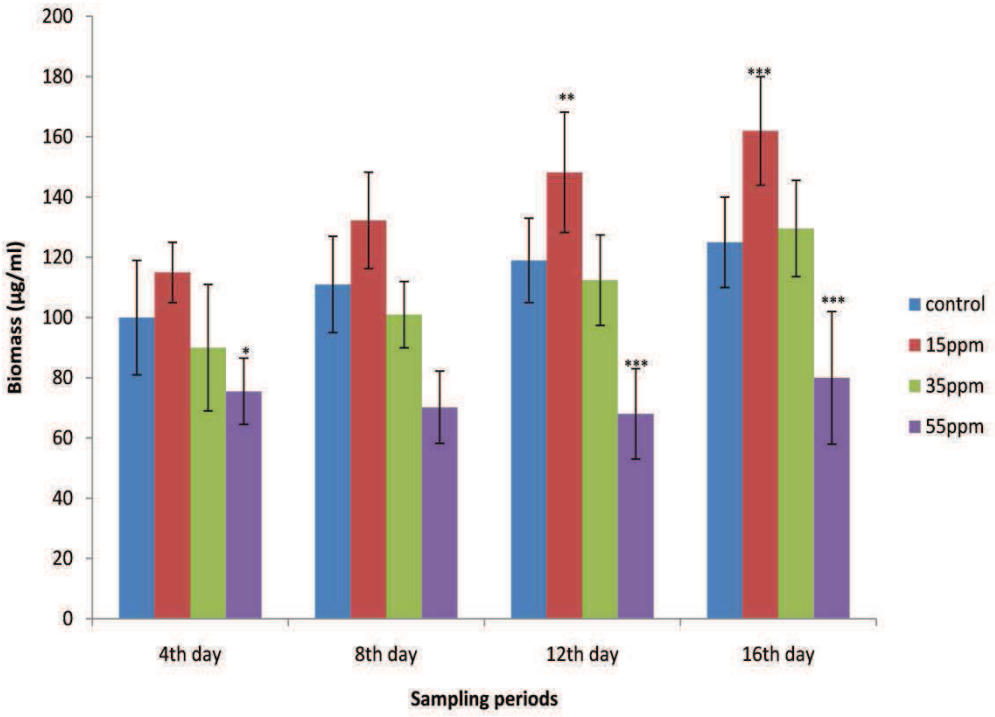


Fig. 25. Effect of different concentrations of Deltamethrin on the biomass content of *W. indica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.8.2 Chlorophyll-a

The effect of Deltamethrin on the chlorophyll-a content of the test organism is depicted in Fig. 26. Deltamethrin contributed in time and dose dependent decrease of the chlorophyll-a content till 16th day from the day of inoculation. On 4th and 8th day, a significant decrease in chlorophyll-a was observed at 35ppm and 55ppm of the pesticide concentration. On 12th day, an insignificant decrease in chlorophyll-a was observed at 35ppm and 55ppm of concentration with an exception at 15ppm where, chlorophyll-a content was found to be higher than that of the control sets. On 16th day, the chlorophyll-a content was found to be decreased by 15% ($p<0.001$) at 15ppm, by 60% ($p<0.001$) at 35ppm and by 73% ($p<0.001$) at 55ppm over the control.

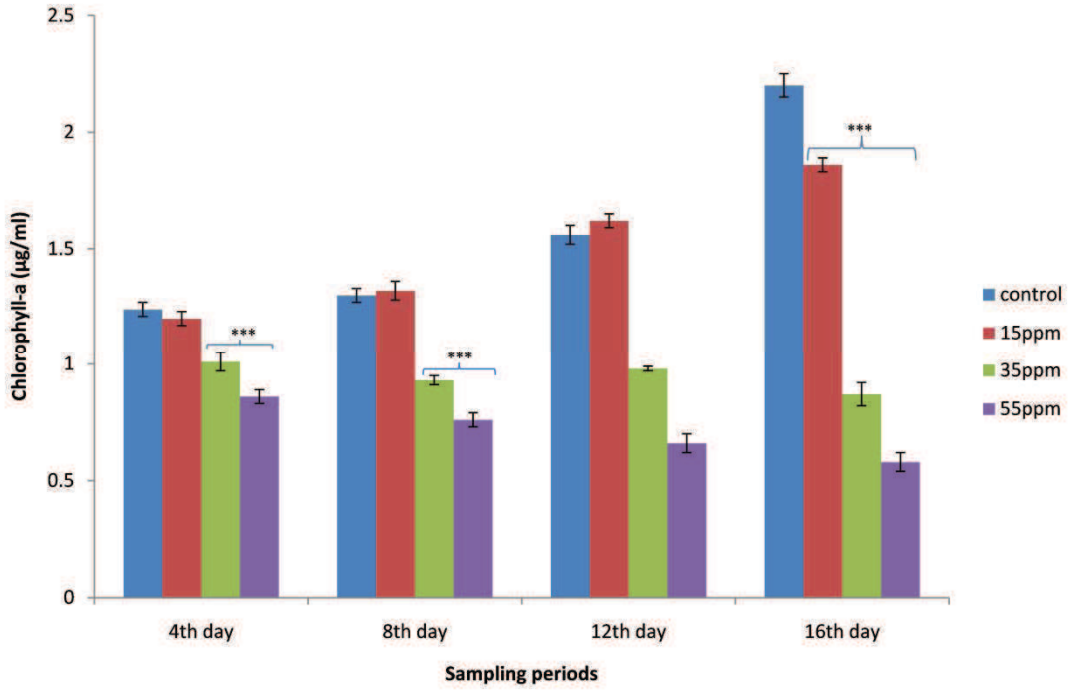


Fig. 26. Effect of different concentrations of Deltamethrin on the chlorophyll-a content of *W. indica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.8.3 Carotenoid

The result depicting the effect of deltamethrin on the carotenoid content of *W. indica* is summarized in Fig.27. The results revealed that there was a decrease in the carotenoid content of the test organism in a time and dose dependent manner. On 4th day, the carotenoid content was decreased by 15% ($p<0.05$) at 15ppm, by 20% ($p<0.01$) at 35ppm and by 28%($p<0.001$) at 55ppm over the control. On 16th day, the control sets showed the highest carotenoid content (1.79 ± 0.08 µg/ml) and 55ppm showed the lowest carotenoid content (0.33 ± 0.08 µg/ml).

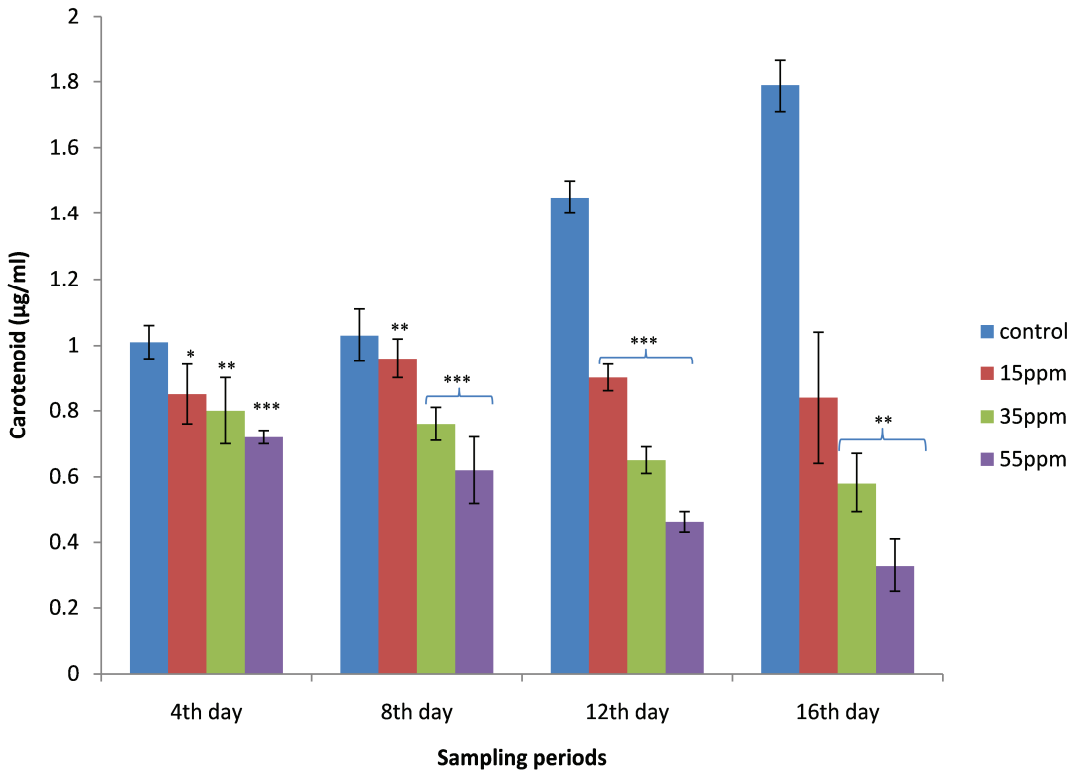


Fig. 27. Effect of different concentrations of Deltamethrin on the carotenoid content of *W. indica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.8.4 Protein

The protein content of the test organism under Deltamethrin stress is depicted in Fig.28. The protein content was seen decreasing till 8th day from the day of inoculation. From 12th day to 16th day, the protein content was observed to be significantly increasing in a time and dose dependent manner. The 4th day and 8th day showed a progressive and significant decrease of protein content with the increase of the pesticide concentrations with time. On 16th day, the protein content was observed to be increased by 6% ($p<0.01$), 9% ($p<0.001$), 20% ($p<0.001$) at 15ppm, 35ppm and 55ppm and on 12th day it was increased by 8% ($p<0.0.001$), 17% ($p<0.001$), 4% ($p<0.001$) at 15ppm, 35ppm and 55ppm of the pesticide over the control respectively.

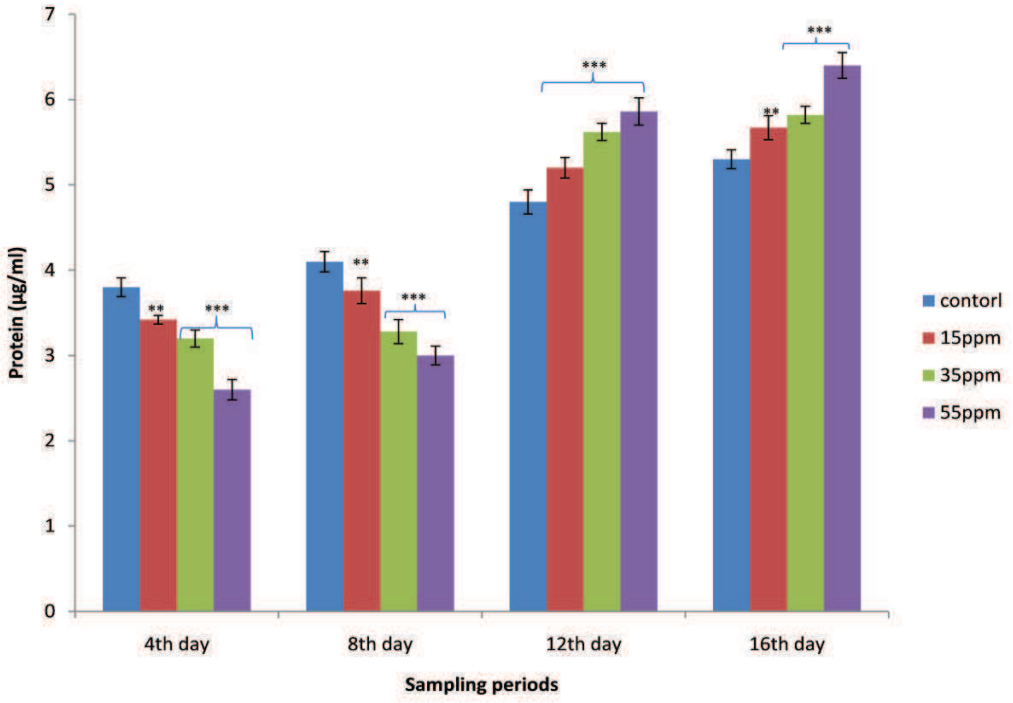


Fig. 28. Effect of different concentrations of Deltamethrin on the protein content of *W. indica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.8.5 Carbohydrate

The effect of Deltamethrin on the carbohydrate content of the test organism is summarized in the Fig. 29. A decrease in carbohydrate content was observed on 4th, 8th, 12th and 16th day when treated with 15, 35 and 55ppm of deltamethrin compared to the control sets. The untreated cultures on 16th day showed the highest carbohydrate content (16.1 ± 0.4 µg/ml) and 55ppm of treated cultures on 4th day showed the lowest carbohydrate content. The fall in carbohydrate content on 12th and 16th day was more than that of the cultures on 4th and 8th day. On 16th day, the decrease in carbohydrate content was registered by 15% at 15ppm, 26% at 35ppm and 33% at 55ppm respectively.

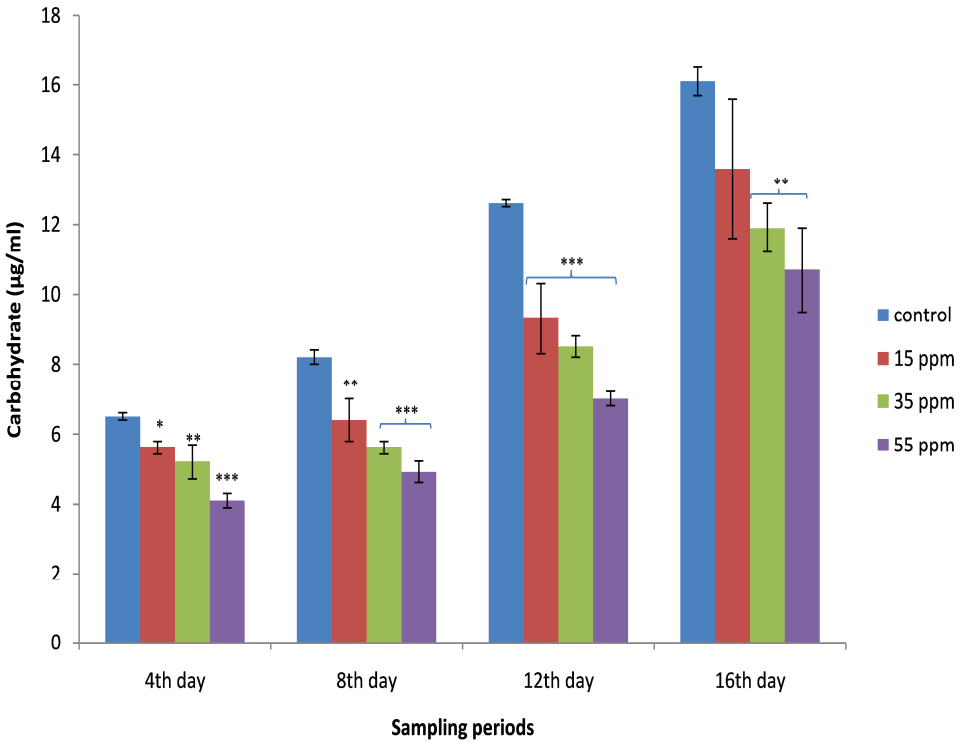


Fig. 29. Effect of different concentrations of Deltamethrin on the carbohydrate content of *W. indica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.8.6 Rate of Nitrogen fixation

The effect of Deltamethrin on the nitrogen fixation by the test organism is depicted in Fig.30. Deltamethrin contributed in time and dose dependent decrease in the nitrogen fixing ability of the test organism till 16th day from the day of inoculation. An insignificant increase of nitrogen fixation was observed at 15ppm on 4th day over the control. At the highest treated concentration i.e. 55ppm, the nitrogen fixing capacity of the test organism decreases by 42% ($p < 0.05$) on 4th day, by 56% ($p < 0.001$) on 8th day, by 75% ($p<0.01$) on 12th day and by 80% ($p<0.001$) on 16th day respectively.

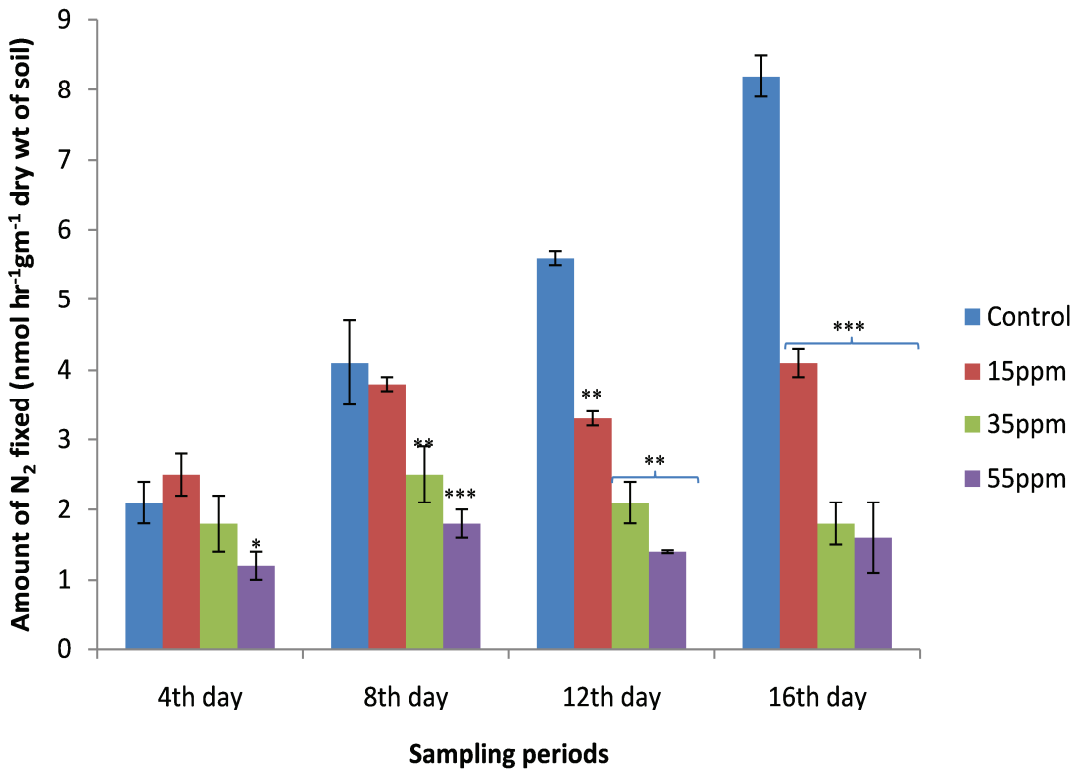


Fig. 30. Effect of different concentrations of Deltamethrin on the nitrogen fixing rate of *W. indica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.9 Effect of Carbofuran on *W. indica*

4.9.1 Biomass

The biomass content of *W. indica* under Carbofuran stress is depicted in Fig.31. The figure clearly shows time and dose dependent decrease of the biomass content with the pesticide application. A steady increase in biomass was observed in the control sets up to 16th day from the day of inoculation. At 62ppm, the biomass content was decreased significantly by 46% on 4thday (p 0.001), 38% on 8thday (p<0.001), 48% on 12th day (p<0.001) and 35% on 16th day (p<0.05) as compared to the control sets. The highest biomass was recorded in the control sets on 16thday (122 ± 2 µg/ml) and lowest was recorded at 62ppm cultures on 4th day (52.2 ± 5 µg/ml).

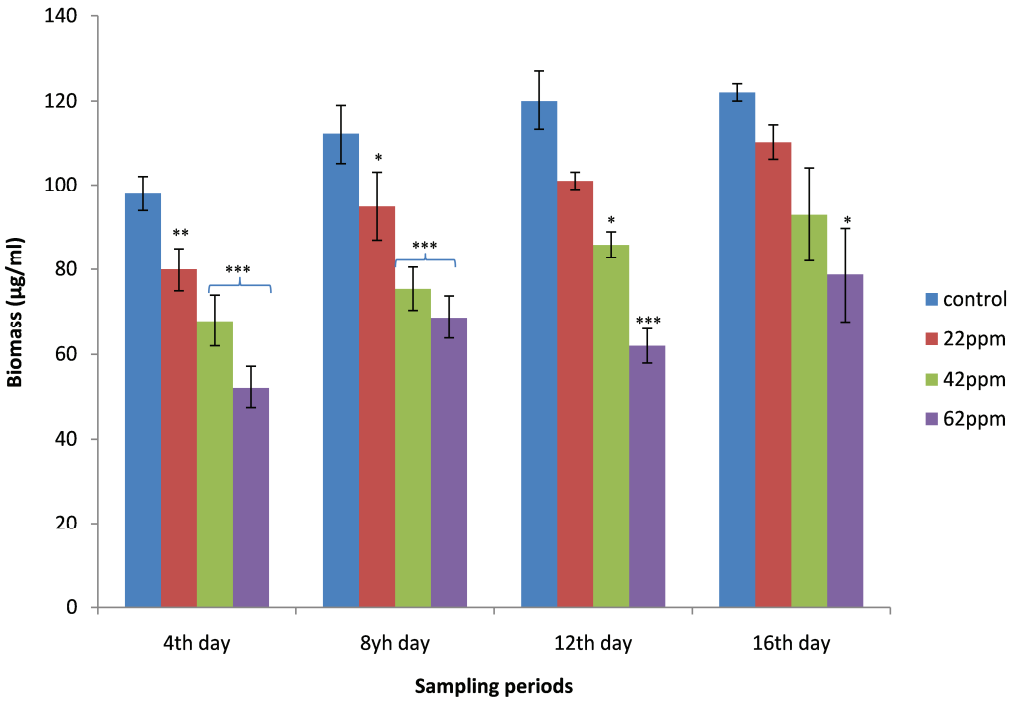


Fig. 31. Effect of different concentrations of Carbofuran on the biomass content of *W. indica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.9.2 Chlorophyll-a

The effect of Carbofuran on chlorophyll-a content of *W. indica* is depicted in Fig.32. The chlorophyll-a content of the test organism was seen to be decreasing in a time and dose dependent manner with the application of Carbofuran. There was a significant reduction in chlorophyll-a content up to 58% ($p<0.001$), 66% ($p<0.001$) and 75% ($p<0.001$) at 22, 42 and 62ppm of carbofuran over the control on 16th day from the day of inoculation. At 62ppm, the chlorophyll-a content was reduced by 26 % ($p<0.01$) on 4thday, by 41% ($p<0.001$) on 8th day and by 57% ($p<0.001$) on 12^{h} day over the control. The overall reduction in the pigment content was found to be significant at all the tested concentrations of carbofuran over the control. On the other hand, a steady increase in the pigment was observed in the control sets up to 16th day from the day of inoculation.

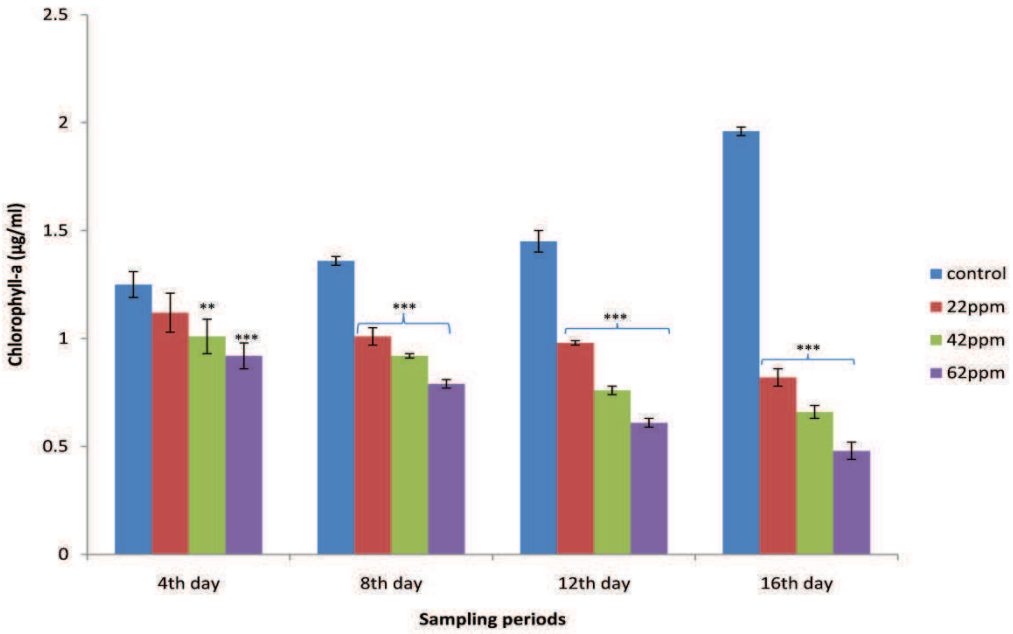


Fig. 32. Effect of different concentrations of Carbofuran on the chlorophyll-a content of *W. indica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.9.3 Carotenoid

The carotenoid content of the test organism with Carbofuran application is depicted in Fig.33. The carotenoid content of the test organism after the application of Carbofuran was seen to be decreasing in time and dose dependent manner till 16th day from the day of inoculation. A gradual increase of carotenoid content was observed in the control sets from 4th to 16th day. A significant fall in carotenoid was observed at all the tested concentrations from 8th to 16th day. The 62ppm cultures showed a reduction of 28% ($p<0.01$) on 4th day, 44% ($p<0.001$) on 8th day, 68% ($p<0.001$) on 12th day and 84% ($p<0.001$) on 16th day respectively over the control. The highest carotenoid was observed in the control sets on 16th day 1.78 ± 0.09 µg/ml) and lowest was observed at 62ppm (0.28 ± 0.08 µg/ml) on the same day.

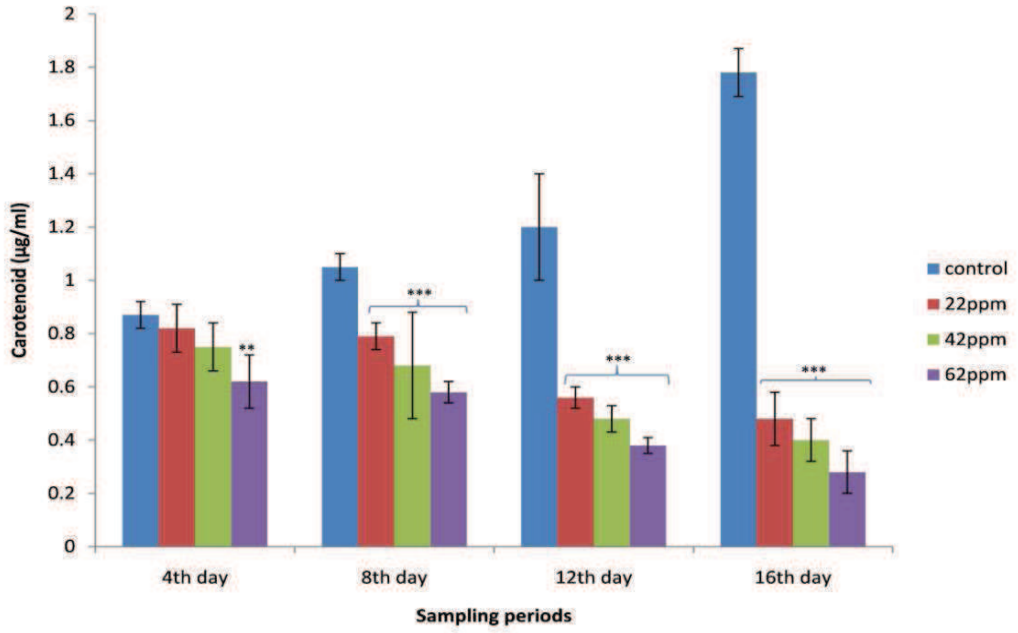


Fig. 33. Effect of different concentrations of Carbofuran on the carotenoid content of *W. indica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.9.4 Protein

The protein content of the test organism was seen to be decreased with the gradual increase of Carbofuran concentration, except at 22ppm where the protein content was increased by 5% ($p<0.05$) on 4^{th} day, by 7% ($p<0.01$) on 8^{th} day and an insignificant increase by 5% on 12^{th} day over the control. At the highest tested concentration i.e. at 62ppm, the reduction in protein content was significant by 22% ($p<0.001$), 11% ($p<0.001$) and 61% ($p<0.001$) on 4^{th}, 8^{th} and 16^{th} day of inoculation. The highest carotenoid was observed in the control sets on 16^{th} day (5.2 ± .16 µg/ml) and lowest was observed at 62 ppm (2 ± .12 µg/ml) on the same day.

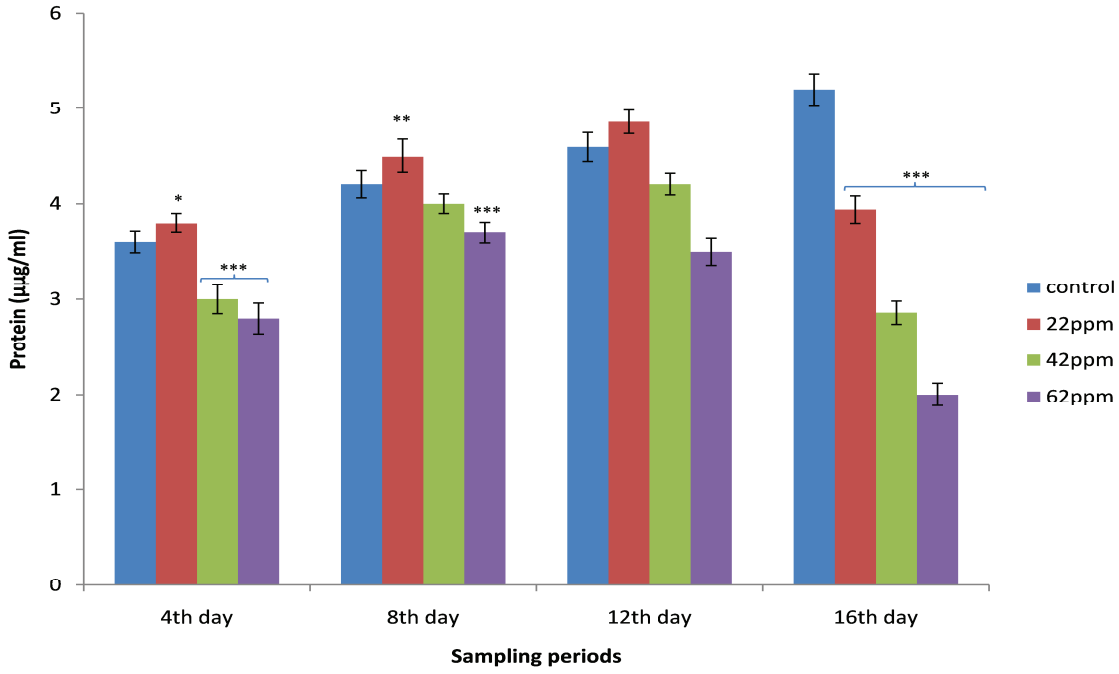


Fig. 34. Effect of different concentrations of Carbofuran on the protein content of *W. indica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.9.5 Carbohydrate

The carbohydrate content of the test organism under Carbofuran stress is depicted in Fig. 35. From the figure, the carbohydrate content was found to be slightly higher than that of the control at 22ppm on the 4thday and on 8th day from the day of inoculation but the increase was not significant as observed from the statistical analysis. There was a gradual reduction in carbohydrate content from lower dose to higher dose of pesticide on the 12th and 16th day. The highest carbohydrate content was found in the untreated set that is in the control, on the 16th day (16.1 ± .9 µg/ml) and the highest inhibitory concentration was found to be 62ppm with lowest reduction of carbohydrate on the 16th day (3.9 ± 0.1 µg/ml) ($p<0.001$).

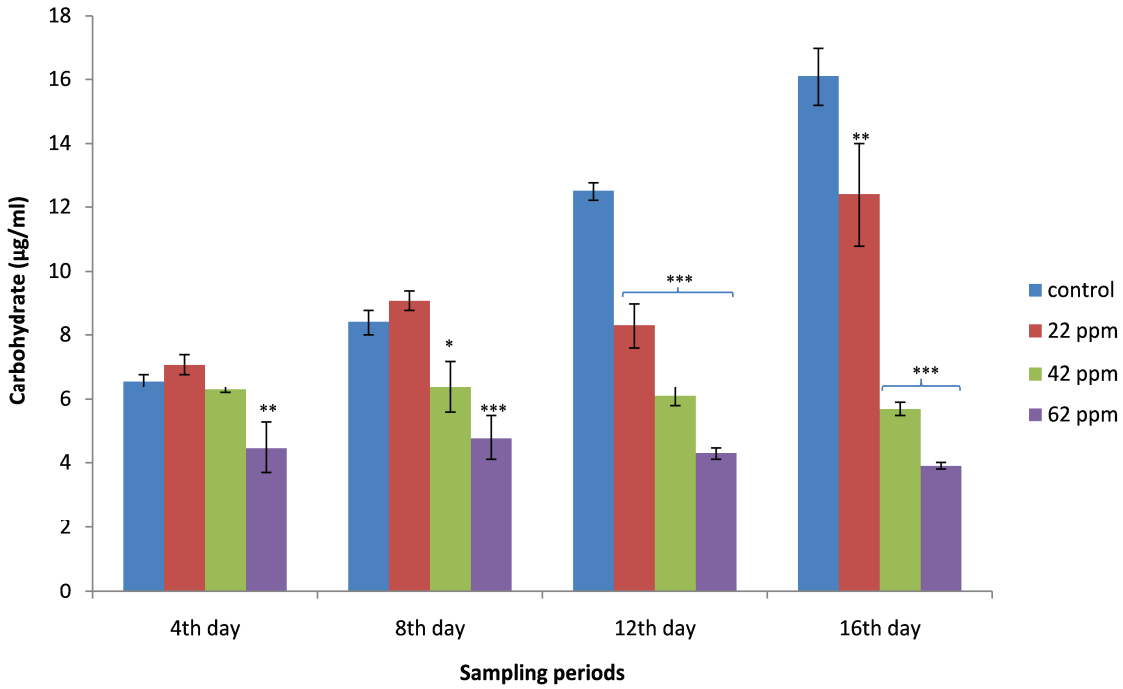


Fig. 35. Effect of different concentrations of Carbofuran on the carbohydrate content of *W. indica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.9.6 Rate of Nitrogen fixation

The rate of nitrogen fixation by the test organism with Carbofuran application is depicted in Fig.36. The nitrogen fixation by the test organism after the application of Carbofuran was seen to be decreasing in time and dose dependent manner till 16th day from the day of inoculation. A gradual increase of nitrogen fixation was observed in the control sets from 4th to 16th day. A significant fall in nitrogen fixation was observed in all the tested concentrations from 8th to 16th day. The 62ppm cultures showed a reduction of 57% ($p<0.01$) on 4th day, 68% ($p<0.001$) on 8th day, 82% ($p<0.001$) on 12th day and 85% ($p<0.001$) on 16th day respectively over the control.

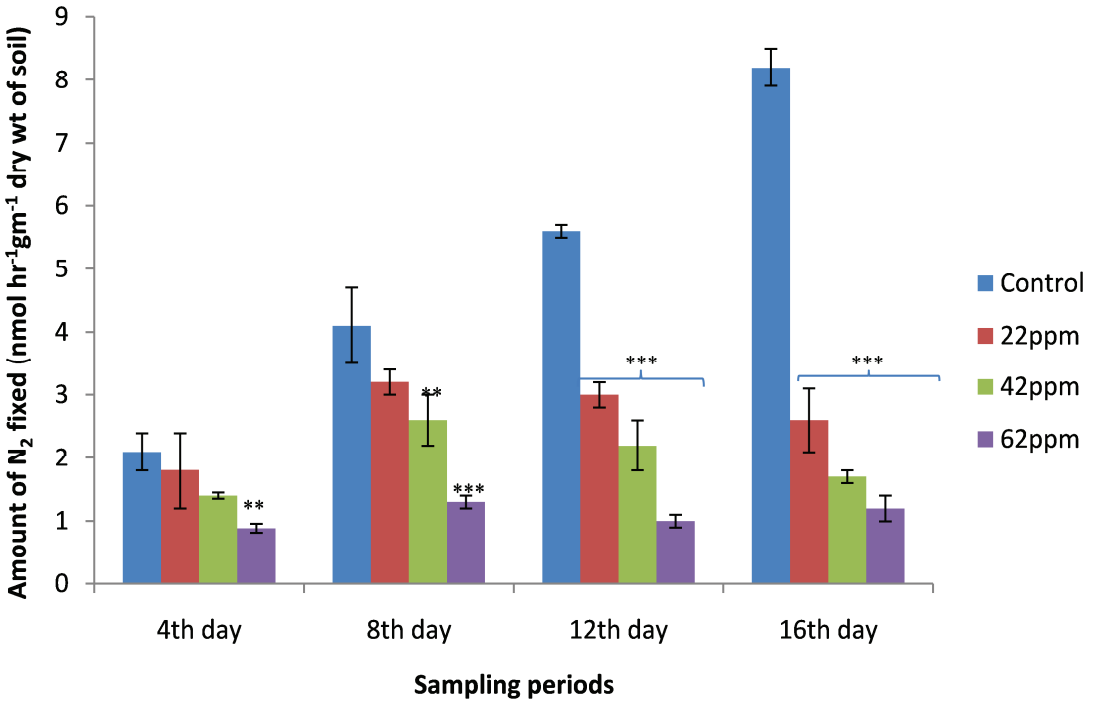


Fig. 36. Effect of different concentrations of Carbofuran on the nitrogen fixing rate of *W. indica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

EFFECT OF MALTHION, DELTAMETHRIN AND CARBOFURAN ON *W. ramosa*

4.10 Effect of Malathion on *W. ramosa*

4.10.1 Biomass

The biomass content in *W. ramosa* was decreased in a time and dose dependent manner from 8th to 16th day with a slight exception on the 4th day where the biomass content was found to be increasing with increase in Malathion concentration with time (Fig. 37). The 56 and 86ppm of concentrations showed a significant increase of 28% ($p<0.01$) and 34% ($p<0.01$) on 4th day as compared to that of the control sets. At the highest tested concentration i.e. 86ppm, the biomass was found to be decreased by 47% ($p<0.001$) on 8th day, by 50% ($p<0.001$) on 12th day and by 49% ($p<0.001$) on 16th day respectively over the control.

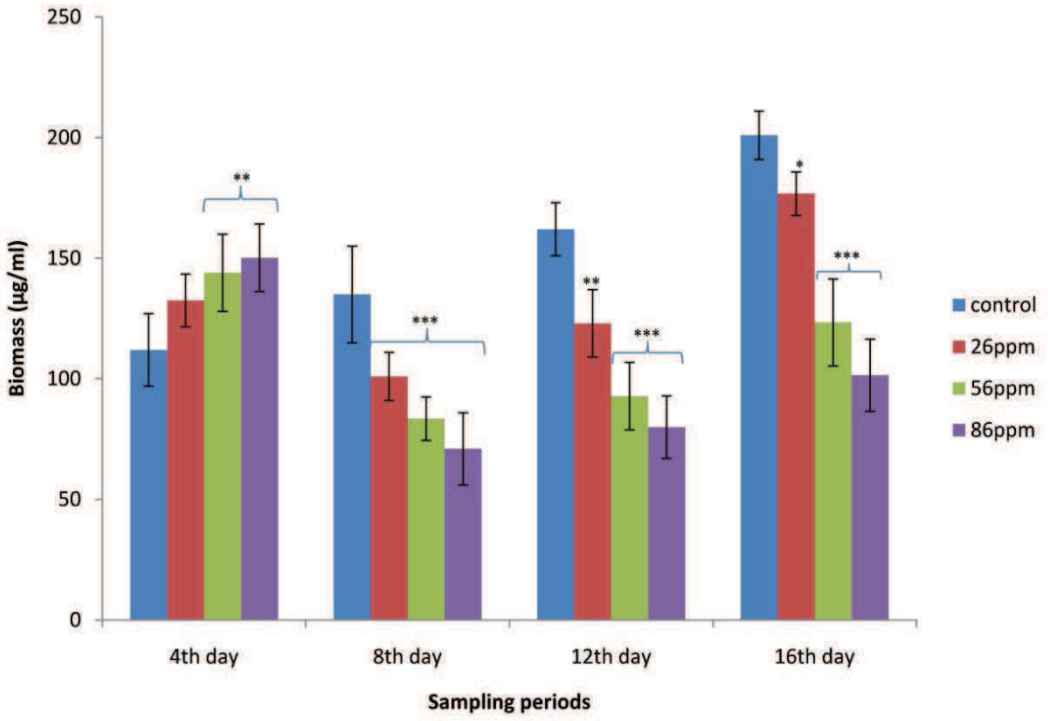


Fig. 37. Effect of different concentrations of Malathion on the biomass content of *W. ramosa* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.10.2 Chlorophyll-a

The growth of the test organism in terms of Chlorophyll-a under Malathion stress is depicted in Fig. 38. From the figure, chlorophyll-a production was found to be higher than that of the control sets at 26 ppm on the 8th day ($p<0.05$), 12th day ($p<0.001$) and 16th day ($p<0.001$) respectively from the day of inoculation. There was a gradual reduction in chlorophyll-content from lower dose to higher dose of pesticide on the 4th day. The highest chlorophyll-a content was found when treated with 26ppm of Malathion on the 16th day (2.8 ± .05 µg/ml) ($p<0.001$) and the highest inhibition was found to be 86ppm with lowest reduction of chlorophyll-a on the 16th day (.48 ± .09 µg/ml) ($p<0.001$).

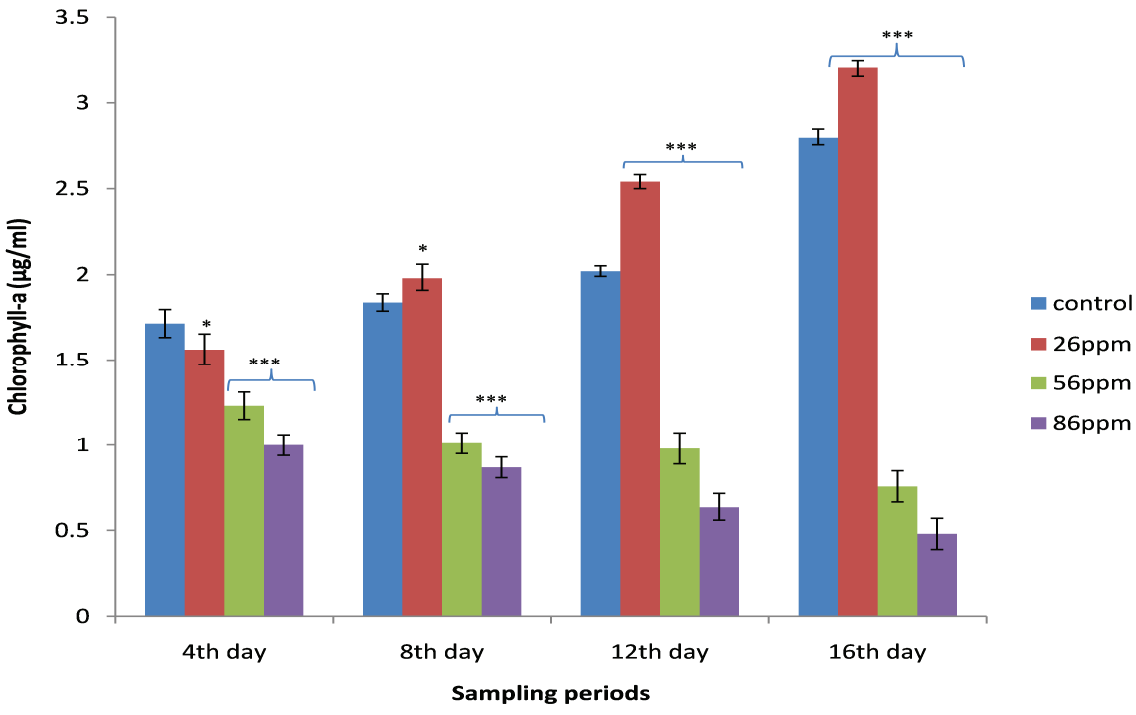


Fig. 38. Effect of different concentrations of Malathion on the chlorophyll-a content of *W. ramosa* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.10.3 Carotenoid

Results obtained after exposing the test organism to different concentrations of Malathion is presented in Fig.39. A remarkable degree of Carotenoid inhibition was observed in *W. ramosa* after exposure to Malathion treatment for a period of 16 days with exception on 12th and 16th day at 26ppm where the carotenoid content was increased by 10% and 77% over the control. Carotenoid content of the test species was observed to be affected by the pesticide in a time dose dependent manner. In the control sets, an exponential growth was observed up to the 16th day from the day of inoculation throughout the experiment period. The highest carotenoid content was observed in the 26ppm treated cultures on the 16th day (1.42 ± .02 µg/ml) and lowest reduction was observed at 86ppm on the same day (0.32 ± 0.1 µg/ml).

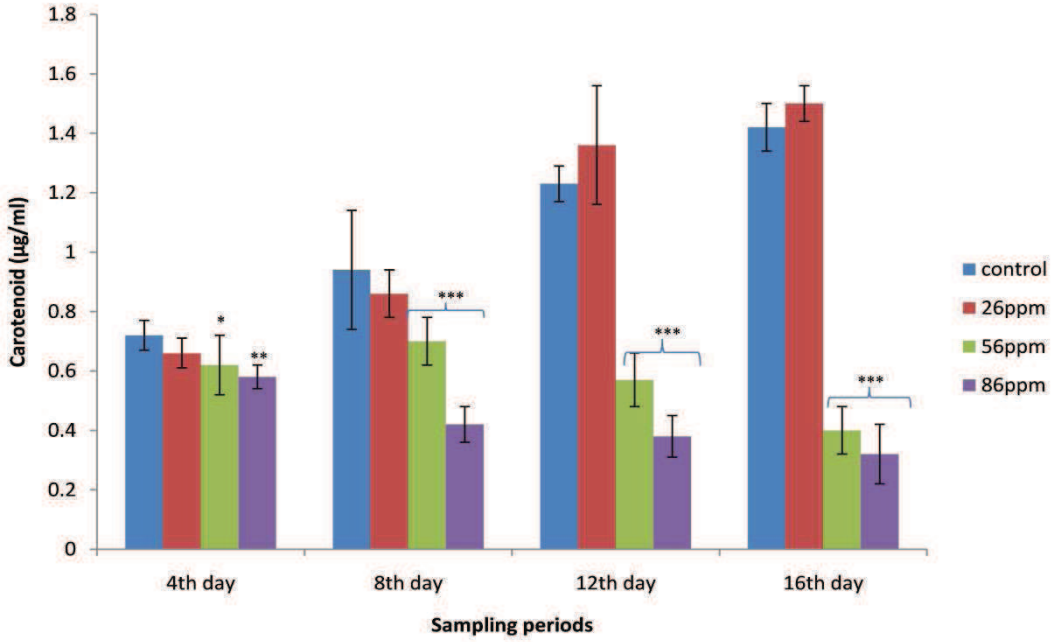


Fig.39. Effect of different concentrations of Malathion on the carotenoid content of *W. ramosa* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.10.4 Protein

The effect on the protein content of the test species treated with Malathion is shown in Fig.40. There was a gradual reduction in the protein content with increase in pesticide concentration in a time and dose dependent manner. The highest protein content was found in the control sets (11.5 ± .14) µg/ ml and lowest at 86ppm (4 ± .19) µg/ml on the 16th day ($p<0.001$). Till 16th day from the day of inoculation, the reduction in protein content was found to be significant at 26, 56 and 86ppm over the control ($p<0.001$). At the highest treated concentration i.e. 86ppm, the protein content was decreased by 28% ($p<0.001$) on 4th day, 41% ($p<0.001$) on 8th day, 57% ($p<0.001$) on 12th day and 65% ($p<0.001$) on 16th day respectively over the control sets.

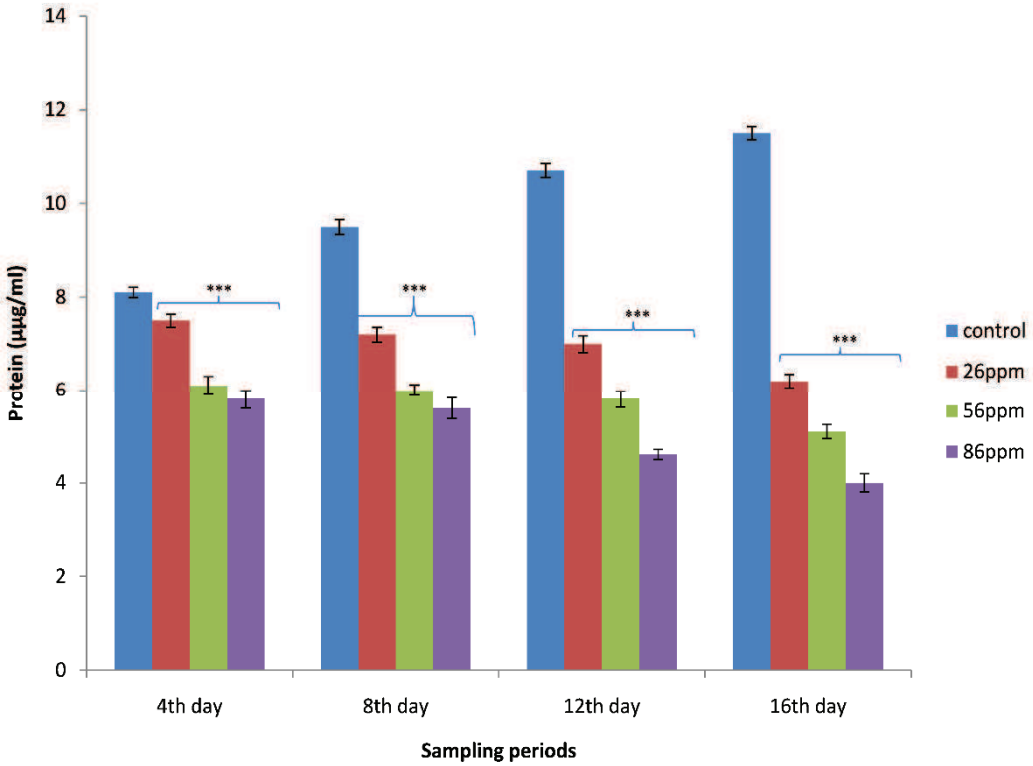


Fig. 40. Effect of different concentrations of Malathion on the protein content of *W. ramosa* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.10.5 Carbohydrate

Results of carbohydrate content obtained after exposing the test organism to varying Malathion concentrations is depicted in the Fig.41. The carbohydrate content was found to be gradually decreased from lower to higher pesticide concentration with the increase in time period of the treatment. A progressive and significant reduction in carbohydrate was observed at 86ppm by 26% ($p<0.001$) on 4th day, by 27% ($p<0.01$) on 8th day, by 34% ($p<0.001$) on 12th day and by 56% ($p<0.01$) on 16th day respectively. The highest carbohydrate content was observed in the untreated i.e., control sets (18.8 ± 0.7) µg/ml on 16th day and lowest was observed in the 86ppm treated cultures on 4th day (6.5 ± .5) µg/ml.

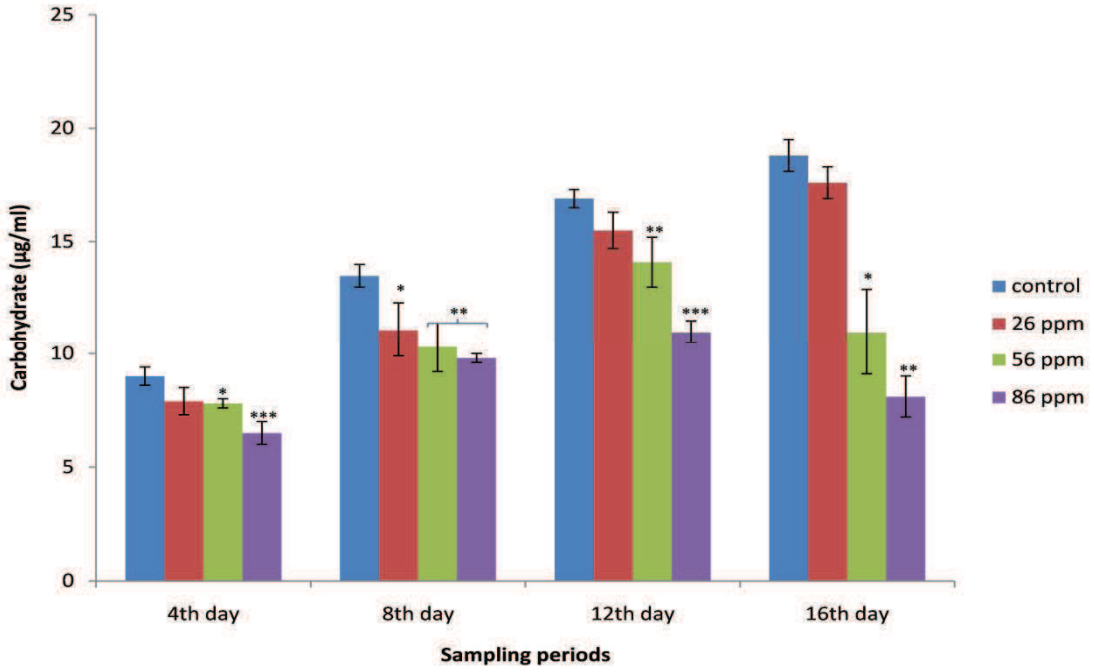


Fig. 41. Effect of different concentrations of Malathion on the carbohydrate content of *W. ramosa* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.10.6 Rate of Nitrogen fixation

Results obtained after exposing the test organism to different concentrations of Malathion is presented in Fig.42. A remarkable degree of reduction in the rate of nitrogen fixation was observed in *W. ramosa* after exposure to Malathion treatment for a period of 16 days with exception on 4th, 8th and 12th day at 26ppm where the nitrogen fixation was increased by 25%, 10% and 38% over the control sets. Rate of nitrogen fixation by the test species was observed to be affected by the pesticide in a time dose dependent manner. In the control sets, an exponential growth was observed up to the 16th day from the day of inoculation throughout the experimental period.

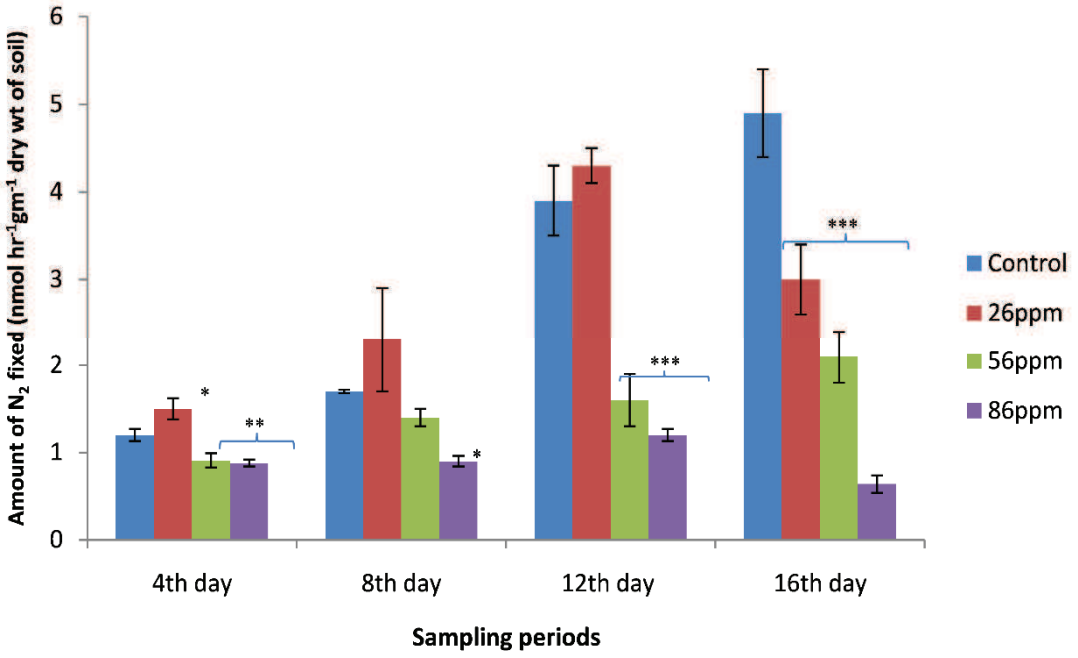


Fig. 42. Effect of different concentrations of Malathion on the nitrogen fixing rate of *W. ramosa* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.11 Effect of Deltamethrin on *W. ramosa*

4.11.1 Biomass

Biomass content of the test organism treated with Deltamethrin is depicted in Fig.43. The overall biomass content of the test organism was observed to be decreased in a time and dose dependent manner up to 16th day from the date of inoculation, 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 21%, by 34% ($p<0.01$) and 47% ($p<0.01$) when treated with 20ppm, 40ppm and 60ppm of deltamethrin solutions respectively on 8th day. On 4th day the decrease was not significant over the control. The highest biomass content was observed in the control sets (200 ± 18) µg/ml on 16th day and lowest was observed at 60ppm (70.5 ± 12) µg/ml ($p<0.001$) on the 8th day.

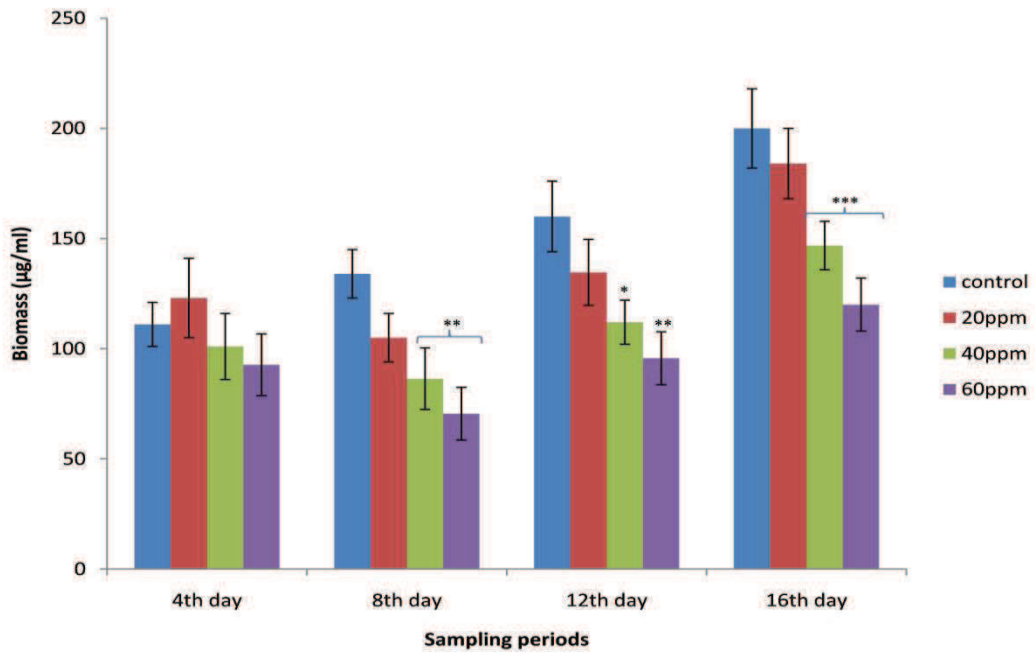


Fig. 43. Effect of different concentrations of Deltamethrin on the biomass content of *W. ramosa* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.11.2 Chlorophyll-a

The chlorophyll-a content of the test organism treated with Deltamethrin is depicted in Fig.44. The chlorophyll-a content was found to be significantly decreased in time and dose dependent manner by the application of the pesticides. There was a significant reduction in chlorophyll-a content up to 43% ($p<0.001$) on 4^{th} day, 58% ($p<0.001$) on 8^{th} day, 68% ($p<0.001$) on 12^{th} day and 79% ($p<0.001$) respectively on 16^{th} day when treated with 60ppm of deltamethrin over the control kept for 16^{th} day. The overall reduction of chlorophyll-a was found to be significant at all the tested concentrations (20ppm, 40ppm, 60ppm) over the control up to 16^{th} day from the day of inoculation. The highest chlorophyll-a content was found in the control sets on 16^{th} day (2.8 ± .05) µg/ml and lowest chlorophyll-a content was found at 60ppm on the same day (.58 ± .02) µg/ml.

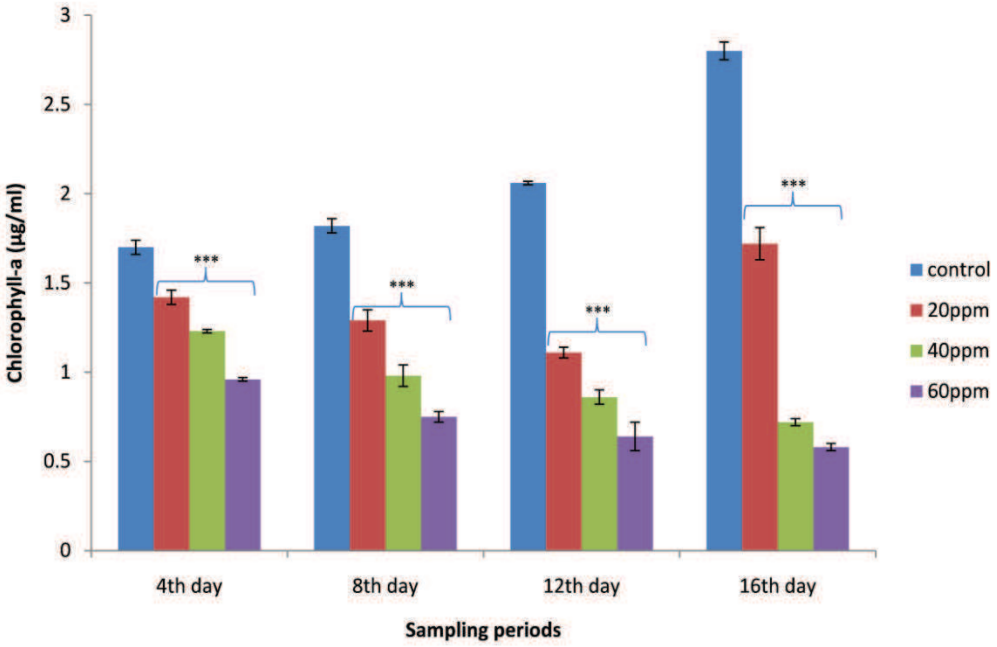


Fig.44. Effect of different concentrations of Deltamethrin on the chlorophyll-a content of *W. ramosa* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.11.3 Carotenoid

Results obtained after exposing the test organism to different concentrations of Deltamethrin is presented in Fig.45. A progressive and significant carotenoid inhibition was observed in *W. ramosa* after exposure to Deltamethrin treatment up to 16 days from the day of inoculation. Carotenoid content of the test species was observed to be affected by the pesticide in time and dose dependent manner. In the control sets, an exponential growth was observed up to the 16th day from the day of inoculation throughout the experiment period. The highest carotenoid content was observed in the untreated cultures i.e. on the control sets on the 16th day (1.44 ± .09) µg/ml and lowest reduction was observed at 60ppm on the same day (0.33 ± 0.6) µg/ml ($p<0.001$). At 60ppm, the carotenoid was reduced by 41% ($p<0.001$) on 4th day, by 26% ($p<0.001$) on 8th day, by 57% ($p<0.001$) on 12th day, by 77% ($p<0.001$) on 16th day respectively.

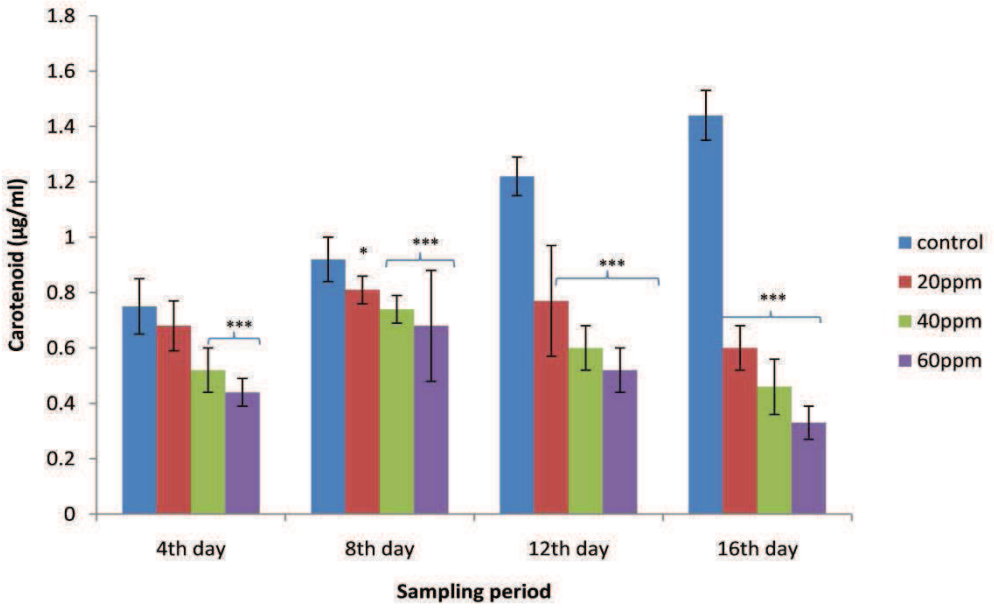


Fig.45. Effect of different concentrations of Deltamethrin on the carotenoid content of *W. ramosa* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.11.4 Protein

The effect on the protein content of the test species with Deltamethrin treatment is shown in Fig.46. There was a gradual reduction in the protein content with concomitant increase of pesticide concentration in a time and dose dependent manner with an exception at 20ppm on 4th and 8th day where the protein content significantly was increased by 18% and 5.3% over the control sets. The highest protein content (11.3 ± .19 µg/ ml) was found in the control set and lowest at 60ppm (4.8 ± .12) µg/ml on the 16th day. At 60ppm, the protein content was reduced by 12% ($p<0.001$) on 4th day, by 34% ($p<0.001$) on 8th day, by 48% ($p<0.001$) on 12th day and by 57% ($p<0.001$) on 16th day respectively.

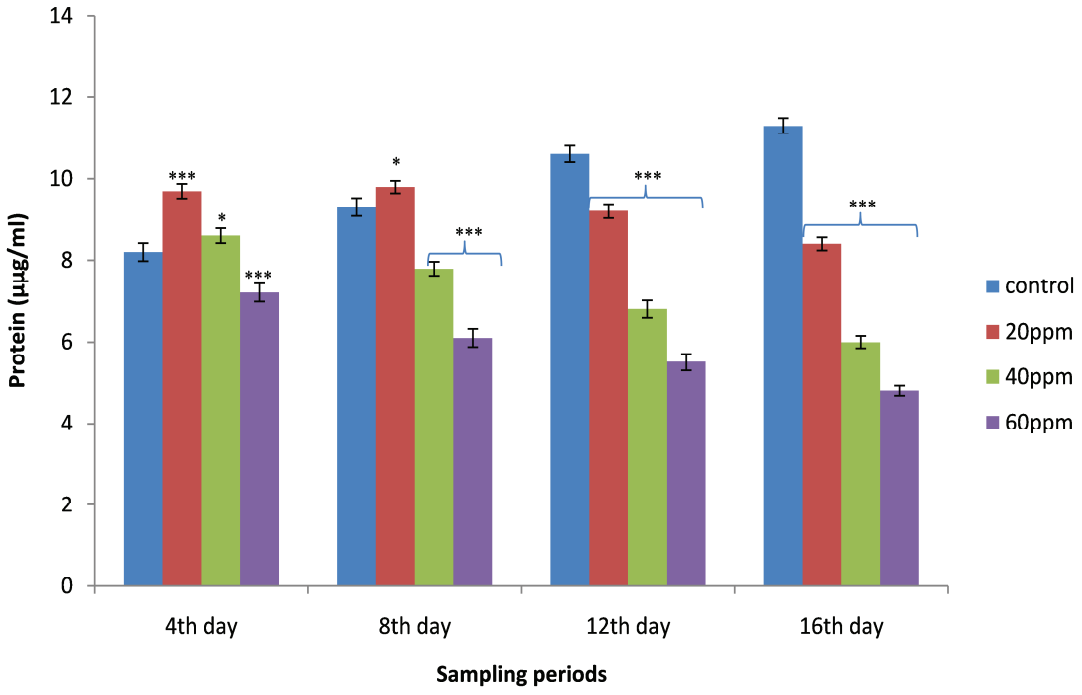


Fig.46. Effect of different concentrations of Deltamethrin on the protein content of *W. ramosa* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.11.5 Carbohydrate

The carbohydrate content under Deltamethrin stress is depicted in Fig.47. The result shows a gradual increase in the carbohydrate content with increase in pesticide concentrations and with the progress of time. However, the increase was observed to be lower than the control sets in all the experimental days. The highest carbohydrate content was observed in the control sets on 16th day (19.5 ± 0.6) µg/ml and lowest was observed 20ppm treated cultures on 4th day (7.5 ± 0.5) µg/ml. At 60ppm the carbohydrate content was reduced by 5.4%, 5.1%, 15% and 8.7% on 4th , 8th 12th and 16th day respectively.

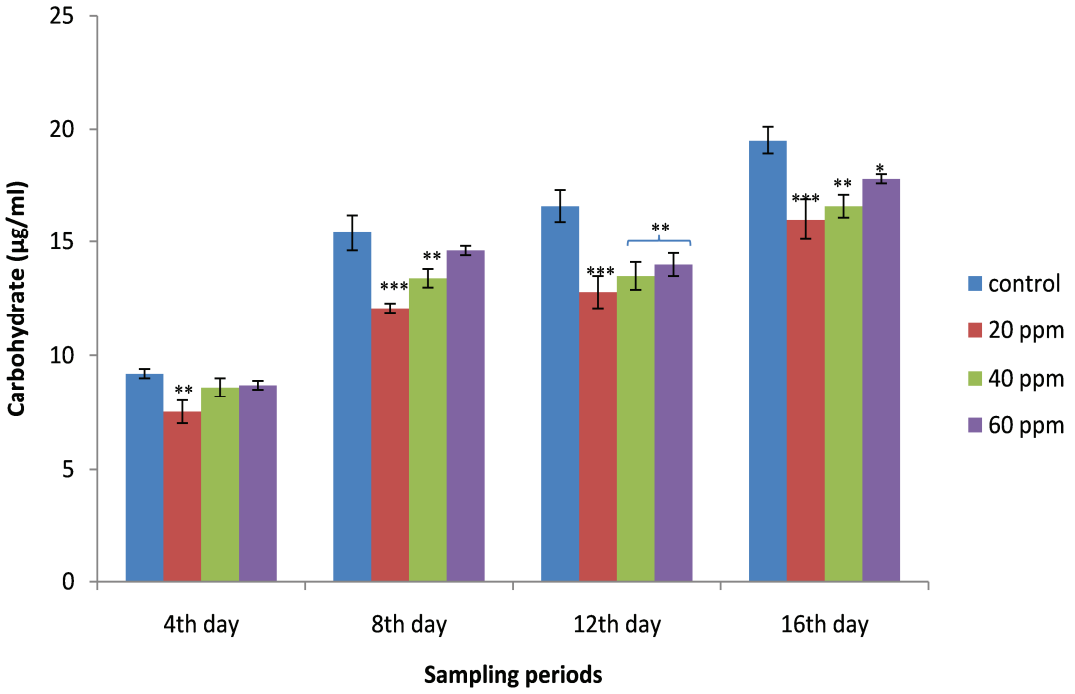


Fig. 47. Effect of different concentrations of Deltamethrin on the carbohydrate content of *W. ramosa* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.11.6 Rate of Nitrogen fixation

Results obtained after exposing the test organism to different concentrations of Deltamethrin is presented in Fig.48. A progressive and significant reduction in the rate of nitrogen fixation was observed in *W. ramosa* after exposure to Deltamethrin treatment up to 16 days from the day of inoculation. Nitrogen fixation of the test species was observed to be affected by the pesticide in time and dose dependent manner. In the control sets, an exponential growth was observed up to the 16th day from the day of inoculation throughout the experiment period. At 60ppm, the nitrogen fixation was reduced by 45% ($p<0.001$) on 4th day, by 47% ($p<0.001$) on 8th day, by 58% ($p<0.001$) on 12th day and by 73% ($p<0.001$) on 16th day respectively.

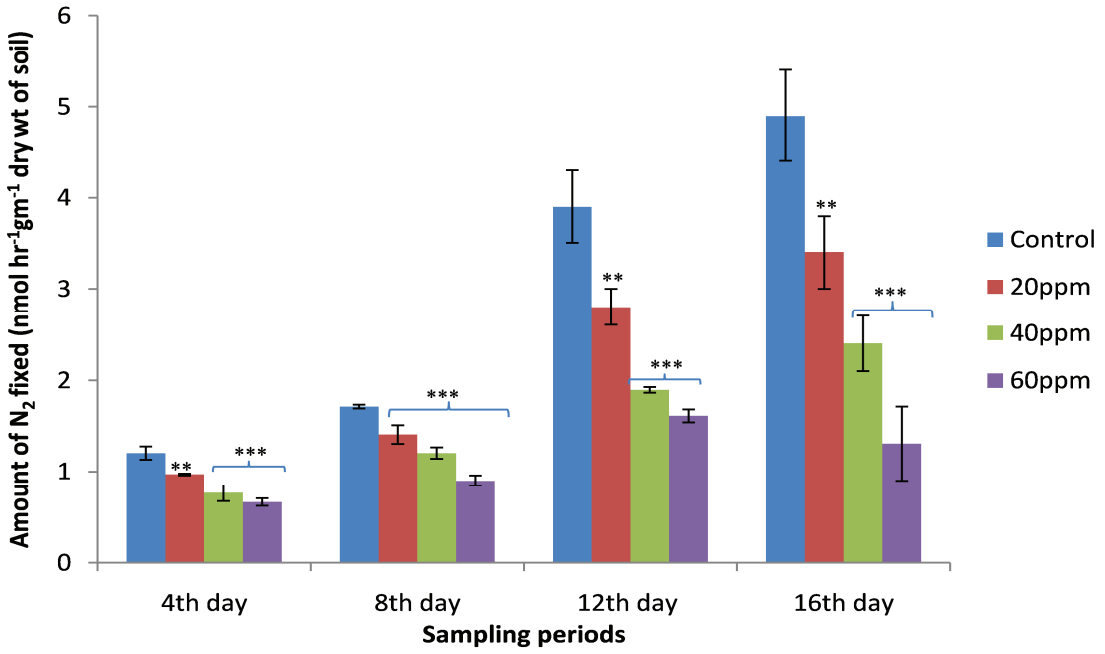


Fig. 48. Effect of different concentrations of Deltamethrin on the nitrogen fixing rate of *W. ramosa* at different time intervals. The values are presented as mean ± SD of three replicates. Asteris ks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.12 Effect of Carbofuran on *W. ramosa*

4.12.1 Biomass

The effect of Carbofuran on the biomass content of *W. ramosa* is depicted in Fig.49. The biomass content was significantly decreased in a time and dose dependent manner up to 16th day from the day of inoculation. The biomass contents were observed to be gradually increased in the control sets on 4th, 8th, 12th and 16th day respectively. At the highest tested concentration i.e. 50ppm, the biomass was found to be decreased by 38% ($p<0.01$) on 4th day, by 46% ($p<0.01$) on 8th day, by 45% ($p<0.01$) on 12th day and by 52% ($p<0.001$) on 16th day respectively over the control.

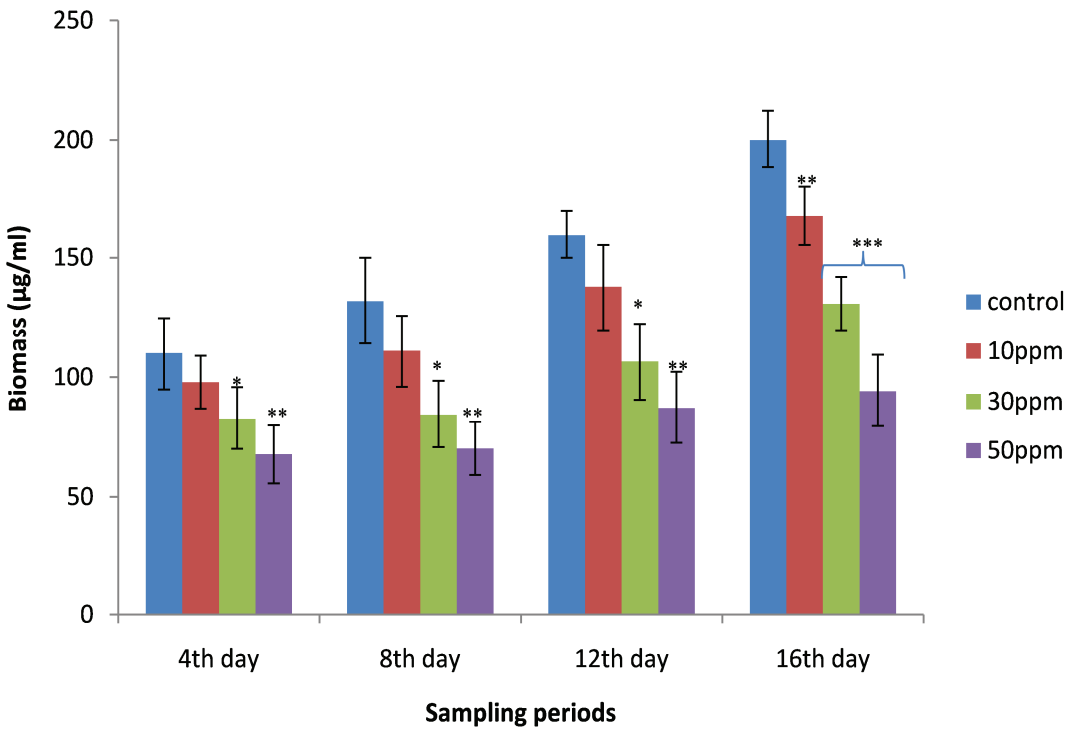


Fig. 49. Effect of different concentrations of Carbofuran on the biomass content of *W. ramosa* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.12.2 Chlorophyll-a

The chlorophyll-a content of the test organism after Carbofuran application is summarized in the Fig.50. The chlorophyll-a content of the test organism was seen to be decreased in time and dose dependent manner with the application of Carbofuran except at 10ppm where the value was found to be increased by 34% on 4th and by 48% on 8th day over the control. At the highest tested concentration i.e. 50ppm, the chlorophyll-a content was significantly reduced by 34% ($p<0.001$) on 4th day, 48% ($p<0.001$) on 8th day, 58% ($p<0.001$) on 12th day and 75% ($p<0.001$) on 16th day respectively.

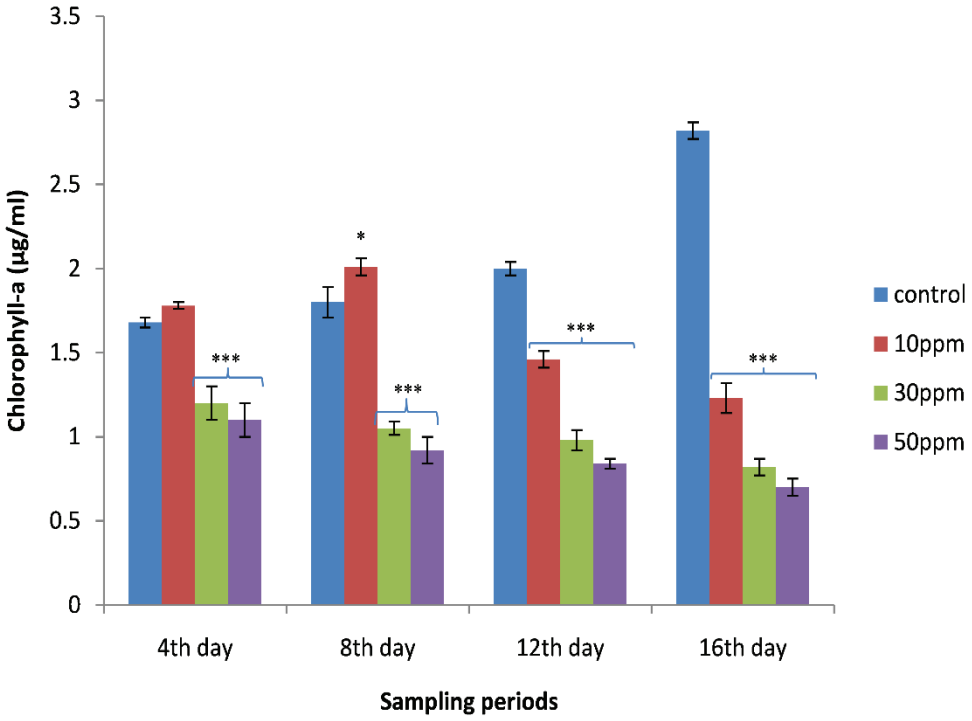


Fig. 50. Effect of different concentrations of Carbofuran on the chlorophyll-a content of *W. ramosa* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.12.3 Carotenoid

Results obtained after exposing the test organism to different concentrations of Carbofuran is presented in Fig.51. A remarkable degree of Carotenoid inhibition was observed in *W. ramosa* after exposure to Carbofuran treatment for a period of 16 days. Carotenoid content of the test species was observed to be affected by the pesticide in a time dose dependent manner. In the control sets, an exponential growth was observed up to the 16th day from the day of inoculation throughout the experiment period. The highest carotenoid content was observed in the control during the 16th day (1.4 ± 0.9) µg/ml. and lowest reduction was observed at 50ppm (0.55 ±0 .09) µg/ml ($p<0.001$) on the same day.

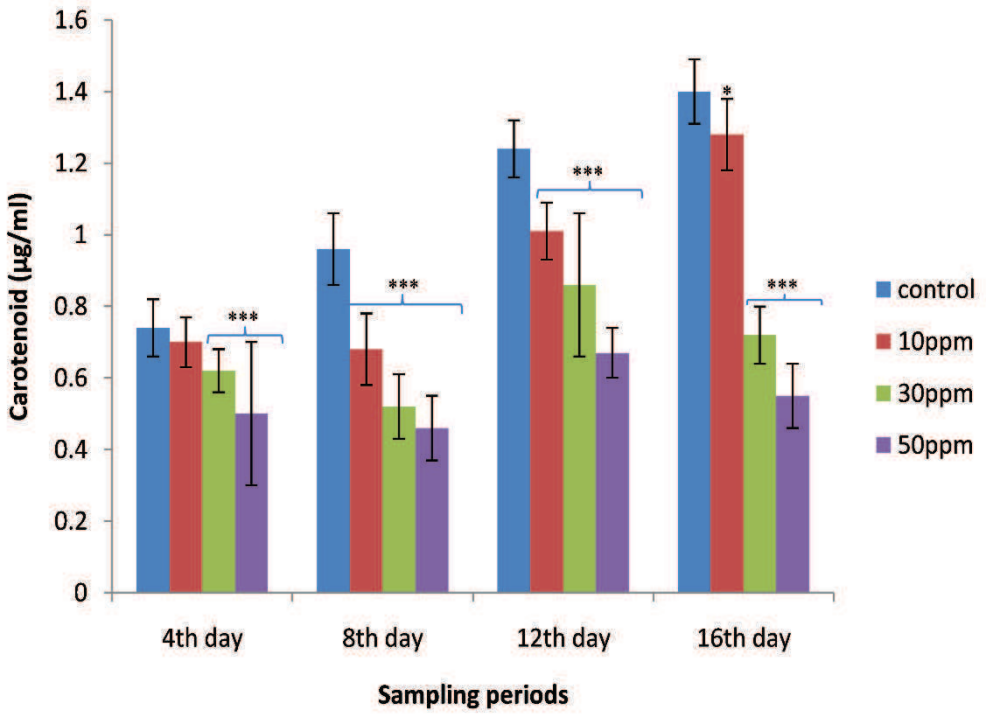


Fig. 51. Effect of different concentrations of Carbofuran on the carotenoid content of *W. ramosa* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.12.4 Protein

The effect on the protein content of the test species with Carbofuran treatment is shown in Fig.52. There was a significant gradual reduction in the protein content with the increase in pesticide concentration in a time and dose dependent manner on 4th and 8th day. The highest protein content was found in the 30ppm experimental set up (12.7 ± 0.24) µg/ ml on 16th day and lowest at 50ppm (5 ± 0.16) µg/ml on the 8th day ($p<0.001$). On 12th and 16th day, the protein content was found to be significantly low at the highest tested concentration i.e. 50ppm by 28.9% and 46% respectively.

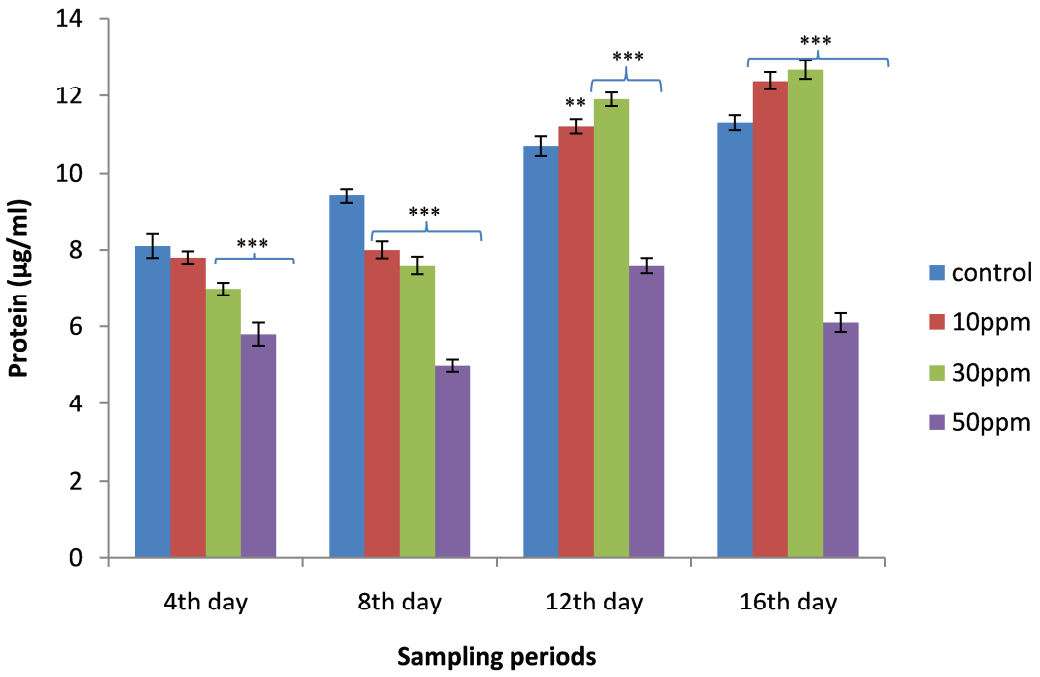


Fig. 52. Effect of different concentrations of Carbofuran on the protein content of *W. ramosa* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.12.5 Carbohydrate

The effect of different concentrations of Carbofuran on the carbohydrate content of *W. ramosa* at different time intervals is shown in the Fig.53. It was observed that when treated with Carbofuran, carbohydrate content gradually increases in a time and dose dependent manner. At 50ppm the carbohydrate content was increased by 56% ($p<0.001$), 38% ($p<0.001$), 52% ($p<0.001$), 52.1%($p<0.001$) on 4^{th}, 8^{th}, 12^{th} and 16^{th} day with Carbofuran application respectively. The highest carbohydrate content was found at 50ppm on 16^{th} day (28.9 ± 1) µg/ml and the lowest was recorded in the control sets on 4^{th} day (9 ± 0.3) µg/ml.

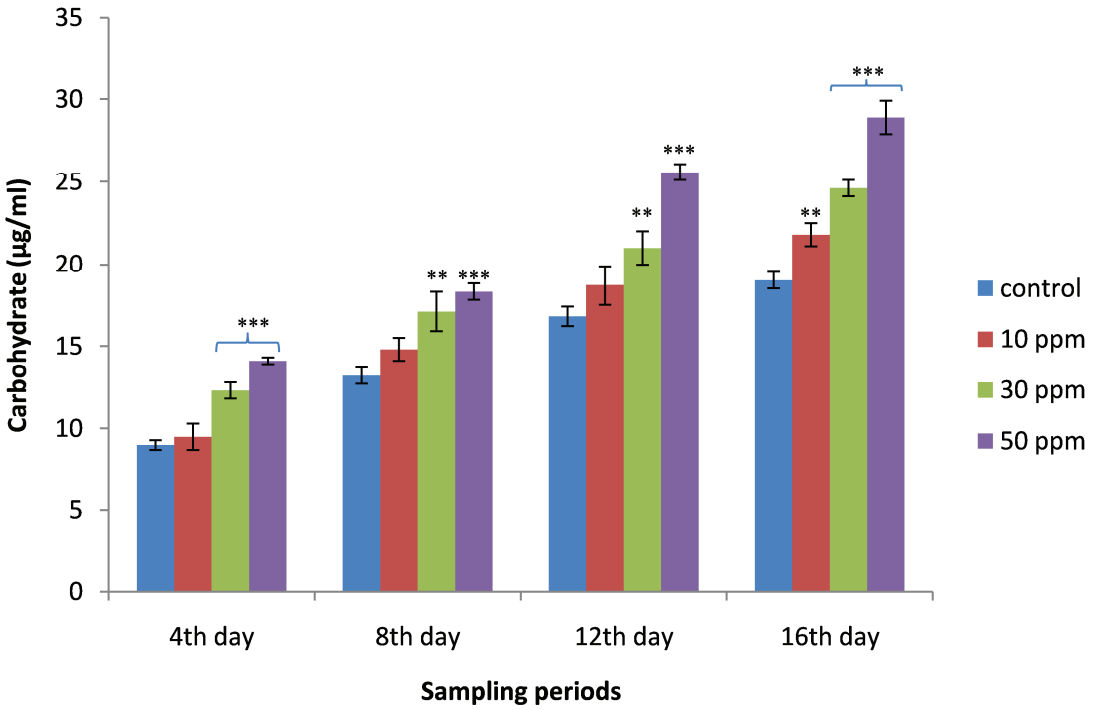


Fig. 53. Effect of different concentrations of Carbofuran on the carbohydrate content of *W. ramosa* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

4.12.6 Rate of Nitrogen fixation

The rate of nitrogen fixation by the test organism after Carbofuran application is summarized in the Fig.54. The nitrogen fixation capability of the test organism was seen to be decreased in time and dose dependent manner with the application of Carbofuran except on 4th day, where the value was found to be increased by 25% at 10ppm, by 83% at 30ppm and by 33% at 50ppm respectively over the control. At the highest tested concentration i.e. at 50ppm, the nitrogen fixed by the test organism was significantly reduced by 66% ($p<0.001$) on 8th day, 76% ($p<0.001$) on 12th day and 81% ($p<0.001$) on 16th day respectively.

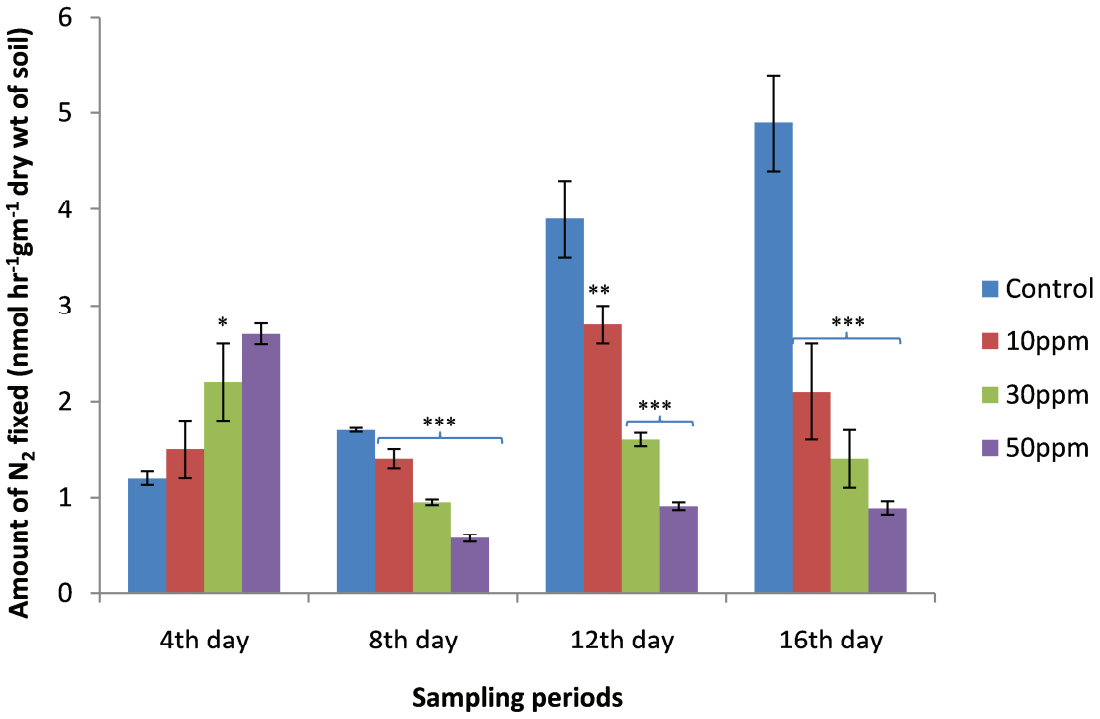


Fig. 54. Effect of different concentrations of Carbofuran on the nitrogen fixing rate of *W. ramosa* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.