CHAPTER V

DISCUSSION

The present study was undertaken to understand the effect of some locally used pesticides of Assam on the growth and biochemical characters of different *Westiellopsis* species isolated from rice field soils of Assam under controlled conditions at Plant Ecology Laboratory of Department of Botany of Gauhati University.

Westiellopsis is a nitrogen fixing cyanobacterial taxa mostly found to be inhabiting the soil ecosystems of tropical countries (Tiwari *et al*., 2005; Sethi *et al*., 2012). *Westiellopsis*, belonging to the family hapalosiphonaceae is now recognized as a distinct genus (www.algaebase.org) with 6 taxonomically accepted species and 2 infraspecific species. *Westiellopsis prolifica* is the type species of the genus with worldwide distribution (Gugger and Hoffman, 2004; Tiwari *et al*., 2005; Ibrahim *et al*., 2013; Yashmin *et al*., 2015) and are highly abundant in tropical rice fields (Bhardwaj and Baruah, 2013). It was first reported from a soil sample of agri horticultural society of Madras (Janet, 2011) and later, has been reported from different agricultural as well as rice fields from different parts of Indian subcontinent (Venkatarman, 1981).

Being members of heterocyst us N_2 fixing cyanobacterial taxa, all species of *Westiellopsis* have been playing a substantial role in maintaining the nitrogen and carbon status in the soil (Roger, 1995; Fernandez-Valiente*et al*., 2000; Irisarri*et al.,* 2001, Dutta and Baruah, 2020). For which, the members of the taxa are being considered as one of the ideal and potential natural biofertilizers especially in rice filed soils of Indian subcontinent (Kumar *et al*., 2010a; Debnath*et al*., 2012; Kumar *et al*., 2012) including Assam (Bhardwaj and Baruah, 2013; Dutta and Baruah, 2020).

The survey conducted during the present study in different organically cultivated rice fields of Assam resulted into isolation and identification of three species of *Westiellopsis* following the standard literatures and monographs of Desikachary (1959), Komarek and Anagnostidis (1989), Komarek (2013), Komarek *et al*. (2014) and authenticated following Algaebase (www.algaebase.org). The three species were *W. prolifica, W. indica and W. ramosa.*

The *W. prolifica* has a torulose shaped filaments with T-shaped branching. The cells in main filaments of this species are barrel shaped with heterocyst of oblong to cylindrical in shape. The *W. indica* has entangled filaments, sometimes fasciculate. The cells are barrel shaped to almost spherical to elongated, heterocyst are of varied shaped. *W. ramosa* has a monoseriate filaments which are constricted at the cross walls with having T- shaped branching with cylindrical to barrel shaped heterocyst in between.

The biochemical contents of cyanobacterial spp. are found to be varying according to time of growth and ecological conditions of the particular place where they used to grow (Vargas *et al*., 1998; Subhashini *et al*., 2003; Maslova *et al*., 2004; Rosales *et al*., 2005). The present study revealed that the chlorophyll-a content of *W. prolifica* in normal laboratory environment ranged around 1.2 (±0.12) µg/ml to 1.8 (±0.01) µg/ml, that of *W. indica* was 1.2 (±0.04) µg/ml to 2.2 (± 0.05) µg/ml and *W. ramosa* was 1.6 (± 0.03) µg/ml to 2.8 (± 0.05) µg/ml after 16 days of incubation period from the date of inoculation in nitrogen free BG-11 growth medium. In contrast, Pattnaik and Samad (2018) recorded around 3 µg/ml to 25 µg/ml of chlorophyll-a in *Westiellopsis sp.* isolated from a sub aerial habitat of Bhubaneswar after 40 days of incubation period with the same growth medium.

The protein content of *W. prolifica* was recorded between 2.1(± 0.11) μg/ml to 5.3 (± 0.18) μg/ml and that of *W. indica* in the range of 3.5 (± 0.1) μg/ml to 5.3 (± 0.11) μg/ml and *W. ramosa* between 8.1 (± 0.11) μg/ml to 11.5 (± 0.14) μg/ml after 16 days of incubation. This result is in conformity with the findings of Kumar *et al.* (2012) who reported the protein content of *W.prolifica* between 4μg/ml to 6.5 μg/ml in 16 days when it were allowed to grow in the same growth media. Similarly, the carbohydrate content of *W. prolifica* was observed within the range of 3.5 (±0.3) μg/ml to 7.8 (± 0.3) μg/ml and that was found between 6.5 (± 0.1) μg/ml to 16.1 (± 0.4) μg/ml in *W. indica.* Whereas in *W. ramosa,* the same was ranged from 9 (± 0.4) μg/ml to 19.5 (± 0.6) μg/ml.

On the other hand, the rate of nitrogen fixed by *W. prolifica* was observed within the range of 0.84 (± 0.3) nmol hr^{-1}gm-1 dry wt. of soil to 5.8 (± 0.3) nmol N_2 fixed hr^{-1}gm-1 dry wt. of soil. In *W. indica* the same was found between 2.1 (± 0.3) nmol N_2 fixed hr^{-1}gm-1 dry wt. of soil to 8.3 (± 0.4) nmol N_2 fixed hr^{-1}gm-1 dry wt. of soil and in *W. ramosa,* it ranged from 1.2 (± 0.07) nmol N_2 fixed hr^{-1}gm-1 dry wt. of soil to 4.9 (± 0.5) nmol N_2 fixed hr^{-1}gm-1 dry wt. of soil.

Majority of the rice grown areas in Assam are either knowingly or unknowingly contaminated with synthetic pesticides. The recalcitrant pesticides applied in the crop fields pass on to the soil systems (Malone *et al*., 2004; Lefranc *et al*., 2013). The effects of different pesticides vary according to concentration of use, properties of the soil where it is applied, ambient condition and targeted or non-targeted organisms on which it is applied (Ecobichon, 1991). Hence, it is imperative to study the influence of various agrochemicals on the non-target soil organisms, particularly the beneficial cyanobacteria which are supposed to be one of the potent groups of natural biofertilizer

(Peterson *et al.*, 1997) in the context of the present day world where synthetic pesticides are deliberately used in the name of enhancing crop yield.

Assam is reported of using an estimated amount of 11.5 kg ha^{-1} of chemical pesticides during last decade of the twentieth century (Barbora and Biswas, 1996) which has been substantially increased by many folds in recent decades. The pesticide groups widely used in different rice fields of Assam are organochlorine, organophosphate, carbamate, pyrethroid and herbicides (Hazarika, 2010). Besides killing or immobilise the targeted pests, pesticide used to affect other insects, bees, nematodes, butterflies, birds, fish, algae and beneficial microbes including nitrogen fixing cyanobacteria (Kalia and Gupta, 2004) residing in the crop ecosystem. Thus it becomes crucial to assess the impact of the synthetic pesticides on the non-targeted and beneficial microbes like, nitrogen fixing cyanobacteria.

Studies undertaken by Nath (2012), Gupta and Baruah (2015) on different agro ecosystems in NE region in general and Assam in particular indicated the detrimental impact of common pesticides on nitrogen fixing cyanobacterial community residing therein. In the present endeavour, the differential effects of three commonly used pesticides of Assam - Malathion(organophosphorous), Deltamethrin(pyrethroid) and Carbofuran(carbamate) on all the three autochthonous nitrogen fixing *Westiellopsis* species isolated from the organic rice field soils of Assam were studied.

All the pesticides used in the present study showed deleterious effects on the biomass content of the test organisms. The biomass decreased considerably with the increase in concentration of the pesticides with time. The highest reduction in biomass content among the three test organisms was observed in *W. indica* at 90ppm of

Malathion concentration (50 ± 4 µg/ml) on 8th day in comparison to the control (110 ± 11 µg/ml). In *W. prolifica*, the highest reduction was observed at 90ppm of Malathion concentration on 16th day (72 ± 8 µg/ml) over the control (210 ± 24 µg/ml) and in *W. ramosa* highest reduction was observed at 50ppm of carbofuran on 4th day (67.6 ± 12 µg/ml) in contrast to the control (110 ± 15 µg/ml). *Anabaena variabilis* also showed the similar results when exposed to Malathion (Manikar *et al*., 2013). The decrease in biomass could be attributed to the inhibition of photosynthesis due to pesticides treatment in the culture medium (Prosperi *et al.,* 1993). Reduction in synthesis of chlorophyll-a, one of the most important pigment required for photosynthesis (Okmen and Ugur, 2011) could be the other reason of inhibition.

With lower dose of treatment, in contrast, a little but insignificant enhancements in biomass were recorded which could be attributed to the adaptability of the test organisms in the slightest chemical stress (Gupta and Baruah, 2015). *Anabaena variabilis* when treated with thiobencarb, a herbicide (Battah *et al*., 2001) and *Nostoc muscorum* with Monocrotophos (Zeeshan and Prasad, 2007) too showed little but insignificant increase in biomass due to the adaptability of the strains in lower concentrations of the chemicals as well as the simultaneous increase of the rate of biochemical activities inside the cells for seeing a probability of disaster ahead. Every organism is adopting similar activities to maintain the homeostasis. Of course, the test organisms couldn’t keep the pace in higher dose (Gupta and Baruah, 2015).

Chlorophyll-a is considered as a major light harvesting pigment in cyanobacteria for carrying out photosynthesis (Eullaffroy and Vernet, 2003). The chlorophyll-a contents of the test organisms after pesticide treatment was seen to be enhanced at low concentration of pesticides as compared to the controls which again could be attributed to their attempts for survival and homeostasis (Battah *et al*., 2001; Zeeshan and Prasad, 2007; Gupta and Baruah, 2015). A significant increase amounting up to 55% in chlorophyll-a content was observed at 15ppm of Deltamethrin on 16th day ($p<0.001$) in *W. indica* which was same in *W. ramosa* at 26ppm for the same time period over the control.

In contrast to above proposition, the chlorophyll-a contents were found to be concomitantly decreased with the gradual increase in pesticide concentrations with time in case of all the test organisms. *W. prolific*a showed highest reduction of chlorophyll-a on 16th day at 60ppm of Carbofuran (0.40 ± 0.02 μg/ml) over the control (1.81 ± 0.1 μg/ml). On the other hand, *W. indica* showed highest reduction at 90ppm of Malathion on the same day (0.43 ± 0.07 μg/ml) over the control (2.01 ± 0.04 μg/ml) and *W. ramosa* showed highest reduction with 86ppm of Malathion treatment on 16th day (0.48 ± 0.09 μg/ml) over the control (2.8 ± 0.05 μg/ml). Malathion usually has stimulatory effect at lower concentration (30ppm) in cyanobacterial and algal population, but sometimes inhibits the growth at higher concentration nearing to 90ppm (Tandon *et al*., 1988; Yamamoto and Tsukada, 2009). Porsbring *et al.* (2009) worked on the effect of a fungicide on marine microalgal communities and observed that the fungicide had deleterious effects on chlorophyll-a, carotenoids and phycobiliproteins of the marine algae. The inhibition of chlorophyll-a were also reported in *Anabaena variabilis* and *Protosiphon botryoides* treated with Simazine, Atrazine (Kobbia *et al.*, 2001) and in *Anabaena cylindrica* treated with Molinate (Galhano *et al*., 2009). The decrease in chlorophyll-a could be attributed to the hindrance as supposed to be put forth by the pesticides in porphyrin ring formation, apart of a chlorophyll molecule that absorbs light energy (Lal and Saxena, 1980).

Carotenoid, an accessory pigment of cyanobacteria plays an important protective role in photo-oxidative damage and also serves as light harvesting pigment during photosynthesis (Schagerl and Muller, 2006). The pigment is usually responsible for transferring energy to chlorophyll-a, light harvesting, photo protection by quenching triplet chlorophyll-a molecule and scavenging singlet oxygen (Shen *et al*., 2009). The carotenoid pigments were seen to be decreasing with time and dose in each of the test organisms throughout the study. The highest reduction of 78 % ($p<0.001$) was observed in *W. prolifica* at 90ppm of Malathion on 16th day whereas *W. indica* showed maximum reduction of 54% ($p<0.001$) at 62ppm of Carbofuran on 16th day and *W. ramosa* exhibited highest reduction of 44% ($p<0.001$) at 86ppm of Malathion on the same day over the control. This result was in consonance with Galhano *et al*. (2009) who reported the significant reduction of carotenoid contents in *Anabaena cylindrica* when treated with Molinate. Chen *et al.* (2013) also recorded time and dose dependent decrease of carotenoid contents in *Anabaena cylindrica* with the application of Bentazon and Molinate and in *Nostoc* sp. when treated with Butachlor. The decrease in carotenoid contents in all the experiments with all the test organisms specified that the pesticides not only accelerated the degradation processes but also blocked their synthesis mechanism (Kapoor and Leenta, 1996). Solubilisation of membrane associated proteins due to pesticides effect (Hirschberg and Chamovitz, 1994) could be one of the prime reasons along with the interaction of pesticides with the membrane bound enzymes in carotenoid biosynthesis (Mohapatra *et al.*, 2003).

In few occasions, however, an insignificant stimulation of carotenoid synthesis was recorded with the test organisms as reflected from the estimation when pesticide concentration was at minimum. An insignificant increase of carotenoids was observed at

20ppm of Carbofuran in *W. prolifica* on 4th (10%), 8th (5%) and 12th (5%) day over the control. In *W. indica,* the carotenoid content was found to be increased by 37% ($p<0.01$) and 63% ($p<0.01$) at 30 and 60ppm of malathion over the control and an insignificant increase of the same was observed in *W. ramosa* at 26ppm of malathion and 10ppm of Carbofuran on 4th day over the control. Such type of results might be due to act of carotenoid as an antioxidant under stress condition (Phukan *et al*., 2019) or might be the slower rate of impact of the applied pesticides on the photosynthetic activity of the test organisms (Azi-zullah *et al*., 2011).

Protein is an important metabolite in cyanobacteria. The data obtained with test organisms highlighted gradual decrease in protein content with the concomitant increase in pesticide dose and in relation to time. In *W. prolifica*, highest reduction of protein was observed at 90ppm of Malathion concentration on 16th day (0.66 ± 0.1 µg/ml) over the control (5.3 ± 0.18 µg/ml). In *W. indica,* the highest reduction was observed at 62ppm of Carbofuran (2 ± 0.12 µg/ml) and in *W. ramosa* at 86ppm of Malathion (4 ± 0.19 µg/ml) for the same day. Such decrease in protein contents with increase in time could be due to the inhibition in the synthesis of enzymes and structural proteins which are necessary for the growth of cyanobacteria (Kapoor and Leenta, 1996). This result is in agreement with Singh *et al*. (2014) and Dowidar (2010). A little but significant enhancement in protein contents in the test organisms were observed with the lower doses of Malathion, Deltamethrin and Carbofuran which could be attributed to the synthesis of stress retarding protein by the test organisms due to influence of the applied pesticide (Fatma *et al*., 2008), of course, not in all occasions. It is well known that stress used to induce synthesis of heats hock proteins (Hsps) and stress proteins in the cells who are confronting with any stress for growth (DeMaio,1999; Feige *et al*., 1996).

In contrast, at higher concentrations of Deltamethrin and Carbofuran, the protein contents of *W. prolifica* were recorded to be around 55 percent ($p<0.001$)lesser at 60ppm of Carbofuran and 75ppm of Deltamethrin respectively on 16th day from the date of inoculation as compared to the control for the same span of time. The result is in conformity with the findings of Battah *et al.* (2001) who recorded significant reduction in protein contents in *Anabaena variabilis* when treated with Thiobencarb. *Westiellopsis prolifica* when treated with Tebuconazole (Kumar *et al.*, 2010) showed gradual reduction of protein contents with the increase in pesticide concentrations which is similar to the present study where the percent reduction of 87, 57 and 55 percent of protein in *W. prolifica* was observed on 16th day at 90, 75 and 60ppm of Malathion, Deltamethrin and Carbofuran respectively. A reduction of 36%, 20% and 61% in *W. indica* was observed on 16th day at 90, 55 and 60ppm of Malathion, Deltamethrin and Carbofuran and a reduction of 65%, 57% and 46% was observed in *W. ramosa* on 16th day at 86 and 50 ppm of Malathion, Deltamethrin and Carbofuran respectively. The reduction in protein content might be due to the inhibition of formation of structural proteins and enzymes essential for the survival and growth of the cyanobacterium (Kapoor *et al.,*1996) or might be due to the retarded growth and concomitant increase in protease activity with the influence and impact of pesticides (Babu *et al*., 2001).

The impact of pesticides on the carbohydrate content of an organism depends on the type of the test organisms, duration and dose of the pesticides used (Moreland, 1980). It was recorded that increase in concentration of pesticide drastically retarded the carbohydrate content of *W. prolifica* at 90ppm of Malathion on 4th day (2.9 ± 0.4 µg/ml) as compared to the control (3.8 ± 0.7 µg/ml). In *W. indica,* with Carbofuran the value was lowest (3.9 ± 0.1 µg/ml) at 62 ppm on 16th day over the control (16.1 ± 0.9

µg/ml) and in *W. ramosa* that was recorded to be 8.1 (± 0.9) µg/ml at 86ppm of Malathion on 16th day in comparison to the control (18.8 ± 0.7) µg/ml. These results were in agreement with the findings of Galhano *et al.* (2009). With higher concentration of Endosulfan, *Aulosira fertilissima, Anabaena variabilis, Nostoc muscorum* too showed lower rate of production in carbohydrate (Fatma *et al.,* 2008) Kumar *et al*. (2012) reported reduction in carbohydrate content in *Anabaena fertilissima, Aulosira fertilissima* and in *W. prolifica* with concomitant increase of Endosulfan and Tebuconazole pesticides. The reduction in carbohydrate could be attributed to the inhibition of the photosynthetic process due to influence of pesticides (Kumar, 1991; Mansour *et al.*, 1994). In some occasions, conversion of sugars into other metabolites were also noticed which might have negative impact on the carbohydrate assimilation (Chai and Chung, 1975).

It is also to be noted that in a few occasions, pesticides stimulated the synthesis of carbohydrate in the test organisms. At 60ppm of Carbofuran treatment, 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 4th , 8th , 12th and 16th days respectively from the date of inoculation in *W. prolifica.* Similarly, at 50ppm of Carbofuran concentration, the same was increased by 56% ($p<0.001$), 38% ($p<0.001$), 52% ($p<0.001$), 52.1% ($p<0.001$) on 4th, 8th, 12th and 16th day respectively over the control in *W. ramosa*. Both the test organisms showed the results against Carbofuran only which could be attributed to the specific extracellular adaptive mechanisms of the test organisms through synthesis of extra- cellular polysaccharides (EPS), an adaptive strategy usually utilized by any cyanobacterium for its survival under stress conditions (Ehling Schulz and Scherer,1999; Nicolaus *et al*., 1999). The pesticides might also influence the ROS scavenging mechanism (Van Den Ende and Valluru, 2009; Matros *et al.*, 2015) at some specific carbofuran concentrations to increase the rate of carbohydrate synthesis under abiotic stress. Battah *et al*. (2001) and Habib *et al*. (2013) while working on *Anabaena variabilis* treated with carbamate thiobencarb and *Calothrix brevissima* treated with Carbaryl also reported the gradual increase in carbohydrate contents in a specific concentration of the applied pesticide in relation to time.

Nitrogen is considered as one of the most important element for growth in plants (Das and De, 2018) for natural supplement in soil. As the plants are not in a position to directly intake the nitrogen from the atmosphere, they used to depend on the nitrogen fixing soil microbes (Das and De, 2018). All the three test organisms were natural nitrogen fixers in soil (Tiwari *et al*., 2000; Singh *et al*., 2017), hence could be considered as natural biofertilizers particularly in rice field soils. The rate of nitrogen fixation is obviously varied from organism to organism, which too, was reflected in case of the test organisms of the present study. In control laboratory condition, the rate of nitrogen fixed by *W. prolifica* was recorded between 0.84 (± 0.3) nmol hr^{-1}gm-1 of dry soil to 5.8 (± 0.3) nmol hr^{-1}gm-1 of dry soil. In case of *W. indica* the same was between 2.1 (± 0.3) nmol hr^{-1}gm-1 of dry soil to 8.3 (± 0.4) nmol hr^{-1}gm-1 of dry soil and in *W. ramosa,* from1.2 (± 0.07) nmol hr^{-1}gm-1 of dry soil to 4.9 (± 0.5) nmol hr^{-1}gm-1 of dry soil.

In the present study, the rate of nitrogen fixation in the test organisms were seen to be decreased significantly at the higher side of pesticide concentrations with time. In *W. prolifica*, the reduction of the rates were overall highest at 90, 75 and 60ppm of malathion, deltamethrin and carbofuran concentrations particularly on the 16th day of incubation from the date of inoculation. The reduction rates could be accounted as 67%, 78%, and 91% in relation to Deltamethrin, Malathion and Carbofuran respectively. On the same day after the inoculation, reduction rates were 74%, 80% and 85% respectively at 90, 55 and 62ppm of Malathion, Deltamethrin and Carbofuran in case of *W. indica*. *W. ramosa* also showed similar trend of reduction in the rate of nitrogen fixation amounting to 73%, 81% and 86% on 16th day from the date of inoculation at 60, 50 and 86ppm of Deltamethrin, Carbofuran and Malathion concentration respectively. The decrease in the rate of nitrogen fixation in the test organisms with higher dose of pesticides concomitantly with time could be attributed to the inhibition in the photosynthetic processes of the test organisms due to lack of reducing agent pool (Leganes and Fernandez-Valiente, 1992) against pesticide stress. Interference of the applied pesticides on ferredoxin production (Singh *et al*., 2014) could also be the other reason in decreasing the rate of nitrogen fixation in case of few organisms. The result is in conformity with the findings of Debnath *et al*. (2012) who observed the decreased rate in nitrogenase activity of a few cyanobacterial strains with the increase of pesticide concentrations. *Nostoc muscorum*, the another nitrogen fixing cyanobacterial strain showed lesser rate of nitrogen fixation when treated with Carbaryl (Bhunia *et al*., 1994). Similar results were also reported by Kumar *et al*. (2009) with *Anabaena fertilissima* under Endosulfan stress.

In contrast to the above, at the lower doses of each of the treatment concentration of the pesticides used in the present study revealed slightest increase in the rate of nitrogen fixation with time up to 8th days from the day of inoculation. Beyond that, of course the rates were drastically reduced. The slight increase in the rate of nitrogen fixation at initial days with lowers dose of pesticides could be attributed to increase in photosynthetic activity leading to production of reduced ferredoxin (Flores *et al*., 1983a and Flores *et al*., 1983b) which, on the other hand, is required for conversion of N_2 to NH_3 in nitrogen fixation. Such activity is usually related with the amount of the chlorophyll-a contents in the test organisms. It has already been discussed that the chlorophyll-a contents of the test organisms were seemed to be enhanced at low concentration of pesticides as compared to the controls which again could be attributed to their attempts for survival and homeostasis (Battah *et al*., 2001; Zeeshan and Prasad, 2007; Gupta and Baruah, 2015).

The present work thus highlighted the impact of three most commonly used pesticides (Malathion, Deltamethrin and Carbofuran) on the three autochthonous nitrogen fixing *Westiellopsis* spp. growing in the rice grown soils of Assam. As rice is the staple crop in the region, most of the farmers of Assam are habituated with unabated application of the synthetic pesticides without having any knowledge on the adverse affects of pesticides on the beneficial soil micro flora. From this study, it was evident that, exposure to higher dose of pesticides retardate growth and rate of nitrogen fixation in all the three test organisms with varied magnitudes. From the outcome of the present study it may be concluded that carbofuran caused maximum and significant inhibition in comparison to malathion and deltamethrin. Hence, use of carbofuran is to be totally reduced as far as possible in the rice fields of Assam. The other major finding is that *W. prolifica* could synthesize more of its metabolites under stress conditions in comparison to *W. indica* and *W. ramosa* hence, it could be promoted as a suitable autochthonous candidate for rice field bio fertilization program in the region. As the present world is now looking forward for sustainable agriculture, use of bio pesticides could be the ultimate answer to mitigate pest menace.