CHAPTER II

REVIEW OF LITERATURE

According to FAO, pesticides are the chemical substances which are use to control, repel or kill pest that interferes in growth, storage and transport of agricultural commodities. These are toxic and are either "selective" or "broad-spectrum" based on their extent of toxicity to the applied organisms (Bopape *et al*., 2014).

Pesticides are being used by the human beings since the beginning of the civilisation (Saeedi Saravi and Shokrzadeh, 2011). Though a few evidences of use of chemicals to protect plants were available prior to 2500 BC, it was only from the mid decades of the last century that chemical protection method was widely accepted as a defence mechanism for the crop plants (Hock *et al*., 1991). In early days, most of the pesticides were either inorganic compounds or the products derived from plants. Hellebore, Nicotine and Pyrethrin are some of the insecticides used in early times to control body lice, aphids and insect pest (Saeedi Saravi and Shokrzadeh, 2011). Around last decades of nineteenth century, lead and arsenic, the two heavy metals were used as an orchard spray in different parts of Europe. Later, lime and copper mixture which is commonly known as "Bordeaux mixture" was widely used to control the downy mildew disease, a fungal infection in grapes (Hock *et al*., 1991) in Europe.

Pest repelling compounds are initially classified into different groups based on the target group of organisms as insecticides, herbicides, fungicides, rodenticides and fumigants (WWW. Humpath.com). Later on, origin of the compound was considered and accordingly, the compounds were classified as chemical pesticides and biopesticides (Council on Scientific Affairs, American Medical Association, 1997) etc.

The approach of using synthetic compounds began with the introduction of DDT by a Swiss chemist Paul Hermann Muller in the year 1939. It was an insecticide having high efficacy to combat insect-borne diseases (Newman, 1978). It was the most widely used insecticide until banned by Environmental Protection Agency (EPA) in 1972. As of 2008, 12 countries including India were reported of using DDT as IRS (World Malaria Report, WHO 2009).After DDT, thousands of synthetic pesticides and chemicals have been formulated, manufactured and transported to be used in the crop fields throughout the globe (Bhatia, 1986).

Due to recent discovery and release of synthetic compounds like Indoxacarb (Lahm *et al*., 2001), Dinotefuran (Wakita *et al*., 2003), Pyridalyl (Sakamoto *et al*., 2003), Flubendiamide (Seo *et al*., 2007), Imidacloprid (Kagabu, 2011) etc. which could either kill or physiologically inactivate the unwanted pest and insects (Gupta, 2011), further introspection involved and the pesticides were started to classify exclusively on their chemical nature as organic, inorganic and synthetic (Kamrin, 1997).

In recent years, a few well-known synthetic chemical pesticides are widely used throughout the tropic which are belonging to any group under Organophosphate, Organochlorine, Carbamate and Pyrethroid compounds (Gupta and Baruah, 2015; Pavela, 2016). These are complex chemical compounds and are either stimulatory or inhibitory, or neutral depending on the nature of the chemical compositions, its concentration when used and the time of treatment (Maly and Rubber, 1983).

It has been observed that there is a great lose in the agriculture due to interference of pest like different weeds, insects and plant pathogens which can be considered as one of the major challenges that farmers encounter during cultivation

(Singh *et al*., 2014). According to an estimate, 8,000 species of weeds, 9,000 species of insects and pests and 50000 species of plant pathogens destroy the crops throughout the globe (Pimentel, 2009). In such cases, pesticides is a relief to the farmers, as estimate said that the crop lose by the pest has been declined by 7% with its application (Singh *et al*., 2016).

Since last few decades of the 20thcentury, it has been observed that the use of pesticides had become an integral part of modern agricultural practices in India as well as throughout the globe. Global cereal production was doubled in the past 40 years, mainly due to increase inputs of pesticides, hybrid crop strains along with the use of other technologies of the 'Green Revolution' as stated by WHO and FAO in their reports published in 1990 and 2001 respectively (Tilman *et al*., 2001). It is further estimated that the extent of use of the pesticides will increased by many folds in 2050 when the human population on earth is expected to rise over nine billion people. Feeding to such a huge gathering will surely be a challenging task (Ash *et al.,* 2010), until and unless the production rate is not being gradually increased with introduction of high yielding hybrid varieties and disease resistant varieties (Anonymous, 2010) along with the application of pesticides to get rid of unwanted foes. The pesticide therefore becomes an essential part of the agricultural system in recent years and has been in use in one-third of the agricultural system (Liu *et al*., 2002) in the entire world including India.

Agriculture can be regarded as the backbone of the Indian economy as more than 70% of the population depends on it and a gross domestic production of around 27% comes from it (Singh *et al.*, 2016). Indian food production has been increased from 51 million tonnes in 1950-51 to 212 million tonnes in 2001-02 and then 244.78 million tonnes in 2010-11 which could be attributed to the introduction of modern agricultural strategies like high yielding seeds with disease resistant variety, better irrigation and protection of crops using pesticides and herbicides (Mukherjee, 2006; Aktar *et al*., 2009; Singh *et al*., 2016). In India, pesticides that are used for crop protection and public health maintenance goes up to a total of 85,000 tons per year (Chaurasia, 2014). Out of this, 77.8% are insecticides, and the rest are fungicides, herbicides, rodenticides, fumigants and miscellaneous pesticides (Chaurasia, 2014). Among the crops, rice alone accounts for about 17.2% of it (Maruthanayagam and Sharmila, 2004). As rice is the one of the staple crop of Indian subcontinent, the agricultural scientists are more concerned with the rice field ecosystem in the entire region and used to keep vigil on the enemies of rice to increase production or to maintain stability in the rice ecosystem.

Though pesticides are being used for killing harmful pests, they have been reported affecting the non targeted and beneficial organisms in the vicinity of their application. These are found to be mixed with or flown into the nearby ecosystems through air, soil and water leading to jeopardise human health and normal environment functioning (Singh *et al*., 2016; Malone *et al*., 2004; Lefranc *et al*., 2013). The continuous application in the crop fields affects the dynamic equilibrium of the soil environment (Bambaradeniya and Amerasinghe, 2003) leading to hamper ecological services of the soil borne organisms. To understand the impact of these hazardous substances, different biological subjects, species, individuals etc. have been in use as model for so many years (Lushchak *et al*., 2018).

The universal use of pesticides in agriculture makes it essential to study the effect of these synthetic chemicals on this group of non targeted beneficial soil microorganisms (Gillberg, 1971). The continuous application of pesticides/herbicides is seen to affect beneficial microbial flora of soil, air and water (Singh and Datta, 2006). Among the beneficial microbes, cyanobacterium occupies an important position in both the soil and aquatic ecosystem (Dutta and Baruah, 2019). A few of them have the dual capacity of fixing carbon and nitrogen simultaneously in the substratum (Irisarri *et al.,* 2001) which makes them an important sources of natural N_2 supplement to any crop fields (Roger, 1995; Fernandez-Valiente *et al*., 2000) and thus, contribute to a greater extent in maintaining soil fertility therein (Dutta and Baruah, 2020).

Cyanobacteria in total or as a group is also one of unique and important members of any rice agro ecosystem (Song *et al.,* 2005) from the perspective of fertility of soil is concerned. The cyanobacteria used to reflect broad spectrum of physiological properties and are found to be tolerance capacity to environmental stresses including pesticidal toxicity (Singh *et al.,* 2013). The fate of the nitrogen fixing cyanobacteria in the pesticide treated rice fields is therefore a major concern in recent days. It is estimated that about 30 kg/ha/yr of nitrogen are fixed by the nitrogen fixing cyanobacteria in the paddy fields (Watanabe, 1965). It affected, amount of N_2 supplement may be reduced, hence study necessitated. The interaction between microalgae and pesticides from the year 1946 to 1975 has been reviewed by Butler in 1977 and the cyanobacterial response to various pesticides has been hence reviewed by Pipe (1992), Das *et al.* (2015), Singh *et al*.(2016) and Kumar *et al.* (2018) in different occasions.

Algae and cyanobacteria show variable degree of response to pesticides from complete inhibition to satisfactory growth by building resistant capacity against pesticides (Ravindran *et al*., 2000). The extent of contamination of Atrizine, a Triazine herbicide in Louisiana's estuaries from Mississippi River water depicted that phytoplankton which are exposed to low to moderate range of atrazine toxicity were able to recover after an extended acclimation period (Starr *et al*., 2017) in contrast to higher stress. Studies revealed that pesticides deleteriously affected the growth, photosynthesis, nitrogen fixation, biochemical composition and metabolic activities of some cyanobacterial species too (Mohapatra and Schiewer *et al.,*2000; Jha and Mishra *et al.,* 2005; Caceres *et al.,* 2008; Bhattacharyya *et al.,* 2011; Prasad *et al.,* 2011; Singh *et al*., 2011;Singh *et al*., 2013).

The cell density, chlorophyll-a and protein content of *Microcystis aeruginosa* and *Mirocystis viridis* decreases after exposure to fenoxaprop-p-ethyl, commonly known as FPE (Yuping Du *et al*., 2017) whereas Galhano *et al.* (2009) reported residual inhibitory effects on growth, photo pigments and photosynthesis of *Anabaena cylindrica* with the excessive application of bentazon and molinate. The effect of furadon on the nitrogen fixation efficiency of soil born blue green algae *Calothrix javanica* de Wilde showed decrease in nitrogen content over the control at higher dose of treatment (Shinde *et al.,* 2010). The growth, carbohydrate, protein and amino acid contents of *Oscillatoria pseudogeminata* was decreased with increasing concentration of Carbaryl pesticides (Bakiyaraj, 2014). At higher dose of Lamdacyhalothrin, *Calothrix* spp. showed significant decrease in biomass (54.5%), chlorophyll-a (68%), carotenoids (38%), phycocyanin (80%), and nitrogen contents (55%) over the control. On the other hand, at lowest concentration of pesticides, there was a little but insignificant increase in growth and chlorophyll-a content of the test organism (Gupta and Baruah, 2015).

Pesticides and herbicides have different levels of toxicity based on their concentrations and organisms of application. Several reports have been published on the comparative toxicity of herbicides, fungicides and insecticides towards microorganisms including blue-green algae (Abou-Waly *et al.,* 1991; Chaurasia, 2014). Herbicides are mostly toxic against the phototropic microorganisms by disrupting their photosynthetic mechanism (Kumar *et al*., 2010). Along with photosynthesis, they not only affect the respiration to cell division, but also put hindrance in the synthesis of carotenoids compounds, protein, carbohydrate and lipid of the organism (Ecobichon, 1991). Chlorophenoxy herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) ethyl ester, one of the commonly used agricultural herbicides are found to affect the protein, carbohydrate, amino acid and phenol along with nitrate reductase and glutamine synthetase enzyme in *Anabaena fertilissima* Rao (Kumar *et al*., 2010).

The recent addition of Chinese sulfonylurea herbicide Monosulfuron-ester which has been widely used in recent years against pest and herbs in rice, wheat, corn, millet fields (Wang, 2006), on the other hand, accelerate the growth rate of *Anabaena flos-aqua* and *Anabaena azotica* in lower dose of 0.03-300 nmol/L. At higher dose of about 30-300 nmol/L, the compounds drastically affect both the species inhibiting the protein and Acetolactate synthase (ALS) activity together (Shen *et al*., 2017). Insecticide and fungicide sometimes inhibits the nitrogenase and glutamine synthetase (GS) activity of cyanobacteria by blocking their metabolic pathway. It has been reported that Fungicides (Bagalol, Mancozeb)and Insecticides (Thiodan and Phorate) inhibited the nitrogenase and Glutamate synthetaseactivity in *Nostoc ellipsosporum*, *Scytonema simplex, Tolypothrix tenuis,* and *Westiellopsis prolifica* (Debnath *et al*., 2012). Bagalol incurred maximum inhibition of nitrogenase and GS activity on *N. ellipsosporum* and *S. simplex* while Thiodan and Phorates howed maximum effect against *T. tenuis,* and *W. prolifica* (Debnath *et al*., 2012).

Most of the non targeted organisms are affected with application of pesticides (Anton *et al*., 1993; Dobsikova, 2003; Shrivastave *et al*., 2011). Insecticide such as Cypermethrin, Lindane, Carbofuran, Endosulfan and Chlorpyrifos deleteriously affected the growth, photosynthesis, nitrogen fixation, biochemical composition and metabolic activities of cyanobacteria (Mohapatro *et al.*, 2003; Prasad *et al.*, 2011; Singh *et al*., 2011; Suresh *et al.*, 2001). Singh *et al*. (2014) evaluated the toxicological impact of cartap hydrochloride on some physiological activities of a non-heterocystous cyanobacterium *Leptolyngbya foveolarum*. They observed that the test microorganism could tolerate insecticide dose up to 80 ppm. Their studies revealed that lower concentration (20 ppm) of Cartap supported good growth with dry biomass, protein, pigments, photosynthesis, respiration compared to the control (without pesticides). On the other hand higher concentrations (40 and 60 ppm) inhibited these parameters in a time dose dependent manner. Alpha-cypermethrin (ACy), a pyrethroid insecticide caused increase in ROS production, lipid peroxidation, pigment reduction and altered enzyme activity in a *Chlorella* species (Baruah and Chaurasia, 2020).

At higher dose of Malathion, there is a reduction in the biomass, protein and carbohydrate content of a nitrogen fixing rice field cyanobacterium, *Westiellopsis prolifica* (Dutta and Baruah, 2019). In another study made by Dutta and Baruah (2020) on *W. prolifica* to understand the differential effects of Deltamethrin and Carbofuran showed that low concentrations of both the pesticides stimulated the growth of the test organism while at higher concentrations the growth characters were significantly inhibited with an exception in carbohydrate content which gradually increased with the increase in carbofuran concentrations. The increase in carbohydrate is considered as an adaptive strategy of the cyanobacterium by synthesising extracellular polysaccharides

(EPS) to overcome the stress of Carbofuran (Dutta and Baruah, 2020). *Calothrix* sp. (GUEco 1002), one of the nitrogen fixing cyanobacterium strains isolated from the rice fields also showed a significant inhibitory effect in the formation of dry biomass, chlorophyll-a, carotenoids and nitrogen content when treated with Deltamethrin, a pyrethroid group (Gupta and Baruah, 2020).This result signifies the inhibitory affects of pesticides on nitrogen fixing cyanobacteria of rice field ecosystem.

Nitrogen is one of the essential macro elements for plant growth and all plants including cultivated crops depend on soil for nitrogen. Rice, as a crop alone utilizes about 50% of the soil nitrogen for their growth (Fernandez-Valiante *et al*., 2000). Most of the soil nitrogen in the rice fields is maintained by the soil autotrophs and heterotrophs through biological nitrogen fixation (Roger and Ladha1992; El-Shahed, 2005; El-Shahed and Abdel- Wahab, 2006; Thamida Begum *et al*., 2011) and cyanobacteria is one of them (Roger, 1995; Fernandez-Valiente *et al.,* 2000; Irisarri *et al.,* 2001). The nitrogen fixing cyanobacteria are therefore, advised to use as biofertilizers in crop fields including the rice fields (Singh *et al*., 2014). As cyanobacteria are one of the most beneficial non targeted microbes in the rice field ecosystem, any harm to it through application of pesticides imbalance the ecosystem as a whole (Gangwar *et al*., 2014).

Nitrogen fixing cyanobacteria grows abundantly in different soil ecosystems (Whitton and Roger, 1989). The water logged rice fields of Thailand, Japan, China, Phillipines, Bangladesh and India are found to be more suitable for their growth (Okuda and Yamaguchi, 1952; Watanabe and Yamamoto, 1971; Roger and Kulasooriya, 1980; Venkataraman, 1981; Song *et al*., 2005). De (1936) was the first person in India who highlighted the importance of nitrogen fixing cyanobacteria in maintaining the nitrogen status of the tropical rice field ecosystem through their growth. The nitrogen content in the rice field soil gradually increases with increase in growth of cyanobacteria (De and Mandal, 1950). Cyanobacteria also play a significant role in the growth of rice. It has been reported that application of cyanobacteria enhances around 20 to 30% in rice production per hectare (Irisarri *et al*., 2001) besides protecting the crops from soil borne diseases (Mishra and Pabbi, 2004).

Along with the growth of rice plants (Saadatnia and Riahi, 2009) the soil properties were also seen to be enhanced up to 19% with cyanobacterial inoculation (Prasanna *et al.,* 2012). Report revealed that the growth, ammonia production and nitrogenase activity within the root zone of rice seedlings are sufficiently increased with the inoculation of *Anabaena azollae* (Kannaiyan *et al*., 1997), a symbiotic stain of cyanobacteria. *Tolypothrix tenuis and Nostoc muscorum* too have influence on growth performance of rice (de Mulé *et al*., 1999).

The Indian rice fields are very rich in nitrogen fixing cyanobacterial diversity (Pandey, 1965; Sinha and Pandey, 1972; Singh, 1975; Bendre and Kumar, 1975 Kolte and Goyal, 1986). Out of which, about 50% of the soil borne nitrogen fixing cyanobacterial genera belongs to either Nostocales or Stignomatales in India (Venkateraman, 1993) and they are either unicellular and filamentous in forms (Ahluwalia *et al.,* 2014). *Aphanothece, Chroococcidiopsis, Dermocarpa, Gloeocapsa, Myxosarcina, Pleurocapsa, Synechococcus, Xenococcus* are some of the important unicellular nitrogen fixing cyanobacterial genera (Vaishampayan *et al*., 2001). The filamentous form of cyanobacteria is divided again into two groups, heterocystous and non heterocystous (Ahluwalia *et al.,* 2014). *Lyngbya, Microcoleus chthonoplastes, Myxosarcina, Oscillatoria, Plectonemaboryanum, Pseudoanabaena, Schizothrix,* *Trichodesmium* belong to the non heterocystous group of algae (Vaishampayan *et al.*, 2001; Pereira *et al.*, 2008). The heterocystous group includes *Anabaena, Anabaenopsis, Aulosira, Calothrix, Camptylonema, Chlorogloea, Chlorogloeopsis, Cylindrospermum, Fischerella, Gloeotrichia, Haplosiphon, Mastigocladus, Nodularia, Nostoc, Nostochopsis, Rivularia, Scytonema, Scytonematopsis, Stigonema, Tolypothrix, Westiella* and *Westiellopsis (*Vaishampayan *et al.,* 2001; Pereira *et al.,* 2008; Rana *et al.,* 2012; Prasanna *et al.,* 2013).

The ecological conditions of The North Eastern states of India (Reddy *et al.,*1986; Devi *et al.,* 1999) usually support a good growth of the nitrogen fixing cyanobacteria including Assam (Brahul and Biswas, 1922; Parukutty, 1939; Bordoloi, 1973; Devi, 1981; Hazarika, 1988; Saikia and Bordoloi, 1994; Bhuyan, 1996; Ahmed and Kalita, 2001; Singh, 2001;Yasmin, 2003; Baruah *et al*., 2009; Bhardwaj and Baruah, 2013). The members belonging to heterocystous nitrogen fixing cyanobacterial taxa viz., *Anabaena*, *Calothrix, Cylindrospermum, Fischerella, Hapalosiphon, Microchaete, Nostoc* and *Westiellopsis* are most common in the rice grown soils of entire North East India (Syiem *et al.,* 2010; Rout *et al.,* 2016, Yashmin *et al*., 2015).

As organophosphate, organochlorines, carbamate and other synthetic pyrethroid insecticides have been in use in different rice grown areas North East India for the last 100 years (Gurusubramanian *et al*., 2008), these heterocystous groups are being frequently affected by exposure to different pesticides, *Anabaena*, one of the common nitrogen fixing cyanobacteria of the rice fields of NE region of India (Syiem *et al.,* 2010) was seen to be adversely affected in its growth, pigments and protein synthesis mechanism when treated with fungicide Mancozeb and insecticide Profenofos (Chaurasia, 2014). The pyrethroid Lamdacyhalothrin and Deltamethrin impart inhibitory effect on the growth of *Calothrix* sp. (GUEco1001) (Gupta and Baruah, 2015; Gupta and Baruah, 2020) which was isolated from the rice fields of Assam.

Westiellopsis (Komarek, 2013) belonging family hapalosiphonaceae under the order Nostocales is one of the prominent heterocystous nitrogen fixing taxa commonly growing in the rice grown soils in entire tropics (Tiwari *et al.,* 2005; Sethi *et al*., 2012) including Assam (Dasgupta and Ahmed, 2013; Bhardwaj and Baruah, 2013; Dutta and Baruah, 2019). *Westiellopsis prolifica* is the main representative species of the genus *Westiellopsis* distributed throughout the world. It was first reported from the soils sample of agri-horticultural society of Madras (Janet, 2011). Later members under the taxa were reported from different agricultural fields in general and from rice grown fields in particular in India (Venkatarman, 1981; Tiwari *et al.,* 2005; Debnath *et al*., 2012).

Apart from rice fields, it has also been reported from the desert soils in Rajasthan (Tiwari *et al*., 2005), fresh water habitats in Madhya Pradesh (Singh *et al*., 2017), wet soils of Loktak lake of Manipur (Thingujam *et al*., 2016) and wet soils of Kaziranga National Park (Yashmin *et al*., 2015). Being members of heterocystous N_2 fixing cyanobacterial group, 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; Dutta and Baruah, 2020). Though the majority of the rice grown areas in India are either knowingly or unknowingly contaminated with unabated use of synthetic pesticides, a very meagre works have been found on the impact and effect of pesticide on *Westiellopsis prolifica* isolated from rice field soils (Dutta and Baruah, 2019; Dutta and Baruah, 2020). Being a potential natural biofertilizer, the taxa invites an in depth study to understand the behaviour of different natural strains of *Westiellopsis* recorded from rice grown soils of NE in general and Assam in particular.