CHAPTER I

INTRODUCTION

In the present-day world, the total arable land is decreasing due to the drastic increase of human population. To balance this situation, there is a need of growing more crops within the limited area. Introduction of high yielding and disease resistant variety is being used to tide over the situation throughout the globe. Pests are uninvited guests to any crop field irrespective of natural and hybrid varieties. In modern agricultural practices, pesticides therefore, are widely used throughout the globe and have become an integral part of the crop management system in recent years.

Pesticides are synthetic organic compounds that are applied in crop fields in order to remove the pests that hamper the growth of crops (Venkatarman, 1975; Mellanby, 1978). According to the Environmental Protection Agency (EPA), pesticides are substances used for protecting plants by killing or repelling different types of pest such as plant parasitic viruses, bacteria, nematodes, fungi, insects, weeds, rodents and birds (Anonymous, 2007).

Pesticides help in killing or inactivating pests which in turn save the crop in different stages of growth (Aktar *et al.*, 2009). Pesticides thus help poor farmers to have good harvest at minimal labour cost. It reduces unnecessary wastage of time in searching, weeding or removing of the foraging substratum of known or unknown pests (Gangwar *et al*., 2014). As we all know, a farmer has to bear a good amount of money throughout the year (Lum *et al*., 2005) for the above-mentioned activity to get rid of pests. People have therefore been in search of suitable and specific agent or compounds.

Historical and mythological literature also mentioned about the use of decoction, mixture and fume to protect plants from unwanted pests. The first introduction of pesticides took place around 4500 years ago, when elemental sulphur dusting was done to repel pest in the ancient Mesopotamia. The more than 4000 years old sacred Hindu text – the Rig Veda also mentioned the use of poisonous plants to combat harmful pest (Rao *et al*., 2007). During the 15th century, chemicals like arsenic, mercury, and lead were used to control pest. Literature reveals that during 17th century AD, an insecticide called nicotine sulfate was extracted from the leaves of tobacco plant and used widely in crop fields. The 19th century marked the introduction of two natural pesticides – pyrethrum and rotenone (Miller, 2002). Gradually, increase of pesticide uses have been observed and showed sharp increase since the later part of the 19th century in the entire globe leading to massive escalation of pesticide pollution.

Considering the impact upon the environment, the first pesticide regulating authority was constituted by the legislation in the United States in 1910 (Goldman, 2007). In the global level however, application persisted. In fact it was increased in the early decades of the 20th century leading to introduction of large amount of synthetic pesticides since 1940 throughout the world (Daly *et al*., 1998). The period from 1940 to 1950 has therefore been considered as the beginning of the pesticide era in the world (Murphy, 2005).

Introduction of synthetic pesticides led to the beginning of the third agricultural revolution i.e. green revolution in the late 1960s (Hazell, 2009). This revolution adopted new technologies like use of hybrid variety of crops in association with pesticides and chemical fertilizers along with the use of modern farm machineries. Remarkable agricultural production has been achieved throughout the globe with the practice. People

started considering pesticides as an important element in food production until it was known for its side effect on human health and related to some environmental issues (Hazarika, 2010). But it is a matter of concern that pesticides are still in use in crop fields.

Pesticides cover a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators and others (Aktar *et al*., 2009). There are four major groups of synthetic pesticides still in use. They are organochlorine, organophosphate, carbamate and pyrethroid groups (Kumar, 2010). These are complex chemical compounds and are stimulatory, inhibitory or neutral depending on the nature of their constituents, concentration, time of treatment and contact (Maly and Ruber, 1983).

Organochlorine is known as the first-generation synthetic pesticide having chlorinated hydrocarbon group. They are widely used in both agricultural and non-agricultural practices (Patil and David, 2008). This group of pesticides has been banned by many advanced countries due to their negative impact on health and environment (ATSDR, 2018). Common examples are DDT, Endrin, Toxaphene, BHC (lindane), Mortal, Thiotax, Endosulfan, etc.

Organochlorine insecticides were gradually replaced by the Organophosphate group. The Organophosphate based pesticides were first reported to be used in the year 1937 (Dragun *et al.* 1984). Blandan is known to be the first organophosphate insecticide which contained TEPP (tetraethyl pyrophosphate) and received wide commercialized acceptance during that period. A German chemist Gerhard Schrader synthesized the Blandan during late 30s and later in 1944, he released another insecticide parathion

(Gallo and Lawryk 1991). Malathion, Monocrotophos, Durmet, Rogor and Metasystox, Match, Temephos and Fenitrothion are a few examples of synthetic pesticides of this group.

Carbamates, which were widely used throughout the world in controlling pest due to their effectiveness and broad-spectrum biological activity (insecticides, fungicides and herbicides) were introduced in 1970 (Aktar *et al*., 2009) and are still in use. They were mostly used to control insects and nematodes in soil. The important features of carbamates are high polarity, solubility in water and thermal instability. Examples are Propoxur, Carbofuran, Sevin and Temik.

Pyrethroids were introduced to the world around 1980s (Aktar *et al*., 2009). Pyrethroids are a group of insecticides similar to natural pyrethrins produced from the flowers *Chrysanthemum cinerariaefolium* and *C. coccineum*. This group of insecticide is characterized as moderately persistent, having high potential for control of insect pests and with low mammalian and bird toxicity (Smith & Stratton, 1986). About 25% of the insecticide market worldwide now belongs to synthetic pyrethroid pesticides (Zhang *et al*., 2010). Common examples are Cypermethrin, Deltamethrin, Lambdacyhalothrin, Fenvalerate and Permethrin.

During 1970s–1980s, herbicides and fungicides were introduced (Aktar *et al*., 2009). Herbicides were introduced with an aim to get rid of the unwanted plants that competes and destroys the growth of beneficial crops. Captan, Mefenoxam, Mancozeb etc. are some of the commonly used herbicides around the world. On the other hand, fungicides are chemicals which have been used in agriculture and horticulture to protect

the crops from fungal infections. Atrazine, Paraquat, Glyphosate etc. are some of the commonly used fungicides (Benson, 1969) in the recent years.

Though all synthetic pesticides tremendously contributed to control the annual pests in the crop fields and took part in enhancing agricultural output, scientists and policy makers were initially unaware of its persistent and recalcitrant nature. They remain accumulated in the soil for long due to their slow natural degradation rate (Da Silva *et al*., 1975) leading to bio magnifications which in turn, affected the dynamic equilibrium of the soil environment in any crop field (Bambaradeniya and Amerasinghe, 2003). Some of them are used to volatilize and to get blow away by wind to nearby areas posing threat to the plants and animals including humans inhabiting in the vicinity.

It is estimated that only around 0.1% of the pesticides reaches the target organism, and the remaining 99.9% is found to be dispersed through air, soil and water to nearby ecosystems somehow (Pimentel, 1995). The pesticide residues are therefore, frequently reported from the surface water in agricultural fields and nearby in most of the tropical countries (Hoysater, 1994; Larson *et al.,*1999; Ul en *et al*., 2002; Maruthanayagam and Sharmila, 2004). It is also revealed that the concentration of pesticides has been increasing day by day particularly in the wetlands and crop fields of South Asian and African countries (Zhang *et al.,* 2011). Aldrin, DDT, Dieldrin, Endrin, etc. are some of the very common persistent organic pollutants widely used in tropical and subtropical region (FAO, 2005; Gupta, 2004; Lallas, 2001).The fate of these non-degradable organic compounds is now becoming a matter of grave concern (Coupe *et al.,* 2000).

In spite of the different problems associated with synthetic pesticides, they are seen to be used in the modern agricultural practices in a regular manner throughout the

tropics (Aktar *et al.,* 2009).During the last few decades, consumption of pesticide has been found to be increasing globally. About two million tons of pesticide is consumed by the world, out of which Europe alone consumes 45%; USA consumes 24% and rest of the world consumes 31% (Gupta, 2004).The amount of pesticides that the world used in the later part of the last decade includes 44% insecticides, 30% herbicides, 21% fungicides and 5% other chemicals. India is using around76% insecticides, 13% fungicides, 10% herbicides and 1%other miscellaneous pesticides (Dey *et al*., 2013) in recent years. As on today, India is leading the Asian countries in terms of pesticide production (90,000 tones) and in consumption level, India is ranked in twelve position globally (Mathur, 1999).

India has a population of more than 1000 people with an area of 328.7 million hectare. To feed the large group of people residing here, about157.35 million hectares of the area is found to be used for cultivation (Eliazer Nelson *et al.,* 2019). It has also been reported and recorded that different types of pesticides are being frequently used in the fields. Records revealed that the first pesticide was used in a field near Calcutta city in 1952 where a production plant for BHC (Benzene hexachloride) was established (Mathur, 1999).The use of pesticides gained momentum in the rest of India during the green revolution. As a result, the technical grade pesticide production in India increased from 5,000 metric tons in 1958 to 102,240 metric tons in 1998 (Aktar *et al*., 2009).The agricultural productivity particularly of rice production was doubled during the green revolution with the use of different synthetic pesticides (Ameen and Raza, 2017) and chemical fertilizers (Shiva, 1998).

Rice is the second largest staple cereal crop in the world (Koesukwiwat *et al*., 2014). In tropical countries, cultivation of rice extends from 8^0 to 35^0 N latitudes across

different ecosystems such as rain-fed lowland, upland, semi deep and deep water, irrigated systems, including coastal saline regions (Krishnaiah and Varma, 2013). Being a tropical country, India is also one of the major rice production hubs in the globe (Smith, 2000).To cater to the needs of the inflated population in India, rice fields have been deliberately sprayed with different types of pesticides to get rid of insects and pests since the inception of the 'Green revolution' in India (Shafer *et al*., 2008). It is estimated that about 24 percent of arable area in Indian subcontinent are occupied by rice (Bishwajit *et al*., 2014) and about 17.2% of the amount of pesticide used in India in total has been sprayed or mixed in rice grown areas (Maruthanayagam and Sharmila, 2004).Though use of pesticides in rice fields acted as a boon to the Indian economy, it has become a bane to the environment (Agnihotri, 1999) which needs intervention.

Pesticides not only contaminate the immediate environment or ecosystem, but also act as blunt weapons to kill the non- targeted organisms occupying the different level of trophic structures in connection with that rice field ecosystem. Even though a pesticide enhances agricultural productivity, its ill effect on the non-targeted organisms is becoming a matter of concern (Thajuddin and Subramanian, 2005). Along with the targeted pests, it affects other insects, bees, nematodes, butterflies, birds, algae, fish, beneficial microbes including nitrogen fixing cyanobacteria (Kalia and Gupta, 2004).

The effects of different pesticides vary according to the concentration of the pesticides, properties of the soil where it is applied, ambient condition and organisms on which it is applied (Ecobichon, 1991). The organisms at T_1 level of any food chain are more likely to get affected by the applied pesticides hampering the equilibrium of nature. The best examples of organisms in T_1 level includes plants, algae and cyanobacteria which have the capacity to synthesize carbohydrate for all organisms of

all successive level trophic structures of any ecosystem, irrespective of terrestrial or aquatic. Being positioned in the base level of any trophic structure of any ecosystem including crop or rice ecosystem, effects on the T_1 organisms due to application of pesticides will obviously have tremendous impact in ecosystem functioning (Gangwar *et al*., 2014). Hence, it is imperative that the influence of various agrochemicals on these organisms, particularly on the beneficial cyanobacteria should be thoroughly understood (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.

Cyanobacteria are a group of prokaryotes containing unicellular to multicellular microorganisms with great diversity and carrying out oxygenic photosynthesis (Carr and Whitton, 1982; Vermaas, 2001). A few of them have the dual capacity of fixing carbon and nitrogen simultaneously in the substratum, which makes them an important source of natural N_2 supplement to any crop fields (Roger, 1995; Fernandez-Valiente *et al.,* 2000; Irisarri *et al.,* 2001) and thus contribute to maintaining soil fertility. They are abundantly found in all agricultural fields in general and rice fields in particular (Venkatarman, 1981). They are therefore known as 'diazotroph biofertilizer' (Singh and Dhar, 2010). These microorganisms have been reported of being used in rice fields as natural biofertilizers for better yield of crops (Irisarri *et al.*, 2001; Mishra and Pabbi, 2004).

Cyanobacteria are widely distributed throughout the globe, which, in a way, reflects its capacity to tolerate environmental stresses including toxic effects of different synthetic compounds (Singh *et al*., 2013). Their occurrence in diverse habitats make them excellent materials for investigation by ecologists, physiologists, biochemists, microbiologists, bacteriologists in different aspects of life science, biotechnology and

biochemistry. Available literature revealed that insecticide such as Cypermethrin, Lindane, Carbofuran, Endosulfan and Chlorpyrifos had deleterious effect on growth, biochemical composition, rate of photosynthesis, nitrogen fixation activities and metabolic activities of cyanobacteria (Mohapatro *et al.,* 2003; Prasad *et al*., 2011; Singh *et al.*,2011; Suresh *et al.,* 2001).

As non-targeted organisms are being unintentionally affected most by the toxic effects of pesticides (Anton *et al*., 1993; Dobsikova, 2003; Shrivastave *et al*., 2011) particularly in tropical rice fields, study on the effect of these persistent and recalcitrant pesticides on all types of beneficial soil microorganisms become extremely essential. In fact, we can say that this is precisely the 'need of the hour' for better management of rice fields in any tropical countries including India. Considering the great economic and ecological significance due to their ability in fixing elementary nitrogen (Singh, 1961; Irisarri *et al*., 2001), *Westiellopsis*, an important member of filamentous nitrogen fixing cyanobacteria belonging to the family Hapalosiohonaceae (Komarek, 2013) which are growing profusely in the rice field agro ecosystems in entire tropics (Tiwari *et al*., 2005; Sethi *et al*., 2012) was selected as the test genus to understand the systemic effects of pesticides on the species or strains belonging to the genus.

The contribution of the species belonging to the genus in the field of nitrogen biofertilizer was extensively reviewed by Venkatarman (1975) and Tiwari *et al*. (2000). Though sufficient work has been done on the diversity and distribution of *Westiellopsis* in different agro ecosystems (Deka and Sarma, 2011), studies on the effect of pesticides upon the genus is still limited. Keeping in view the ecological services provided by the members of the taxa, the present study was conducted to ascertain the impact of some of

the most commonly used pesticides viz: Malathion, Deltamethrin and Carbofuran on the growth and nitrogen fixing capacity of *Westiellopsis* spp. isolated from rice-grown areas of Assam with the following objectives.

Objectives:

1. To isolate *Westiellopsis* spp. from different rice fields of Assam.
2. To study the effect of pesticides on growth characteristics and biochemical constituents of *Westiellopsis* spp.
3. To analyse the nitrogen fixing capacity of *Westiellopsis* spp. exposed to pesticides.