Chapter 1
INTRODUCTION

Rice (*Oryza sativa* L.) is the seed of the monocotyledonous plant, belonging to the grass family (Poaceae). As a cereal grain, rice is the most important staple food for a greater part of the world's human population especially in Asia. India is the world's second largest producer only after China. Rice being the chief staple food crop of India, is grown in most of the states and occupies more than thirty per cent of the total cultivated area as reported by (Adhikari *et al*., 2012; Chakravorty *et al*., 2013). According to (Chakravorty and Ghosh, 2013), its cultivation is mostly concentrated in the river valleys, low-lying coastal areas and deltas of north eastern and southern India, especially in the states of Andhra Pradesh, Assam, Bihar, Chhattisgarh, Karnataka, Kerala, Odisha, Tamil Nadu, Uttar Pradesh and West Bengal, which together they count about ninety seven per cent of the country's rice production. West Bengal is called as 'bowl of rice' with over 450 rice landraces according to (Deb, 2005; Chatterjee *et al*., 2008). Rice is cultivated in West Bengal on over 65 per cent area under agricultural crops as reported by (Adhikari *et al*., 2012) in three different seasons i.e., Aus (autumn rice), Aman (winter rice) and Boro (summer rice).

Since ages India is home to a wide range of varieties of rice cultivars, landraces and many lesser known types that have been under cultivation by indigenous farmers as well as local entrepreneurs according to (Vinita *et al*., 2013). Also as according to (Roy *et al*., 1985; Paroda and Malik, 1990; Khush, 1997) more than 75,000 local cultivars/landraces of rice are present in here. Moving towards the north-eastern region of India where also rice occupies the most important position of staple food with estimated 10,000 indigenous cultivars of rice landraces grown in this region. Since rice is the most important crop and also of the known fact that it earns a lot of money for the country and also for the state, the rate of production of rice need to remain constant. So to meet up to that level of production a lot experiments are done on rice plant to improve its disease resistance and life span.

Among the most important limiting factors that affect rice production is diseases and fungal disease infection is one of the most serious rice diseases in the rice sector. It results in poor production, poor quality, poor milling returns and reduced income. Rice brown spot is a chronic disease that affects millions of hectares of rice every growing season, grown by some of the most resource-poor farmers. Still much needs to be understood about brown spot disease despite of its widespread occurrence and impact. It causes an annual yield loss conservatively estimated at 5%. Brown Spot is conventionally perceived as a secondary problem that reflects rice crops that experience physiological stresses as for example drought and poor soil fertility, rather than a true infectious disease especially occurring in environments where water supply is low.

Brown spot has been associated with two major epidemics in India, the first in the Krishna Godavari delta in 1918 to 1919, and the second, during 1942 in today's India and Bangladesh according to (Chakrabarti, 2001). The latter of which has been associated with the Great Bengal Famine as according to (Chakrabarti, 2001; Padmanabhan, 1973). However one needs to remember that the relationship between plant disease epidemics and famines probably is never simple, as because so many other factors lead to major social consequences as reported by (Chakrabarti, 2001; Zadoks, 2008). According to (Reddy *et al*., 2010), rice brown spot is still widely reported across India and more often in the South and South-East Asian countries as per (Savary *et al*., 2000a). Also according to (Savary *et al*., 2000b, 2006) it causes a huge loss in the yield that, on an average, are in the range of 10 % of the attainable yield wherever it occurs in the lowlands of tropical and subtropical Asia. Therefore, Rice Brown Spot is by far one the strongest yield reducers amongst rice diseases today. Further, there is indication that BS (Brown Spot) is becoming more frequent and severe as drought is becoming more frequent as per (Savary *et al*., 2005), perhaps due to increased variability in rainfall. Also (Bedi and Gill, 1960) reported that the range of reported yield losses to brown spot, often articulated in absolute terms, is variable from 4 to29 %, and about 12 %, as per (Aluko, 1975), from 8 to 23 % as per (Fomba and Singh, 1990) from 26 to 52 % according to (Chakrabarti, 2001). The latter figures represent a broader and extreme range as because it is responsible for losses that have been caused by grain infection. Heavily infected grains are not suitable for consumption of humans, which partially explain the effect of BS in the Great Bengal Famine.

Brown spot symptoms initially show a small oval to circular spots on the first seedling leaves. Leaf spots are seen all throughout the growing season and may differ in shape, size and colour as per the environmental conditions, age of the spots, and the degree of susceptibility of the rice cultivars. Small brown spots are dark brown to reddish brown while large brown spots are light, reddish-brown or gray in the middle surrounded by a dark to reddish-brown in the margin .Older spots may have a bright yellow halo surrounding the lesion. Disease development is also favoured by optimum temperature between 16 and 36°C and high relative humidity (86-100%). Leaves must be wet for at least 8-24 hours for infection to occur. Decrease in yield of rice due to brown spot epidemic in Bengal in 1942 was attributed to constant temperature of 20-30°C for two months, abnormally cloudy weather, and higher-than-normal temperature and rainfall at the time of flowering and grain-filling stages.

Originally the causal pathogen of brown spot in rice was classified in the *Helminthosporium* group, but was subsequently transferred to *Bipolaris*, which is characterized by bipolar germinating conidia. However (Della and Madonna, 2005) reported that it might be caused by *Drechslera* due to observance of intercalary germination in some of the isolates. *Drechslera* and *Bipolaris* species are important plant pathogens and are associated with symptoms of dark spots on leaves, and also root rot of seedlings. These fungi contain dark pigmentation which is due to the presence of a substance called melanin in their cell walls, which is measured as an important factor of virulence. The agent responsible for brown spot in rice (*Oryza sativa* L.) is *Bipolaris oryzae* (Breda de Haan) according to Shoemaker, 1959 [syns. *Drechslera oryzae* (Breda de Haan) as reported by Subramanian and Jain, 1966 and *Helminthosporium oryzae* (Breda de Haan) as reported by Miybabe & Hori, 1901]. According to (Ito & Kurib, 1942) the teleomorph of this species is *Cochliobolus miyabeanus* The generic name *Helminthosporium* is deeply entrenched in the literature of phytopathology, and separation of the graminicolous species into *Drechslera*, *Bipolaris,* and *Exserohilum* has not been accepted universally according to (Alcorn, 1988). It has been suggested that knowledge of the systematics of the two genera, *Drechslera*, and *Bipolaris*, is insufficient as per (Hawksworth, 1986). The classification of the causal agents of "*Helminthosporium*" diseases is based mainly upon morphological and, to a lesser degree, on biochemical and physiological characteristics according to (Matsumura *et al*., 1998). Some authors have also used molecular markers to study the relationship among these species according to (Bakonyi *et al*., 1995; Abadi *et al*., 1996) for more understandings.

Natural enemies that are used to control disease are called biological control. Biological control is an innovative, cost effective and eco friendly approach. Biological control is also an alternative to the use of harmful chemical pesticides. Biological fungicides may also be used to repress the population of the pathogenic organisms through competition with pathogenic organisms and also stimulate plant growth, which allows plants to quickly drive away any pathogen effects, or damage the pathogen by means of toxins produced according to (Cook, 2000; Gilreath, 2002). Bio control agents come from natural materials such as animals, plants, bacteria, fungi and certain minerals also. Pathogenic microorganisms affecting plant health are a major and chronic threat to food production and ecosystem stability worldwide. As agricultural production intensives over the past few decades, producers became more and more dependent on agrochemicals as a relatively reliable method of crop protection helping with economic stability of their operations. However, according to De Weger *et al*., 1995 and Gerhardson, 2002, increasing use of chemical fertilizers causes several negative effects such as, development of pathogen resistance to the applied agents and their non target environmental impacts. Furthermore, the growing cost of pesticides, particularly in less- affluent regions of the world, and consumer demand for pesticide-free food has made it necessary to a search for substitutes for these products. There are also a number of fastidious diseases for which chemical solutions are few, ineffective, or nonexistent as per (Gerhardson, 2002). Biological control is therefore being considered as an alternative way of reducing the use of chemicals in agriculture as reported by (De Weger *et al*., 1995, Gerhardson, 2002, Postma *et al*., 2003 and Welbaum *et al*., 2004).

There have been only a few reports on the improvement of Brown Spot control involving biological control agents. However, the use of antagonistic microbes for plant health management has emerged as a viable technology in the recent past. According to (Singh *et al*. 2005) commercially available antagonistic microbes, mostly belonging to the genera *Pseudomonas* and *Trichoderma*, can reduce the damage by direct effects on the pathogens (mycoparasitism, antibiosis, competition for iron) or by improving plant immunity (induced resistance, IR). Direct antagonism has been the key factor in suppression of many soil-borne pathogens, while IR is active against diverse foliar pathogens including both bacteria and fungi as reported by (Shoresh *et al*., 2010). Seed treatments with *Trichoderma viride* or *T. harzianum* have yielded up to 70 % disease reduction according to (Biswas *et al*., 2010b). Over 70 % disease reduction has been achieved from the use of selected *Pseudomonas* sp. Isolates according to (Joshi *et al*., 2007; Ludwig *et al*., 2009).

Not only fungi but research on Plant Growth-Promoting Rhizobacteria (PGPR) with non-legumes such as rice have shown beneficial effects through biological nitrogen fixation according to (Malik *et al*., 1997), increased root growth as per (Mia *et al*., 2012) with enhanced nutrient uptake as per (Yanni *et al*., 1997), phytohormone production according to (Chabot *et al*., 1996), plant growth enhancement stimulation by other beneficial bacteria and fungi according to (Saharan and Nehra, 2011) and disease control as per (Ramamoorthy *et al*., 2001). Peng *et al*. (2002), however, reported that rhizobial inoculation known for their symbiotic relationship with legumes, could also increase rice grain yield, but little is known about the mechanisms involved. The beneficial effects of the selected rhizobial isolates could be due to their plant growth-promoting abilities namely biological Nitrogen fixation, phosphate solubilization and plant growth regulator or phytohormone similar to the known valuable effects of PGPR according to (Boddey *et al*. in 1997; Verma *et al* in 2001; Araujo *et al*.in 2013 and Kloepper *et al*. in 1980). Also Yanni *et al.* (1997) have shown beneficial interactions of rhizobial isolates on growth of rice, which was believed to be due to increased root efficiency in water and nutrient uptake.

The role of soil organisms that are present below the ground and which interact with plant roots has gained increased attention in recent years according to (Reynolds *et al.*, 2003;van der Putten, 2003; Callaway *et al.,* 2004), and the interactions between beneficial and harmful pathogenic organisms have been known and identified as being particularly relevant due to their important implications for plant fitness according to (Schippers *et al.*,1987; Fitter and Garbaye, 1994;Bever, 2003).Penetration and establishment of fungus in the roots of host plant involves a complicated series of events and intracellular modifications according to (Bonfante-Fasolo and Perotto,1992).Symbiosis between plants and beneficial soil microorganisms like Arbuscular Mycorrhizal Fungi (AMF)are known to support plant growth and help plants to manage with biotic and abiotic stresses. Intense physiological changes take place in the host plant when roots are colonized by AMF affecting the interactions with different organisms below- and above-ground. Defensive effects of the symbiosis against pathogens, pests, and many parasitic plants have been reported for many plant species that includes agriculturally important crops. Other than those mechanisms such as improved plant nutrition, pathogenic competition and experimental proof supports a major role of plant defences in the observed defence mechanism. During establishment of mycorrhiza, accent of plant defence responses occurs thus attaining a functional symbiosis. As a result of this modulation, a gentle, but effective establishment of the plant immune responses are seen to occur, not only in local defence but also systemically. This activation leads to a prepared state of the plant that allows a more competent activation of defence mechanism in reaction to attack by potential enemies. Maiti *et al*. in 1995 earlier reported colonization by native AMF in rice plant. Earlier workers like Saha *et al*. in 1999 also reported incomplete dependency of upland rice on native AMF for phosphorus acquirement. Dubey *et al*. in 2008 also studied the occurrence of VAM fungi at altering stages of growth of rice plants.

Generally a plant responds to a pathogen by mobilizing a composite network of active defence mechanisms. The success of the plant in driving off the pathogenic attack depends upon the organization among the different defence strategies and the quickness of the response. It is usually believed that plants defend themselves against pathogenic fungi by producing certain fungitoxic substances such as phytoalexins, pathogenesis related (PR)-proteins, oxidized phenols and several other components. Also in most cases the role of a single defence component has been reported at a time while working on disease resistance of a host–pathogen system according to (Bera and Purkayastha**,** 1999).Plants usually display an antibiotic reaction (hypersensitive reaction) when they come across with a pathogenic fungi, and are known to produce phytoalexins which shows antifungal activity against the pathogenic fungus in the tissue around the reaction spot. Examples of phytoalexins include momilactones A and B, oryzalexins A, B, C, D, E, F, and S, sakuranetin, oryzalic acids A and B, oryzalides A and B, and phytocassanes A, B, C, and D (Japanese Patent Application No. 7-43520/1995) revealed by the present inventors.

The present investigation was undertaken taking into consideration many factors but the focus was mainly on study of defence response of rice plants with bioinoculants. The reason being that the on field observation as related to the symptomology on preliminary assessment indicated that the degree severity was more due to fungal pathogen causing brown spot causing crop loss. The goal of this investigation as apparent was to initiate an understanding of the dynamics of micro-flora dwelling in the rhizosphere of the rice plants and their state of interaction with prevailing pathogens affecting the rice plants. The present study was designed with an aims to explore the possibility of using beneficial bio inoculant, plant growth promoting fungi (PGPF) and arbuscular mycorrhizal fungi (AMF) isolated from rhizosphere of rice plants (*Oryza sativa* L.), specially cultivars grown in West Bengal and Sikkim hills for management of brown spot disease caused by fungal pathogen (*Drechslera oryzae*).

To evaluate and corroborate the goal certain objectives were outlined:

- Screening of resistance of rice cultivars towards *Drechslera oryzae*.
- Raising polyclonal antibody against *Drechslera oryzae* and their immunological detection and identification using immunoassays.
- Determination of level of phenolics and ascertaining their antifungal activity associated with rice cultivars to infection against *Drechslera oryzae*.
- Induction of resistance in rice plants against *Drechslera oryzae* using using bioinoculants (AMF, PGPF and PGPR).
- Evaluation of defence enzymes [Phenylalanine ammonia lyase (PAL), Chitinase (CHT), β-1,3 Glucanase (β GLU) and Peroxidase (POX)] in rice cultivars following induction of resistance.
- Cellular localization of chitinase and glucanase in rice leaf following induction of resistance using bioinoculants.
- Extraction of phenolics and antifungal compound and their evaluation in rice cultivar following induction of resistance against *D. oryzae* using bioinoculants.