Chapter 2
REVIEW OF LITERATURE

Rice being one of the most important cereal crops for all over the world is the seed of the grass species *Oryza sativa* (Asian rice) or *Oryza glabarrima* (African rice). Almost half of the population of the world feeds on rice and it also adds for more than 50% of the regular calorie intake (Maclean *et al*., 2002). It is the staple food for more than three billion peoples all around the world. Rice is grown in almost 114 countries all over the world and more than 50 countries have an annual production of 100,000 tons or more. In comparison to South America and Africa where rice is consumed in equal quantity to wheat and maize but here in Asia rice is the most highly consumed staple food in their daily life. In worldwide production on agricultural commodities rice stands out to be in the third highest position just after sugarcane and maize according to FAO (2012). Grains such as maize and wheat serves for other various purposes other than human food but rice serves as a valuable grain that adds up to the nutritional value and input of calorie accounting for almost one fifth of the calorie taken by humans worldwide.

In the pass thirty years due to advancement in the cultivation techniques of rice farming and availability of much improved high yielding rice varieties the rate of production has been almost doubled but unfortunately it is still being difficult to meet up to the needs of the worldwide rapidly increasing population (Fischer *et al*., 2000; Sasaki and Burr, 2000).

In India almost all the state produces rice but the cultivation is mainly carried out in low lying coastal areas, deltas and river valleys. Seven major rice producing states are: West Bengal, Uttar Pradesh, Andhra Pradesh, Punjab, Tamil Nadu, Odissa and Bihar. These states produce more than half of the total rice produced in the whole country. West Bengal is the leading producer of rice in India and accounts for 14 % of total rice produced in the country. The major rice producing districts in the state are Bardhaman, Medinipur, North and South 24 Parganas. East and West Midnapur, Jalpaiguri, Bardhaman, Bankura, Birbhum, North and South Dinajapur etc. Around the world there are nearly 40,000 different varieties of rice under the species name *Oryza sativa* with a total of about 74 varieties are from India. Most of the varieties in the

hands of the farmers have not yet been studied which still the potential to compete with the now has advanced varieties with organic mode only.

Table1 . Nutrient content of rice per 100 gm portion (Nutrient data laboratory).

Nutrient components	**Value per 100 gm**
Water (gm)	12
Energy (KJ)	1528
Protein (g)	7.1
Fat (g)	0.66
Carbohydrate (g)	**80**
Fibre (g)	1.3
Sugar (g)	0.12
Calcium (mg)	28
Iron (mg)	0.8
Magnesium (mg)	25
Phosphorus (mg)	115
Potassium (mg)	115
Sodium (mg)	5
Zinc (mg)	1.09
Copper (mg)	0.22
Manganese (mg)	1.09
Vitamin (mg)	0.1

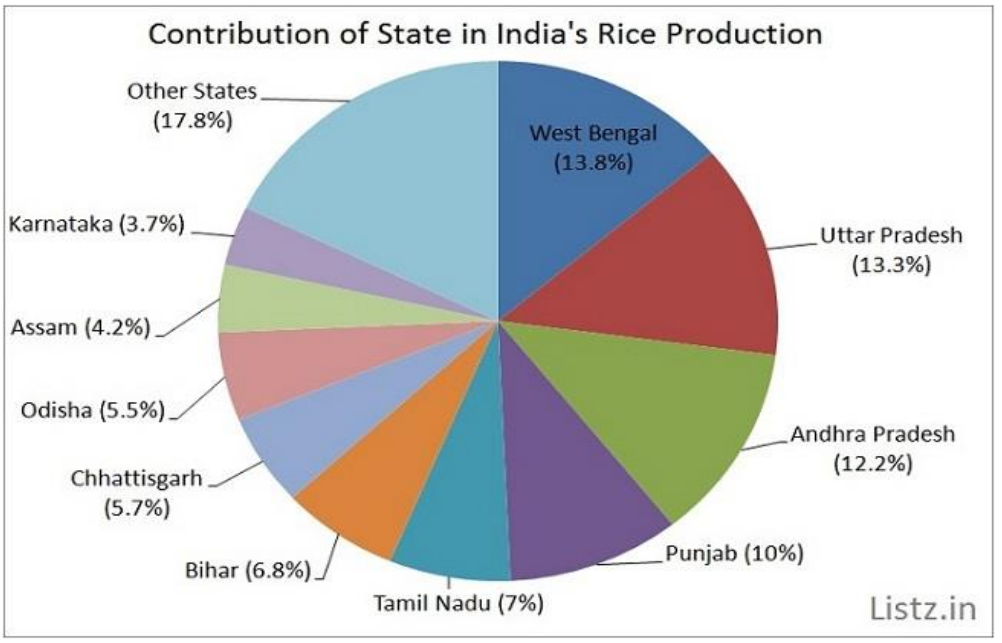


Figure 1. Contribution of State in India's Rice Production. Data collected from: www.wikipedia.com

Diseases

The geographical distribution and the seasonal development of any plant diseases is mostly influenced by the climatic condition, weather and the soil factors like humidity, moisture, temperature, pollutant, pH of the soil, light etc.(Jones, 1924).Severe damage can be caused to the rice plant by the pathogens that often may result in the reduced productivity of food grain. Some of the rice diseases commonly found in nurseries and in the field are given.

Table 2. Some common rice disease

Nursery diseases	**Main Field Diseases**
Blast – *Pyricularia grisea*	Brown Spot – *Helminthosporium oryzae*
Bacterial Leaf Blight – *Xanthomonas oryzae*	Sheath Rot – *Sarocladium oryzae*
Rice Tungro disease – *Rice tungro virus*	Sheath Blight – *Rhizoctonia solani*
	False Smut – *Ustilagonoidea virens*
	Grain discolouration – Fungal complex
	Leaf Streak – *Xanthomonas oryzae pv. oryzicola*

Data collected from:Crop Protection, agritech.tnau.ac.in.

Rice Brown Spot

Rice brown spot is the most aggressive and important rice disease in the world affecting millions of hectares of land every year (Chakrabarti, 2001; Padmanabhan, 1973; Savary *et al.*, 2000a; Zanao Junior *et al*., 2009). Brown spot prevails in almost every place where rice is grown especially in China (Singh, 2005). It can reach upto such a severe state in cool summer and soil deficiency of nitrogen that it may cause a loss in the yield and reduction in the weight of the kernel and the total number of grains the panicle bears (Mew and Gonzales, 2002).This conditions are usually associated with poor farmers with much less resources (Ou, 1985; Zadoks,1974).High humidity level (> 92.5%),leaf wetness and favourable temperature (24-30^{O} C) are favourable condition for disease development (Picco and Rodolfi, 2002).Spores can be transported to other parts of the same plant or other plants by wind and rain. Brown lesions formed in the leaf can reduce nutrient uptake and areas of photosynthesis which ultimately results in the decrease of tillering nodes. Brown spot of rice may be caused by a combined effect of physiological disorders, processes involved in the disease development and mechanisms for disease resistance which brings about a genuine questions regarding the involvement of different metabolic pathways their bases of genetics and interacting genes and cluster of genes (Igawa *et al*., 2005; Timmusk and Wagner, 1999).

Importance of Brown Spot

Two major epidemics has been associated with Brown spot in India: First in Krishna-Godawari delta 1918-1919 and second in today's India and Bangladesh in 1942 (Chakrabarti, 2001). The latter case is associated with Great Bengal Famine (Chakrabarti, 2001; Padmanabhan, 1973). It is a point to note that the disease epidemics and the famines caused are never simple as because the whole process is a combined effects of many consequences (Chakrabarti, 2001; Zadoks, 2008).

Rice brown spot is still reported widely from all across India (Reddy *et al*., 2010) and also more frequently in South and South- East Asian countries (Savery *et al*., 2000a) .It also causes losses in the crop yield upto a limit of 10% in whichever area it occurs (Savery *et al*., 2000b, 2006) especially in the lowlands of tropical and Sub-tropical Asia. This result proves Brown spot to be the strongest disease resulting in the decrease of yield of rice so far. Also as because nowadays the drought conditions all over the world is becoming very serious problems the indication of increase of severity

of brown spot disease has also been found(Savary *et al*., 2005) which is due to the variations in rainfall.

A rapid decrease in the grain yield due to brown spot has been reported which may vary from 4 to 29% (Bedi and Gill, 1960) 12 % (Aluko, 1975) about 8 to 23% (Fomba and Singh, 1990) and about 26 to 52% as reported by (Chakrabarti, 2001).The increase in the range of the later figures shows the effects in the yield losses due to the infection. Grains that are intensely infected are not at all ready for the human intake which almost explains the effect of brown spot on the Great Bengal Famine. Rice brown spot caused by *Drechslera oryzae* (Breda de Haan), Subram. And Jain has two distinct types of symptoms, first is leaf spot and the other one grain discolouration (Drechsler, 1934).

Host plant Resistance

In nearly all natural habitats, plants are surrounded by an massive number of potential enemies (biotic) and a variety of abiotic environmental stress. Roughly all ecosystems contain a wide variety of bacteria, viruses, fungi, nematodes, mites, insects, mammals and other herbivorous animals, significantly responsible for heavy diminution in crop yield. In natural systems, plants face a surplus of antagonists and thus posses a innumerable defence and have evolved multiple defence mechanisms by which they are able to deal with various kinds of biotic and abiotic stress according to (Ballhorn *et al*., 2009). Moreover antimicrobial nature, some of which are performed and some of which induced by infection. There are various other modes of defence including the building up of polymeric barriers to pathogen penetration and the synthesis of enzymes that degrade pathogen cell wall according to (Hammond *et al*., 1996). A very powerful and cost effective method to deal with Brown spot is development of disease resistance in plants. Despite of the severity of brown spot and its importance not much consciousness has been given towards this disease rather than leaf blast and bacterial blight (Savery *et al.*, 2011).

Various Source of resistance

It has been a very long period of continuous efforts regarding the search for different sources of resistance towards Brown spot (Chakrabarti, 2001; Nagai and Hara, 1930). Indentified fifteen different Oryza sativa entries out of one hundred and twenty four were classified as resistant (> 5% severity) according to Satija *et al.* (2005). Also

Hossain *et al*. (2004) identified one resistant variety out of 29 entries. After screening of upland rice germplasm which are exotic and indigenous to Eastern India has proved that several field genotypes has expressed partial and complete resistance to the brown spot pathogen (Shukla *et al.*, 1995). It appears that the sources of disease resistance is very few in case of *Oryza sativa* entries and as per recent studies such as Goel *et al.* (2006) search for other pools is recently being practised specially *Oryza nivara*.

Biological control of Brown spot

A very few research works has been done for the advancement of the use of bio control agents in suppression of brown spot. Moreover, there has been a rapid emergence of viable technology regarding the application of antagonistic microbes for the management of plant health in recent years. *Pseudomonas* and *Trichoderma* are the most commercially used antagonistic microbes that has the power for the reduction of the disease by direct effects on the pathogen such as antibiosis, mycoparasitism ,competition for iron or by the improvement of plant immunity system such as induced resistance (IR) (Singh *et al*., 2005).Many soil borne pathogens are suppressed by direct antagonism whereas Induced resistance is effective against broad range of foliar pathogens which includes both bacteria and fungi (Soresh *et al.*, 2010).

The use of fungicides to control the disease causes several unpleasant effects i.e. development of resistance in the pathogen, residual toxicity, pollution in the environment, high cost etc. Therefore, it has become essential to adopt eco friendly approaches for better crop health and for yield. According to (Mason and Mathew, 1996; Singh, 1994), the practical use of natural compounds as control agents is receiving increased attention and this is partly due to their non-toxicity to humans, their systemicity and biodegradability. Investigations on mechanisms of disease suppression by plant products have suggested that the active principles present in them may either act on the pathogen directly as per (Amadioha, 2000) or induce systemic resistance in host plants resulting in reduction of disease development according to (Narwal *et al*., 2000; Olivieri *et al*., 1996; Paul and Sharma, 2002; Schneider and Ullrich, 1994). Also (Hammerschmidt and Kuc, 1995) reported that, induction of plant's defence genes by prior application of inducing agents is called induced resistance When plants are treated with non-pathogenic or some less harmful pathogens [eg. rhizobacteria-induced systemic resistance (RISR)], it then go onto triggers the production of defence-related gene products according to (Harman *et al.*, 2004). The defense gene products include

peroxidise (PO), polyphenol oxidase (PPO) that catalyze the formation of lignin and phenylalanine ammonia-lyase (PAL) that is involved in phytoalexins and phenolics synthesis according to (Ramamoorthy *et al*., 2002) . So, these are the enzymes whose activity needs to be increased to prevent fungal diseases like brown spot and this can be done by application of various bioinoculants.

Plant growth promoting rhizobacteria (PGPR)

According to (Glick, 1995; Hallman *et al*., 1997; Rovira, 1965; Sturz *et al*., 2000; Welbaum *et al*., 2004), biocontrol agents are liable to interact with other disease management elements, especially host plant resistance, as well as plant growth related or abiotic stress related, genes: research leading to an perceptive of such interactions at the molecular level could in itself provide light on the physiology of environmental stresses, of disease and their interactions. There has been a huge amount of literature describing potential uses of plant associated bacteria as agents stimulating plant growth and managing soil and plant health .Plant growth-promoting bacteria (PGPB) according to (Saha *et al*., 1999) are associated with many plant species and are commonly present in many environments. The most widely studied group of PGPB are plant growth-promoting rhizobacteria (PGPR) as reported by (Kloepper and Schroth, 1978) colonizing the root surfaces and the closely adhering soil boundary, the rhizosphere as per (Kloepper and Schroth, 1978; Kloepper *et al*., 1999). As reported by Kloepper *et al*. (1999) or, more recently, by Gray and Smith (Gray and Smith, 2005), some of these PGPR can also enter root interior and establish endophytic populations. Many of them are able to go beyond the endodermis barrier, crossing from the root cortex to the vascular system, and subsequently flourish as endophytes in stem, leaves, tubers, and other organs according to (Bell *et al*., 1995; Compant *et al*., 2005; Gray and Smith, 2005; Hallman *et al*., 1997).

According to (Gray and Smith, 2005; Hallman *et al*., 1997), the degree of endophytic colonization of host plant organs and tissues shows the ability of bacteria to selectively adapt to these specific ecological niches. Accordingly, close associations between bacteria and host plants can be formed as reported by (Compant *et al*., 2005; Hallman *et al*., 1997, Kloepper *et al*., 1999) without harming the plant as per (Hallman et al.,1997, Kloepper *et al*.,1992; Kloepper *et al*.,1999; Lodewyckx *et al*., 2002; Whipps, 2001). Even though, it is generally believed that several bacterial endophyte communities are the result of a colonizing process initiated in the root zone according to

(McInroy and Klopper, 1995; Sturz *et al*., 2000; Van Peer *et al*., 1990; Welbaum, 2004), they may also instigate from other source than the rhizosphere, such as the phyllosphere, the anthosphere, or the spermosphere according to (Hallman *et al*., 1997). Regardless of their different ecological niches, free-living rhizobacteria and endophytic bacteria use some of the same mechanisms to promote plant growth and control phytopathogens as reported by (Bloemberg *et al*., 2001; Dobbelaere *et al*., 2003; Glick, 1995; Hallman *et al*., 1997, Lodewyckx *et al*., 2002; Sturz *et al*., 2000).

The broadly recognized mechanisms of bio control mediated by PGPB are competition for an ecological niche or a substrate, production of inhibitory allele chemicals, and induction of systemic resistance (ISR) in host plants to a broad spectrum of pathogens according to (Glick, 1995; Haas *et al*., 2000; Haas *et al*., 2002; Lugtenberg, 2001; Ryu *et al*., 2004a) and/or abiotic stresses (Mayak, 2004; Nowak and Shulaev, 2003). According to (Thomashow, 1996) despite their potential as low-input practical agents of plant protection, relevance of PGPB has been hampered by incompatible performance in field tests; this is usually attributed to their poor rhizosphere proficiency as reported by (Schroth, 1981 and Weller, 1988). Rhizosphere competence of biocontrol agents comprises effective root colonization combined with the ability to survive and proliferate along growing plant roots over a considerable time period, in the presence of the indigenous microflora according to (Lugtenberg and Dekkers, 1999; Parke, 1991; Weller, 1988; Whipps, 1997). Given the importance of rhizosphere competence as a precondition of effective biological control, understanding root-microbe communication as per (Bais *et al*., 2004), as affected by genetic according to (Kilic-Ekici and Yuen, 2004; Okubara *et al*., 2004) and environmental as per (Pettersson and Baath, 2004) determinants in spatial as per (Bais *et al*., 2004) and temporal as per (Ping and Boland, 2004) contexts, will significantly contribute to improve the efficacy of these biocontrol agents.

According to (Kerry, 2000; Ping and Boland, 2004; Ramamoorthy *et al*., 2001; Ryu *et al*., 2004b; Thomashow, 1996) biopriming plants with some PGPB can also endow with systemic resistance against a broad spectrum of plant pathogens. Diseases of fungal, bacterial, and viral origin and in some instances even damage caused by insects and nematodes can be reduced after application of PGPB. Certain bacteria trigger a phenomenon known as ISR phenotypically similar to systemic acquired resistance (SAR). SAR develops when plants successfully activate their defence

mechanism in response to primary infection by a pathogen, notably when the latter induces a hypersensitive reaction through which it becomes limited in a local necrotic lesion of brown, desiccated tissue as reported by (Van Loon, 1998). As SAR, ISR is effective against different types of pathogens but differs from SAR in that the inducing PGPB does not cause evident symptoms on the host plant according to (Van Loon, 1999).

Plant growth promoting fungi (PGPF)

Free-living fungi such as *Trichoderma* spp. are common in soil and root ecosystems. Recent discoveries show that they are opportunistic, avirulent plant symbionts, as well as being parasites of other fungi. At least some strains establish robust and long-lasting colonizations of root surfaces and penetrate into the epidermis and a few cells below this level. They generate or liberate a diversity of compounds that induce localized or systemic resistance responses, and this explains their lack of pathogenicity to plants. These root–microorganism associations cause substantial changes to the plant proteome and metabolism. Plants are protected from numerous classes of plant pathogen by responses that are similar to systemic acquired resistance and rhizobacteria-induced systemic resistance. Root colonization by *Trichoderma* spp. also frequently enhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients.

The induction of resistance in plants by *Trichoderma* spp. has been inadequately studied compared with the responses that are induced by rhizobacteria, possibly because the *Trichoderma* research community has focused on factors that are connected with direct effects on other fungi, especially mycoparasitism and antibiosis. That was probably the first clear expression of induced resistance by *Trichoderma* (Bigirimana *et al.,* 1997*)* .They showed that treating soil with *Trichoderma harzianum* strain T-39 made leaves of bean plants resistant to diseases that are caused by the fungal 41 pathogens *B. cinerea* and *Colletotrichum lindemuthianum*, even though T-39 was present only on the roots and not on the foliage. The same group extended their findings from *B. cinerea* to other dicotyledonous plants reported by (De Meyer *et al*., 1998). Analogous studies have now been carried out with a wide range of plants, including both monocotyledons and dicotyledons, and with different *Trichoderma* species and strains. The ability of *T. harzianum* strain T-22 to induce systemic resistance is particularly notable to pathogens in maize as there are, so far no similar reports of

resistance being induced in this crop by any other root associated commensal or symbiotic microorganism. As a result, the capacity to induce resistance to a range of diseases — which are caused by diverse classes of plant pathogen (including fungi, bacteria and viruses) — in a variety of plants seems to be widespread in this fungal genus.

Constant, improper and non-discriminative use of chemicals is known to cause unwanted effects such as residual toxicity, development of pathogen resistance to fungicides, environmental pollution, health problems to humans and animals and increased expenditure for plant protection. As an alternative, plant pathologists have focused their attention to developing environmentally safe, long-lasting and effective biocontrol methods for the management of plant diseases. Among various fungal and bacterial biocontrol agents, *Trichoderma* spp. was most frequently used against various plant diseases. Research during the previous two decades has led to the possibility of biological control as an increasingly realistic option for rice disease management according to (Tsahouridou and Thanassoulopoulosh, 2002). This organism has been shown to be efficient for the control of brown spot disease and the increase of plant growth on rice according to (Harish *et al.*, 2007). Rice plants sprayed with spore suspension of *T. harzianum* obtained a significant reduction in the sternness of disease under greenhouse conditions as reported by (Abdel-Fataah *et al*., 2007). Also, these species are able to colonize the root surface and rhizosphere from the treated seeds, protecting them from fungal diseases and stimulate plant growth and productivity according to (Baker, 1988).

As reported by (Abdel-Fattah *et al*., 2007) direct foliar application of *T. harzianum* was also found to reduce the disease severity and appreciably improve grain yield, total grain carbohydrate and protein, in addition to a significant improvement in the total photosynthetic pigments in rice leaves .The use of *Trichoderma* spp., well-known mycoparasites, can help by improving nutrients uptake and mobilization, enhancing nitrogen use efficiency, promoting root growth and plant biomass, and improving tolerance to various physiological stresses, including soil salinity and drought through the reduction of oxidative damage that stresses cause according to (Harman, 2011; Shoresh *et al*., 2010). Use of these microbes could suppress disease through direct antagonism against the pathogen because imbalanced plant nutrition and drought stresses are predisposing factors for Brown Spot development. This effect

would be combined with improved plant nutrient supply and delayed onset of water shortage in plant tissues according to (Bae *et al*., 2009) varying plant physiology to the disadvantage of the pathogen.

Arbuscular Mycorrhizal Fungi (AMF)

Arbuscular mycorrhizas are mutualistic associations which are formed in between the roots of 80 percent of terrestrial plant species and fungi belonging to the small phylum Glomeromycota (Schubler *et al.*, 2001). The word symbiosis is named after the Greek word "mycos" and "rhiza" which means "fungus-root" and it is probably the oldest and most widespread plant symbiosis on Earth. However, through the fossil records and phylogenetic evidence it shows that their existence is more than 459 million years as in accordance to (Smith and Read, 2008), indicating a selection advantage for both the partners. The fungi forming the arbuscular mycorrhiza (AMF) are obliging biotrophs requiring the host plant for the completion of their life cycle. The fungus colonizes the root cortex and forms intracellular structures called arbuscules (from the Latin "arbusculum", meaning bush or little tree) where the exchange of nutrients between the partners takes place. The extracellular hyphal network spreads widely into the surrounding soil, thereby reaching out of the nutrient depletion zone and improving the supply of inorganic nutrients, especially phosphate and nitrate (Smith *et al*., 2011). In return, receiving the photosynthates from the heterotrophic fungal partners or the host plant (Smith and Smith, 2011).

According to (Mohadevan *et al*., 1988) vesicular arbuscular mycorrhizal fungi forms symbiotic association with roots of most terrestrial plants including many agricultural crops. These are known to occur worldwide in a broad range of unlike environments from artic to tropic and occupy a wide range of ecological niches as per (Shrivastava *et al*., 1996). The role of VAM fungi in the improvement of crop plant is well recognized as reported by (Krishna and Bagyaraj, 1982; Katiyar *et al*., 1994; Rao *et al*., 1995). According to (Bagyaraj and Varma, 1995), VAM fungi are known to improve the nutrient status of the plants, increase growth and development protects plant against pathogen and gives fight to drought and salinity.Colonization by native AMF in rice plant has been reported earlier by (Maiti *et al*., 1995). Partial dependency of upland rice on native AMF for phosphorus acquisition has also been reported by earlier worker such as (Saha *et al*., 1999). The occurrence of VAM fungi at altering stages of growth of rice plants has been studied by (Dubey *et al*., 2008). In recent years,

the application of artificially produced inoculum of VAM fungi has increased its significance in the field of agriculture, horticulture and forestry. Application of mycorrhizal inoculum increased the soil nutrients and root colonization in rice plants as reported by earlier worker such as (Yeasmin *et al*., 2008).

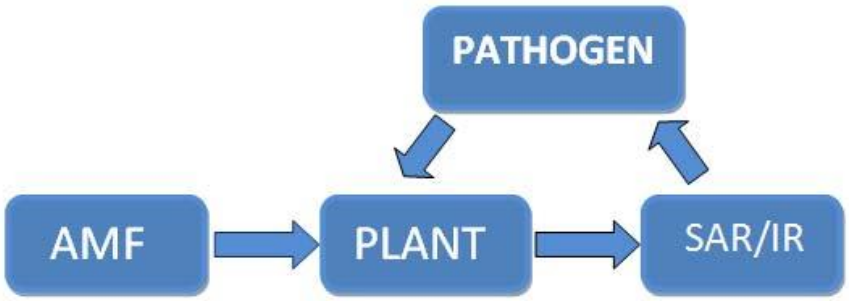
. The ancient mutualist and biotroph of plants, Arbuscular mycorrhizal fungi (AMF), has been found to improve the supply of water and nutrients, such as phosphate and nitrogen to its host plant. In its exchange, it takes a part of photosynthate sugar to complete its life cycle. Although having its own immune system, the plant upon pathogen attack gets weaken and needs reinforcement to fight back and become stabilize in the battle ground. AMF fulfils the need of host plant and provides with support in many ways by induction of attenuated defence signaling for fighting against phytopathogen. This increase not only makes plant more tolerant towards the attack of phytopathogen but also, enhances the genetic, biochemical and signaling factors responsible for its defence purpose.

According to (Kachroo and Kachroo, 2009), it is well known that the plants fights with phytopathogens by means of their own immune system (IS). These comprise physical and chemical barriers and several active mechanisms performed. IS is further divided into primar and secondary immune system. The primary immune system involves interaction of strain- specific avirulent (AVR) protein from the pathogen with a associated plant resistance (R) protein according to (Dangl and Jones, 2001). This in turn initiates systemic acquired resistance (SAR) in systemic tissues to provide with immunity against the secondary infections by related and unrelated pathogens according to (Durrant and Dong, 2004; Klessig *et al*., 2009). A further mode of secondary immunity, termed induced systemic resistance (ISR), is activated upon colonization of plant root by non-pathogenic rhizosphere microbes according to (Van Loon *et al*., 1998). Very often it has been observed that despite having their own defence system, plants require support to fight against phytopathogens. This may be due to slow response or low induction level of defence related factors. According to (Schubler *et al*., 2001), AMF have long been known as a dominant among symbionts of plants. They belong to the phylum, Glomeromycota and colonize 70–90% of land plant species according to (Smith and Read, 2008).

Although the colonization specificity does matter but not much, depending upon many factors including the genotype of the host plant as reported by (Koide and

Schreiner, 1992; Meghvansi *et al*., 2008). There are several benefits of AMF colonization in plants, mainly the increase in nutrient uptake according to (Smith and Read, 2008). In spite of this, still there is a doubt that the AMF has any direct involvement in the host's defence signaling against phytopathogens. Although, there are some indirect functions which donate to strengthen the plant defence responses including rise of plant nutrition as reported by (Smith and Read, 2008) and damage compensation. Moreover, it includes anatomical alterations in the root system according to (Wehner *et al*., 2010), microbial changes in the rhizosphere and enhancing the attenuated plant defence responses by altering the host's signaling pathways according to (Pozo and Azcon-Aguilar, 2007). Defense strategies by VAM are carried primarily through modulation in Jasmonic acid (JA) and salicylic acid (SA) dependent pathways (Pozo and Azcon-Aguilar, 2007). AMF is also reported to play a significant role in inducing the hydrolytic enzymes in defense response (Pozo *et al*., 1999), increased levels of pathogenesis- related (PR) proteins, various phytoalexins (Harrison and Dixon, 1993; Morandi, 1996; Larose *et al*., 2002), accumulation of callose (Cordier *et al*., 1998) and generation of reactive oxygen species (Salzer *et al*., 1999). Therefore, several records have been discovered which potrays the ability of AMF in controling and reducing the severity and incidence of phytopathogens for a longer duration. Also it is a well known fact that the knowledge reagarding the mechanism behind it is very little.

Interaction of AMF induced plant defence responses (SAR/IR) with the pathogen



Colonization of the root system by arbuscular mycorrhizal fungi (AMF) can improve plant resistance as well as tolerance to biotic stresses. Moreover this bioprotection has been adequately described in different plant systems, but the mechanisms still remains mainly unknown. Moreover, experimental evidences on mechanisms such as improved plant nutrition and competition, supports the association

of plant defence mechanisms in the observed protection. During the establishment of the mycorrhiza, inflection of plant defence responses occurs in response to the detection of the AM Fungi in order to attain a functional symbiosis. As a result of this inflection, a mild but effective establishment of the response of plant immunity might take place not only locally but systemically also. This establishment provides a protective state of the plant that allows a more effective establishment in response to the danger by the harmful enemies.

The basis of the success behind the interaction that has taken place during the course of evolution is the mutual benefits, which is however ensured through a strictly bidirectional control of the mutualism (Kiers *et al*., 2011). With regards to the plant, this guideline shows vital alteration in both the primary and the secondary metabolism in result regulating the defense mechanism in the plants (Harrison, 1999; Hause and Fester, 2005). However these alterations have n immense impact on the physiology of the plant that alternates the capacity of the plant to handle the stresses. Earlier works on mycorrhizas have shown the increase in the growth as well as the yield of the mycorrhizal plants, which might be considered mostly due to the increased levels of nutrition of the plant (Linderman, 1994). Also, in the later times many other researchers showed an extremely better tolerance of the mycorrhizal plants to abiotic stresses, such as salinity, presence of the heavy metals, or drought (Miransari, 2010; Smith *et al*., 2010). It has also been proved that the mycorrhizal plants provide a better resistance to the soil borne fungal as well as the bacterial pathogens, nematodes, or root- chewing insects (Azcón-Aguilar and Barea, 1997; Whipps, 2004).In the last few years it has

also been recorded that the mycorrhizal plants can develop induced resistance in response to the shoot pathogens(Pozo and Azcón-Aguilar, 2007; Koricheva *et al*., 2009; Campos Soriano *et al*., 2012).

The persistence of mycorrhizas during the course of evolution can be considered as one of the important phenomenon for the need of assistance in overcoming stressfull conditions, even in the plant systems where the symbiosis dosenot provide any growth benefits(Newsham *et al*., 1995).As because of the properties such as of biofertilizer and its bioprotectivity mycorrhizal symbiosis has proved to become a subject of main focus for researchers as an alternative to the chemical fertilizers and also pesticides with regard to sustainable agriculture(Harrier and Watson, 2004; Mukerji and Ciancio, 2007; Fester and Sawers, 2011).

Despite the obvious benefits of an improved nutritional status for stress tolerance/ resistance, mineral supply experiments have shown that the protective effect observed in mycorrhizal plants cannot be attributed to improved nutritional status alone (Fritz *et al*., 2006; Liu *et al*., 2007). AM associations bring about significant changes in the host plant and its environment: at the rhizosphere level, they influence soil structure, carbon deposition in soil, and microbial diversity, in part through changes in root exudation. These shifts in the microbial communities of the rhizosphere may indirectly influence the out-come of plant interactions with other organisms, including pathogens and beneficial microbes (Berta *et al*., 2002; Barea *et al*., 2005; Artursson *et al*., 2006; Lendzemo *et al*., 2007; Cipollini *et al*., 2012; Effmert *et al*., 2012). Apart from the changes in the rhizosphere, multiple modifications also occur within the host plant.

In the roots, changes in architecture, alterations of the metabolic profile, and accumulation of certain defence compounds may occur (García Garrido and Ocampo, 2002; Strack *et al*., 2003; Hause *et al*., 2007; Schliemann *et al*., 2008; Péret *et al*., 2009; López Ráez *et al*., 2010 a, b). For example, the accumulation of apocarotenoids (cyclohexenone and mycorradicin derivatives) can be observed in mycorrhizal roots, which are the main component of the yellow pigment found in many plant species upon colonization by AMF and have been proposed to play a role in control of the degree of colonization and mycorrhizal functionality (Strack *et al*., 2003; Strack and Fester, 2006; Flob *et al*., 2008; Schliemann *et al*., 2008).Qualitative and quantitative changes in flavonoid contents have been observed, the changes depending on the host plant, AMF, and developmental stage of the symbiosis (Vierheilig and Piché, 2002; Akiyama *et al*., 2002). Changes in phenolic compounds, defence-related phytohormones, and reactive oxygen species also have been reported (Fester and Hause, 2005; López-Ráez *et al*., 2010a, b). Noteworthy, the symbiosis also has a considerable impact on the aerial parts of mycorrhizal plants, some of the reported changes being related to defense or stress tolerance (Liu *et al*., 2007; Kaschuk *et al*., 2009; Fiorilli *et al*., 2009; Pozo *et al*., 2009; Fester *et al*., 2011; Aloui *et al*., 2011).

As for the higher resistance to pests and pathogens of AMF-colonized plants, observations of systemic protection against pathogens in non-colonized root fragments from mycorrhizal plants and enhanced resistance of the aerial parts to certain attackers have pointed out the involvement of plant defense mechanisms (Cordier *et al*., 1998; Pozo *et al*., 2002; Pozo and Azcón-Aguilar, 2007). Defence mechanisms are

coordinated by the plant immune system, strikingly similar in some aspects to the innate immune system in animals (Ausubel, 2005). This system allows the plant to distinguish non-self-alien organisms by recognizing structurally conserved microbe-associated molecules, such as flagellin, lipopolysaccharides, or peptidoglycans, which are collectively, termed microbe-associated molecular patterns (MAMPs, or PAMPs in the case of pathogens). PAMPs are recognized by transmembrane pattern recognition receptors (PRRs), which leads to the induction of the appropriate responses in the host and to PAMP-triggered immunity (PTI) (Ausubel, 2005; Jones and Dangl, 2006; Boller and He, 2009; Thomma *et al*., 2011).

In an evolutionary “arms race," microbes have evolved effector proteins that are secreted into the host and suppress PTI, thus allowing successful host colonization by the pathogen, thus causing effector-triggered susceptibility of the plant to the disease. In some cases, intracellular proteins of the plant recognize pathogen effectors or their modified target proteins and activate immune responses that are quicker, more prolonged, and more robust than those in PTI, resulting in effector-triggered immunity (ETI) (Jones and Dangl, 2006; Boller and He, 2009; Thomma *et al*., 2011). Plant defense responses are coordinated by small molecules that act as signal transducers and tailor the coordinated expression of genes that code for defence-related proteins and compounds (Ausubel, 2005; Jones and Dangl, 2006). Among these molecules, the phytohormones jasmonic acid (JA), salicylic acid (SA), abscisic acid (ABA), and ethylene (ET) play key roles (Pieterse *et al*., 2009). According to the challenger lifestyle, one signalling pathway will prevail over the others.

It is generally assumed that the SA-dependent pathway regulates responses such as programmed cell death, effective against biotrophic organisms, and the JA-dependent pathway regulates responses to necrotrophs and chewing insects (Glazebrook, 2005). However, these hormone signalling pathways do not act independently, but influence each other through a complex network of regulatory interactions, JA and SA pathways in general being mutually antagonistic (Pieterse *et al*., 2009). As biotrophs, mycorrhizal fungi share some similarities with biotrophic pathogens, and are able to trigger plant defence responses at initial stages (Paszkowski, 2006).

Thus, for a successful colonization, the fungus has to cope with these reactions and actively modulate plant responses. We have proposed that this modulation may result in pre-conditioning of the tissues for efficient activation of plant defences upon a

challenger attack, a phenomenon that is called priming (Pozo and Azcón-Aguilar, 2007). Priming sets the plant in an "alert" state in which defences are not actively expressed but in which the response to an attack occurs faster and/ or stronger compared to plants not previously exposed to the priming stimulus, efficiently increasing plant resistance. Thus, priming confers important fitness benefits (Conrath *et al*., 2006; Van Hulten *et al*., 2006; Walters and Heil, 2007).

In the past decade, many priming causing agents have been identified. It has been observed that some chemicals that induce stress responses in plants also induce priming when applied at lower doses, and several fungicides have been shown to prime defenses in treated plants in addition to their primary antifungal activity (Conrath *et al*., 2006; Beckers and Conrath, 2007). Other well-studied examples of priming by chemicals include increased resistance to downy mildew in Arabidopsis thaliana after treatment with the non-protein amino acid Baminobutyric acid (BABA), as well as primed defence responses in tomato and Arabidopsis pre-treated with hexanoic acid and subsequently infected with grey mold (Ton *et al*., 2005; Vicedo *et al*., 2009; Kravchuk *et al*.,2011). Remarkably, priming events occur as a result of inter individual or even inter-species communication. For example, green leaf volatiles released by wounded or infested plants are also able to induce a more efficient activation of defences in neighbouring plants upon subsequent attacks (Kessler *et al*., 2006; Ton *et al*., 2007; Yi *et al*., 2009).

In Arabidopsis seedlings exposed to volatile blends from two Bacillus species, the disease severity caused by a bacterial pathogen was significantly reduced (Ryu *et al*., 2004). Moreover, priming seems to be the mechanism underlying the Induced Systemic Resistance (ISR) observed in plants interacting with beneficial microorganisms (Conrath *et al*., 2006; Goellner and Conrath, 2008; Van Wees *et al*., 2008). Interestingly, priming of the plant immune responses by beneficial microbes is often dependent on a functional JA signaling pathway, as has been described for rhizobacteria and AMF (Verhagen *et al*., 2004; Pozo *et al*. 2004, 2010; van der Ent *et al*., 2009). The molecular mechanisms behind priming of plant defences and its biological relevance in plant resistance are now being uncovered (Pastor *et al*., 2012), and evidence for trans-generational effects of priming have been a major advance in plant research (Luna *et al*., 2012; Rasmann *et al*., 2012; Slaughter *et al*., 2012). Here, we give a summary of the impact of the arbuscular mycorrhizal symbiosis on plant

interactions with other organisms. We give special emphasis to the spectrum of protection against deleterious organisms (Mycorrhiza-Induced Resistance, MIR) and provide an overview of the underlying mechanisms, focusing on the priming of plant defences associated with mycorrhization.

Systemic protection by a mycorrhizal association can even be observed in the aerial parts of a colonized plant, but in contrast to below-ground interactions, reports on AM effects on pests and pathogens attacking shoots are less studied, and the outcome of the interaction is more variable. Early studies described a higher susceptibility of AM plants to viruses, and biotrophic pathogens appear to thrive better on mycorrhizal plants, although an increased tolerance has been observed in terms of plant mass and yield (Gernns *et al*., 2001; Whipps, 2004). Concerning hemibiotrophs, the impact of the symbiosis varies from no effect to reduction of the disease, for example, against *Colletotrichum orbiculare* in cucumber (Lee *et al*., 2005; Chandanie *et al*., 2006). However, pathogens with a necrotrophic lifestyle are hampered in their proliferation, and symptom development is less severe on mycorrhizal plants. Examples are the fungi *Alternaria solani* in tomato (Fritz *et al*., 2006; dela Noval *et al*., 2007), *Magnaporthe grisea* in rice (Campos-Soriano *et al*., 2012), and *Botrytis cinerea* in roses and tomato (Moller *et al*., 2009; Pozo *et al*., 2010).

A functional mycorrhizal association requires a high degree of coordination between both partners. The fungus has to deal with the plant's immune system, contend with the defence mechanisms and overcome them for successful colonization of the host (Kloppholz *et al*., 2011; Zamioudis and Pieterse, 2012). Once established, the plant has to regulate the level of fungal proliferation within the roots to prevent excessive colonization and carbon drainage, thus maintaining the interaction at mutualistic levels. For example, under conditions of high exogenous phosphate supply, the plant actively inhibits proliferation of the fungus within the roots (Breuillin *et al*., 2010). Similarly, plants possess a feedback system that prevents excessive colonization over a critical threshold, a phenomenon termed autoregulation of the symbiosis, described initially in the rhizobium-legume symbioses (Vierheilig, 2004; Vierheilig *et al*., 2008). Mechanistic similarities between the autoregulation of mycorrhization and nodulation and the induction of systemic resistance by beneficial microbes have been pointed out (Vierheilig *et al*., 2008; Zamioudis and Pieterse, 2012). In summary, from presymbiotic stages and throughout a well-established AM association, plant defence mechanisms are

tightly regulated to control the symbiosis. As a side effect, this regulation may directly impact root pathogens.

During the early stages of the interaction, the plant reacts to the presence of AM fungi by activating some defense-related responses that are subsequently suppressed (García-Garrido and Ocampo, 2002; Liu *et al*., 2003). Before penetration of the roots, the fungus seems to trigger the plant's immune system as a biotrophic pathogen would (Guimil *et al*., 2005; Paszkowski, 2006). In response to colonization by AMF, a quick but transient increase of endogenous salicylic acid (SA) occurs in the roots with a concurrent accumulation of defensive compounds, such as reactive oxygen species, specific isoforms of hydrolytic enzymes, and the activation of the phenylpropanoid pathway (Pozo *et al*., 1998; Blilou *et al*., 1999; Dumas-Gaudot 2000; Fester and Hause, 2005; de Román *et al*., 2011). These reactions are temporally and spatially limited compared to the reaction during plant-pathogen interactions, suggesting a role in the establishment or control of the symbiosis (Dumas-Gaudot *et al*., 1996; García-Garrido and Ocampo, 2002). Indeed, SA signaling seems to have a negative effect on AM colonization (de Román *et al*., 2011; Herrera-Medinaetal., 2003), and AM establishment requires inhibition of certain SA-regulated responses (Dumas-Gaudot, 2000) as described for other mutualistic symbiosis (Soto *et al*., 2009).

Defence strategies of plant

Systemic Acquired Resistance (SAR) and Induced Systemic Resistance (ISR)

Almost all the plant when attacked either by a herbivores or by any sort of pathogens locally would react with the production of compounds that would either reduce or inhibit further attack or activity of the pathogens. Local response generally occurs in the plant organ where it is originally attacked and also in the organs at far distance and unaffected ones generally known as systemic resistance. One of these type of resistance used by the plants as defence strategies against the pathogens is Induced Systemic Resistance (ISR) and Systemic Acquired Resistance (SAR).(Hunt *et al*., 1996; Schneider *et al*., 1996; Sticher *et al*.,1997; Mauch-Mani and Metraux, 1998; Hammerschmidt, 1999a).

SAR is an induced defence mechanism providing a long-lasting protection against a huge range of microorganisms. The signalling molecule Salicylic Acid is required in association with pathogenesis related protein which also helps in the

contribution of resistance in plants. In accordance to Vallad and Goodman (2004) who had worked both on positive and negative responses and also in future improvement in the utility of both chemical and biological elicitors of induced resistance in agricultural use along with the incorporation of the potentiality to use genetic variation within the crop species population for the better utilization of SAR and ISR in the field.

Later it was discovered that the isochorismate pathway is the important sourse of Salicylic acid during SAR which was done using the *Arabidopsis* as plant model. The positive regulator protein NPR1, in response to the SA, moves towards the nucleus where it interacts with TGA transcription factors as a result inducing the expression of defence genes ultimately activating SAR. It has been suggested by Durrant and Dong (2004) that the mobile signal for SAR might be a lipid molecule. The search for the mobile signal that travels through the phloem from the site of infection for the establishment of systemic immunity has been going on for a long time. In the past few years several candidate signalling molecules have been emerged which includes the methylated derivatives of well known defence hormone (methyl salicylate), jasmonic acid, glycerolipid derived factor and a group of peptides which is involved in cell to cell basal defence signalling. Signal amplification of systemic SAR appears increasing on parallel salicylic acid dependent defence responses in fine tuning with auxin (Volt *et al.,* 2008).

A very important role is played by Salicylic Acid (SA) (Raskin, 1992) in the signalling pathway that leads to ISR (Mauch-Mani and Metraux, 1998; Cameron, 2000; Metraux, 2001). The endogenous levels of SA in the phloem increase both locally and systemically after the infection before the ISR occurs (Malamy *et al*.,1990; Metraux *et al*., 1990; Rasmussen *et al*., 1991).SA is seen to be produced in response to the infection both locally and systemically therefore *de novo* production of SA in plant parts not infected might however contribute in the systemic expression of ISR (Meuwly *et al.,* 1995).The SA levels is positively correlated with the level of resistance of plants that exhibit constitutive expression of SA which has been proved to be true for natural cultivars of rice (Silvermann *et al.*,1995), and for within plant differences in SA levels in potato (Coquoz *et al*.,1995), and also for arabidopsis plant that can express a noble hybrid enzyme salicylate synthase (SAS) activity so have a increase in the level of SA (Mauch *et al*., 2001).One important experiments showing the role of SA in one or the other forms of the ISR have bought into utilization transgenic plants that express the

bacterial *nahG* gene encoding for naphthalene hydroxylase G. Therefore such types of plants fails to accumulate SA and a blockage occurs in their response of their ISR(Delaney *et al*., 1994; Gaffney *et al*., 1994).

Several experiments done using the combination of nahG and the wild type of shoots grafted onto the nahG and also the wild type plants revealed the elicitation of ISR in the tissue of wild types even when the nahG transformed parts of the plant received the inducing infection, which ultimately suggests that the signal that emanates from the inducing tissue is not SA (Vernooij *et al*., 1994).These nahG plants might suffer defects which is yet to be discovered (Cameron, 2000).According to Rasmussen *et al*.(1991) time duration in the process of induction and the accumulation of SA within the phloem in combination with experiments involving removal of leaf were not l all consistent with SA being the first systemic signal in cucumber. All these evidence throws light upon the understanding that the SA and other systemic signals are involved in the signalling of ISR (Sticher *et al*., 1997).

Moreover local responses in the cell surrounding may include changes in the composition of the cell wall that can ultimately result in the inhibition of entry of the harmful pathogens, and *de novo* synthesis of antimicrobial compounds such as pathogenesis related (PR) proteins and phytoalexins (Kuc, 1995; Hammerschmidt, 1999b). Phytoalexins are mainly associated to the local response whereas the occurrence of the PR proteins is mainly associated with both local and systemic response.

Phytoalexins

Phytoalexins were first conceived as antifungal substances produced by cells of potato tubers as they underwent hypersensitivity to penetration by infection hyphae of incompatible races of *Phytophthora infestans* (Mont.) de Bary (Muller and Borger, 1941). The hypothetical phytoalexins were suggested to prevent further growth of the hyphae in these cells and also to confer cross-protection against infection by compatible races inoculated at the same time or some hours later. Although no chemical entities were isolated from potato at this time, Muller & Borger suggested that phytoalexins were non-specific in their effect on fungi, and that a major difference between hypersensitive and susceptible varieties of plant was the speed of formation of phytoalexins in response to infection. Thus it was considered that the speed of this

reaction was dependent upon the sensitivity of the host cell to the fungus attempting infection. The precise meaning attached to this word 'speed' was not clear in these writings, but it is of current interest as revealed by studies on phaseollin accumulation in beans to be discussed later.

Muller (1958) thought that phytoalexins as envisaged in his earlier work with potato might be of general occurrence in plants. He attempted to demonstrate that such substances formed in bean (*Phaseolus vulgaris* L.) as it underwent hyper- sensitivity in response to attempted infection by the fruit pathogen *Sclerotinia fructicola* (Wint.) Rehm. Droplets of spore suspension were placed in the cavities of opened bean pods from which the seeds had been removed. The spores were observed to germinate, and to cause brown flecks in the underlying tissue within 24 h. Host damage was indicated by uptake of the stain rhodamine B and also by a decrease of pH in affected cells. Infection droplets were collected, combined, made spore-free by centrifugation and tested for their effects on other spores in vitro. They became increasingly antifungal after incubation in seed cavities for 14 h and completely fungistatic after 24 h. Control droplets of water became highly stimulatory to test fungi after incubation in pods. The antifungal principle in the fungi static infection droplets was removed by partition with petroleum spirit leaving a highly stimulatory water-phase. The properties of the anti-fungal fraction suggested that a distinct chemical entity had been extracted as a phytoalexin.

Plants are constantly attacked by many potential pathogens and respond by the activation of defence genes, the formation of reactive oxygen species (ROS), and the synthesis of pathogenesis-related (PR) proteins, localized cell-wall reinforcement and the production of antimicrobial compounds. Low molecular mass secondary metabolites with antimicrobial activity that are induced by stress are collectively named phytoalexin, and are an important part of the plant defence repertoire (Hammerschmidt, 1999b; Pedras *et al*., 2011). Phytoalexins are a heterogeneous group of compounds (Shinbo *et al*., 2006; Schmelz *et al*., 2011; Huffaker *et al*.,2011) that show biological activity towards a variety of pathogens and are considered as molecular markers of disease resistance. The concept of phytoalexins was introduced over 75 years ago (Muller and Borger, 1940) based on the finding that potato (*Solanum tuberosum*) tuber tissue that had previously been infected with an incompatible race of *Phytophthora infestans* induced resistance to a compatible race of *P. infestans*. It was hypothesized

that the tuber tissue, in response to the incompatible interaction, produced substances (phytoalexins) that inhibited the pathogen and protected the tissue against later infection by other compatible races of the pathogen (Coleman *et al*., 2011). Since then, the field has evolved extensively, not only with respect to studying the roles of phytoalexins in defence against pathogens and pests, but also with respect to their health-promoting effects (Boue *et al*., 2009; Ng *et al*., 2011; Smoliga *et al*. ,2011; Holland, *et al*.,2010; Jahangir *et al*.,2009; Yang *et al*. 2009). For example, indole phytoalexins contribute to the antioxidant, anticarcinogenic and cardiovascular protective activities of Brassica vegetables (Jahangir *et al*. 2009). Peanut (*Arachis hypogea*) phytoalexins have antidiabetic, anticancer and vasodilator effects .The biological activities of glyceollin, a soybean (*Glycine max*) phytoalexin, include antiproliferative and antitumor actions (Ng *et al*., 2011). The sorghum (*Sorghum bicolor*) phytoalexins, 3-deoxyanthocyanins, might be useful in helping to reduceincidence of gastrointestinal cancer (Yang *et al*., 2009). The phytoalexin resveratrol from grapevine (*Vitis vinifera*) has anti-aging, anticarcinogenic, anti-inflammatory and antioxidant propertiesthat might be relevant to chronic diseases and/or longevity in humans (Smoliga *et al*., 2011).Phytoalexins accumulate at infection sites and they inhibit the growth of fungi and bacteria in vitro therefore, it is logical to consider them as possible plant-defence compounds against diseases caused by fungi and bacteria. There is a evidence of phytoalexin being taken role in plant defence. Concerning the accumulation of pisatin in pea and phaseollin in green bean, it was apparent that the phytoalexins accumulated to fungitoxic concentrations not only in inoculum droplets placed on opened pea or bean pods but also in the tissues immediately below the inoculum droplets (Cruickshank and Perrin, 1968) . These data supported a role for phytoalexins in plant disease resistance, but there were and still are exceptions. There are also examples that phytoalexins accumulated during compatible plant-pathogen interactions. These include the induction of pisatin by the virulent Oomycete *Aphanomyces eutiches (*Pueppke and Van Etten, 1976) and by the pathogenic strains of the fungus *Nectria hematococca* and induction of spirobrassinin by virulent races of *Leptosphaeria maculans* (Pedras and Seguin-Swartz, 1992). Similarly Glazebrook and Ausubel (1994) reported that the virulent pathogen *Pseudomonas syringae pv. maculicola* elicits the synthesis of high levels of camalexin in *Arabidopsis thaliana*. Mert-Türk *et al*. (1998) also showed that camalexin accumulated during both compatible and incompatible interaction in *A. thaliana* when challenged with an Oomycete, *Peronospora parasitica*. If the results

exemplified above are interpreted, in incompatible interactions, phytoalexin accumulation limits or stops pathogen growth, thereby conferring resistance to the plant. In compatible interactions, the pathogen apparently, tolerates the accumulated phytoalexins, detoxifies them, suppresses phytoalexin accumulation, and/or avoids eliciting phytoalexin production (Mansfield, 1982). To develop disease protection strategies, plant pathogen research in the field of phytoalexins has also focused on interpreting their biosynthesis pathways and regulation in different crop plants by using different cultivars, transgenic plants and mutants, and by applying -omics, molecular biology and biochemical approaches.

Plant defence reactions against pathogens, including fungi, bacteria, and viruses, involve induced synthesis of low molecular weight compounds called phytoalexins. Biotic elicitors that are derived from the cell surface of pathogenic microbes as well as host plants trigger the defence response. lt is considered that an elicitor molecule combines with a plant membrane receptor (Cosio *et al*., 1992; Shibuya *et al*., 1993) and that the complex activates a series of specific genes, resulting in the synthesis of phytoalexins (Brooks and Watson, 1991). It has been suggested that JA could be an integral part of a general signal transduction system regulating inducible defence genes in plants (Farmer and Ryan, 1992; Gundlach *et al*., 1992). In suspension-cultured rice (Oryza *sativa* L.) cells, treatment with an elicitor (N-acetylchitoheptaose) induces production of phytoalexins (Yamada *et al*., 1993). In this report, we present evidence that JA is a key signal transducer between recognition of N-acetylchitoheptaose and the production of a phytoalexin, momilactone A, in the rice cells.

Hypersensitive reaction or response (HR) is the programmed cell death of infected plant cells to restrict pathogen invasion to the initially infected regions and is included in the resistance (*R*) gene–mediated resistance to pathogens. Upon HR, the biosynthesis of low molecular-weight defence signal compounds, such as salicylic acid, jasmonic acid, and ethylene (De Laat *et al*., 1983; Iwai *et al*., 2006; Malamy *et al*., 1990; Seo *et al*., 2001), is induced for the signal transductions of disease resistance. As well as these signal compounds, antifungal molecules called phytoalexins (PA),which are also low molecular-weight compounds produced in host plants in response to parasite infection (Muller and Borger ,1940) or stress (Kuc, 1995), are induced in many plant-microbe interactions. Although the roles for PA in disease resistance were proposed (Kuc, 1995), structures of the PA were found to be diverse, including

flavonoid, isofla- vonoid, diterpenoid, sesquiterpenoid, and indole (Grayer and Kokubun, 2001), and precise studies to reveal the biological significance of each PA for resistance in individual experimental system shave been required.

Pathogen-induced defence mechanisms in higher plants may involve de novo synthesis of antifungal compounds, known as phytoalexins, which play an important role in the disease resistance of various plant species. Plants that are attacked by pathogenic microorganisms respond with a variety of defence reactions. One such reaction is the production of secondary metabolites that serve as plant antibiotics, known as phytoalexins, which are generated through the perception of signal molecules called elicitors, which are mostly derived from pathogens. More than 300 structures of phytoalexins were isolated from approximately 900 plant species, including the rice phytoalexins momilactones A and B,oryzalexins A through F4–and S, phytocassanes A through E (diterpenes), and sakuranetine (a flavanone).Umemura *et al*.(2003) reported that phytocassanes play an important role in disease responses of rice plants.

Elicitors have the property of inducing the production of phytoalexins in rice plants, as well as to agents for controlling rice diseases. More particularly, the present investigation relates to evaluation of phytoalexins characterized in rice plants which are cultivated for tests. Phytoalexins synthesized in the rice plant bodies are extracted so as to screen the property of elicitors of inducing the production of phytoalexins such as phytocassanes and rnornilactones and the like in rice plants. Because such phytoalexins have potent antimicrobial activity against causal organisms of rice plant diseases such as rice blast fungus (*Magnaporthe grisea*, previously designated as *Pyricularia oryzae*), rice sheath blight fungus (*Rhizoctonia solani*), and rice brown spot fungus (*Drechslera oryzae*) elicitors having the property of inducing the production of phytoalexins in rice plants Would be useful as active ingredients in agents for controlling rice diseases.

Fifteen phytoalexin compounds have been identified in suspension-cultured rice cells treated with biotic elicitors such as a chitin oligosaccharide or a cerebroside (Kuc,1995 and Harborne, 1999) and/or from rice leaves that were either infected with the rice leaf blast pathogen Magnaporthe grisea or exposed to UV irradiation (Cartwright *et al*.,1977; Akatsuka *et al*.,1983; Kono *et al*.,1984; Sekido *et al*.,1986; Kato *et al*.,1993; Kodama *et al*.,1992; Koga *et al*.,1995; Koga *et al*.,1997). With the exception of the flavonoid sakuranetin, all of these rice phytoalexins are diterpenoids. These compounds have been classified into four structurally distinct types of polycyclic

diterpenoid phytoalexins based on the structures of their diterpene hydrocarbon precursors: phytocassanes A to E, oryzalexins A to F, momilactones A and B, and oryzalexin S (Kodama *et al*., 1992). The common precursor geranylgeranyl diphosphate is cyclized to ent-copalyl diphosphate (ent-CDP) and then to ent-cassa-12, 15-diene and ent sandaracopimaradiene, leading to phytocassanes A to E and oryzalexins A to F, respectively. Geranylgeranyl diphosphate is also cyclized to syn-CDP and then to 9 _H-pimara-7,15-diene and stemar-13ene, leading to momilactones A and B and oryzalexin S, respectively. Phytocassane A to D are produced upon elicitation by fungal pathogen *Magnaporthe grisea* and isolated from rice stems infected with *Rhizoctonia solani* (Koga *et al*., 1995). Phytocassane E, which is induced by the potato pathogen *Phytophthora infestans* also shows antifungal property against *Magnaporthe grisea* (Koga *et al*., 1997). These studies indicated that the antifungal activities of phytocassane B, C and E (EC50 values, 4–7 μg/mL) are about four-folds stronger than the activities of phytocassane A and D. This higher antifungal activity was attributed to the β-hydroxyl group in C-1 position of phytocassane B, C and E, which can form intra molecular hydrogen bond with the carbonyl group in position C-11 (Koga *et al*., 1997).

PR- proteins

A mechanism of plants defence from pathogen attack is induced resistance (Metraux *et al*., 2002). During induced resistance, a biological or a chemical stimulus is recognised by the plant setting in motion a signal transduction pathway that activates resistance responses, such as programmed cell death and defence gene expression (Edreva, 2004). Induced Systemic Resistance (ISR) is a type of induced resistance which is produced in roots by nonpathogens, like the very important soil-borne plant growth promoting rhizobacteria (PGPR) and beneficial soil-borne plant growth-promoting fungi (PGPF), and is transmitted through the signal molecules of ethylene (ET) and jasmonic acid (JA) (Vallad and Goodman, 2004). In monocotyledonous plants, transcripts related to ET and/or JA signalling pathways have been induced or primed after treatment with beneficial fungi, bacteria band insects associated with ISR (Kanno *et al*., 2005; Muyanga *et al*., 2005; Djonovic *et al*., 2007).

According to van Loon (1999) the PR proteins were originally suggested to have been defined and also detected its accumulation in huge amounts only after infection and absent in healthy plants (van Loon and van Kammen, 1970).They have now been discovered in more than 40 species belonging to 13 families. Two distinct groups of PR

proteins have been recorded. Prevalent in the intercellular spaces are the acidic PR proteins and basic PR proteins that are more or less functionally similar but have different molecular weights as well as the amino acid sequences located mainly in the intracellular vacuoles (Legrand *et al.,* 1987; Niki *et al.,* 1998; van Loon 1999).

Some of the PR proteins have also been reported to have Chitinase (Legrand *et al.,* 1987) or β 1, 3-glucanase activity. Chitinase are basically structurally and functionally different group of enzymes having the capacity to hydrolyse the chitin and many of them are believed to play a significant role in the defence of plant against several fungal pathogens (Sahai and Manocha, 1993; Jackson and Taylor, 1996). Chitinase also shows effective antifungal activities (Schlumbaum *et.al*., 1986) and over-expression of chitinase in the plants shows decrease in the susceptibility towards the infection by the fungus with chitin- containing cell wall (Broglie *et.al*., 1991; Datta and Datta, 1999). In contrary to this the function of many other PR protein is still not known (van Loon and van Strein, 1999) and many PR proteins can be functionally active only when it is combined. Specifically the basic PR proteins are also expressed in specific tissue and in a controlled manner throughout the development as for example during leaf senescence (van Loon, 1999).Expression of specific defence related genes such as *PR-1* and *β-glucanase* 2 often used as ISR markers can be uncoupled from phenotypic pathogen resistance(Greenberg *et.al*., 2000) which ultimately indicates that these compounds are not necessary for an effective resistant phenotype.PR proteins are normally used as ISR markers but yet it has not until been reported any antiviral or antibacterial activity.