CHAPTER - II

REVIEW OF LITERATURE
2.1 Cercospora leaf spot (CLS) disease management

CLS disease, although considered to be of minor economic importance earlier, has recently received greater attention by many researchers all around the world. This is due to its high disease severity with the crop damage estimated up to as high as 70% (Gibbons 1980; Vidyasekaran 1981; Subrahmanyam 1986; Shane and Teng 1992). Considering its worthwhile significance, an urgent need for the effective disease management strategies has been strongly felt. Some of the important disease management strategies are reviewed here.

2.1.1 Fungicide application and its drawbacks

There are several reports demonstrating the use of different fungicide chemicals for the management of CLS. According to Karaoglandis et al. (2000), sugar beet growers in Greece use a successful strategy of always mixing a triazole fungicide with a protectant fungicide to control CLS and manage fungicide resistance. In field trials conducted in Minnesota to determine the efficacy of labeled and experimental fungicides for controlling CLS, it was observed that Tetraconazole and pyraclostrobin, when applied alone, consistently provided effective CLS control and resulted in high sucrose yield (Khan and Smith 2005). Mangandi and Peres (2012) conducted an experiment for the evaluation of commercial fungicides for the control of CLS in shrub rose ‘Fuchhsia Meidiland’ in Alabama and North Carolina, USA. They concluded that the fungicide such as Compass™ and Daconil Ultrex® applied weekly as well as Eagle® and Heritage® applied twice monthly reduced the severity of the mentioned disease to just few
spots on the lower leaves. Daconil Weather Stik®, Immunox®, and Halt® are other examples of fungicide which provide control of CLS.

In Indian scenario, field trials conducted by Tandon and Singh (1968) revealed that Dithane M-22, Cosan, Fycol 8E and Dithane Z-78 were effective in controlling the Tikka disease of groundnut caused by *Cercospora arachidicola*. Similarly, Dubey *et al.* (1995) observed that carbendazim (Bavistin) was better than copper fungicides for control of leaf spots of groundnut caused by *Cercospora arachidicola*. In another study, it was suggested that the management of CLS disease caused by *Cercospora punicae* in pomegranate can be achieved by adopting integrated schedule of the following: avoiding rainy season crop and regulating winter season crop during October-April, sanitation of orchard, pruning of diseased branches and application of Bordeaux paste, and copper dust 4% @ 20 kg/ha or drenching with bleaching powder @ 2.0% and regular spraying of Streptocycline (500 ppm) along with Carbendazim (0.15%)/mancozeb (0.2%)/copper oxychloride (0.25%) at 15 days interval (Anonymous, 2009). Some fungicides can be used with varying application rate and methods for controlling *Cercospora hydrangeae* causing CLS disease in hydrangea plants (Anonymous 2013c). For instance, 1-4 oz of Heritage 50W per 100 gal was recommended for application at first sign of disease and repeated at 14-28 day intervals. It was also recommended by the mentioned handbook that the application of the fungicides such as Chlorothalonil, mancozeb, myclobutanil and thiophanate-methyl should be done when the disease symptoms starts to appear first on lower leaves. Further, the dosage must be repeated every 10 to 14 days as needed. Conditional
application of higher rate of above mentioned fungicides at shorter interval was commented in the handbook when the mentioned disease is severe.

As it is evident from the foregoing discussion that there are myriad of instances presenting the use of chemical fungicide with varying degree of success for controlling the CLS disease in various crops. However, the concerns by various researchers have been raised that the increased use of chemicals for management of plant diseases can create ecological imbalance by causing health hazard to all kinds of life beings.

2.1.2 Application of botanicals and their drawbacks

Many researchers have attempted to explore the bioactivity of different plant extracts against CLS disease. Enikuomehin (2005) carried out a comparative evaluation of Benetex T and the aqueous leaf extracts of *Aspilia africana*, *Chromolaena odorata*, *Musa paradisiaca* and *Tithonia diversifolia* to control CLS of two sesame cultivars (530-6-1 and Pttil No.1). Results revealed that the suppressive effect of leaf extracts of *A. africana*, *C. odorata* or *T. diversifolia* were comparable to Bentex T (20% Benlate+ 20% Thiram) for pseudoCLS disease in sesame cultivars. In another study, Gado (2007) attempted to control CLS disease caused by *Cercospora beticola* in *Beta vulgaris* (Sugar beet) crop under field trial by two plant extracts (Sincocin and Agrispon) and three triazole derivative fungicides (Score, Eminent and Opus) in two successive growing seasons (2004-2005 and 2005-2006). On comparing all treatments, Score appeared to be the best among all treatments followed by Eminent, Opus, mixture of Agrispon and Sincocin, individual treatment of Sincocin and then, Agrispon in a descending order.
Nevertheless the reduction of disease severity was more actively achieved by fungicides than bioactivators, components of yields in bioactivator treated plants, particularly Agrispon, were higher than that of the fungicides. Precisely, root weight and sugar percentage was increased significantly by Agrispon treatment with decrease in potassium, sodium and α-amino acid, leading to increase in sugar quality to a greater extent. Further, dual treatment of Sincocin and Agrispon (1:1 v/v) was found the most efficient (82.49%) among the bioactivator treatments in reducing disease severity followed by individual treatments of Sincocin (81.71%) and then, Agrispon (80.99%). Finally, Gado (2007) recommended bioactivators such as Sincocin and Agrispon can be an alternative solution for disease management of CLS disease of sugar beet and for increasing yield component of sugar.

It is evident from above mentioned studies that the use of botanicals has potential for management of CLS. However, most of the studies evaluated the in vitro efficacies of the extracts and more emphasis is required on development of standard extraction procedures and formulations which can be recommended to the farmers for field application. In addition, the use of different plant extracts requires exploitation of plants which will also lead to ecological imbalance.

2.2 Role of organic amendments in disease management

Organic amendments (OA) such as vermicomposting enrich the soil with oxygen, thus encouraging aerobic microorganisms. These microorganisms perform several important functions like nitrogen fixation, production of enzymes, antibiotics, growth hormones, etc. This process has been known for its potential of
disease suppression. Oka et al. (2000) pointed that organic amendments consistently have beneficial effects on soil nutrients, soil physical conditions, soil biological activity and thereby improving the health of plants and reducing populations of plant parasitic nematodes. A screen house experiment was conducted to evaluate the effect of cow manure, chicken manure and their combinations on nematode destroying fungi, nematode community and growth of tomato (*Solanum lycopersicum* L.) which showed that the OA used, stimulate the occurrence of nematode destroying fungi in the soil and also reduce plant parasitic nematodes.

OA, possessing the ability of disease suppression, produced from various wastes have been tested against several soil-borne plant pathogens. For instance, Szczech and Smolinska (2001) and Wachira et al. (2009), tested animal manures for its ability of disease depressiveness against *Phytophthora nicotianae*. Further, Kannangara et al. (2000) used separated dairy solids against *Fusarium oxysporium* f. sp. *lycopersici*. In another study, a combination of vegetable wastes, bark (*Salix* spp.), and cattle manure (Simsek-Ersahin et al. 2009) was also tested on both *F. oxysporium* and *Rhizoctonia solani*. Further, the disease suppression ability of some commercially available vermicomposts was examined against *Pythium* in cucumbers, and *Rhizoctonia* in radishes in the greenhouse, *Verticillium* in strawberries and by *Phomopsis* and *Sphaerotheca fuliginae* in grapes in the field (Arancon et al. 2004). Sahni et al. (2008) while studying the collar rot disease incited by *Sclerotium rolfsii* demonstrated that substituting the soil with different amounts of vermicompost causes significant reduction in mortality of chickpea compared to control. In a more recent study, Elmer and Ferrandino (2009)
concluded that augmenting earthworm populations can suppress verticillium wilt of eggplant, and strategies that increase earthworm numbers may contribute to disease suppression.

All these studies have established the disease suppressive ability of different types of OA but their underlying mechanisms were less studied. Zhang et al. (1996) found that compost induced resistance in cucumber to both Pythium root rot and anthracnose caused by Colletotrichum orbiculare and that this phenomenon was negated by sterilization. They reported that the effect of compost on peroxidase activity in cucumber was more pronounced after plant infection. Similarly, high glucanase activity was found in Arabidopsis thaliana and cucumber plants grown in compost after infection, compared with that in plants grown in peat (Zhang et al. 1998). They concluded that compost induced systemic acquired resistance in a different way from its induction by pathogens or salicylic acid. These findings suggest that the microflora in the compost had an effect on these PR proteins in both plant types, but that much of the activation resulted from infection by the pathogen. Further in two preliminary tests, the expression levels of the PR proteins PR-Q, chitinase1 and peroxidase were not elevated when melon plants were grown in the suppressive compost, compared with their levels in plants grown in conducive peat in the absence of FOM (Yogev et al. 2010). These results indicate the lacunae of consistent results for suppressive compost against variety of crops. Therefore, the application of suppressive composts and its mechanism needs validation on variety of crops.

In other systems, PR-Q and peroxidase were found to be up-regulated in transgenic tobacco plants treated with suppressive compost expressing viral
movement proteins (Hofius et al. 2001), and in marrow (Cucurbita pepo L.) plants infected with cucumber mosaic virus (CMV) (Tecsi et al. 1996), respectively.

Despite having several application based studies, the less known mechanism behind the disease suppressive property of OA needs attention. Further, the nutrients in the OA applied in the rhizosphere region of soil may percolate down especially in the rainy season or higher application of water or in crops with high irrigation. Therefore to overcome this problem, compost extracts, a liquid form of compost, were developed. These are much easier to handle for applying to the crops than solid comports, which are bulky and heavy and need soil incorporation. These extracts are increasingly being applied, as soil drenches or soil and foliar sprays, to enhance plant growth and control plant diseases and pests (Simsek-Ersahin 2011). Since last decade, the utilization of vermicompost extracts/teas as bio-control agents has accelerated (Simsek-Ersahin 2011).

**Vermiwash**

Vermiwash (compost extract or worm-tea or compost-tea) is one of the by-products of vermicompost. It is the organic fertilizer decoction obtained from the units of vermiculture/vermicompost as drainage. It is used both as foliar spray and in the root-zone of the plants (Ranganathan 2006). It is also called Vermi-Tea or Vermi-liquid. It was found to develop resistance to diseases in plants and was beneficial in nurseries, lawns and orchards (Ranganathan 2006). As a foliar spray, it was reported to have yielded good results, especially initiating flowering and
long lasting inflorescence of Anthuriums (Rao 2005). It could also be used as a liquid fertilizer applied to the rhizosphere (Rao 2005).

Compost teas are reported to control plant pathogens through different mechanisms. The most reported factor influencing the efficacy of compost teas in inhibiting the development of plant disease is their microbial content. The microorganisms present in the tea may act as pathogen antagonists through their ability to compete for space and nutrients (Al-Mughrabi et al. 2008), to destroy pathogens by parasitism (El-Masry et al. 2002), to produce antimicrobial compounds, or to induce systemic resistance in plants (Zhang et al. 1998). Other work hypothesized that the physicochemical properties of the compost teas, namely nutrients and organic molecules such as humic or phenolic compounds (Hoitink et al. 1997; Siddiqui et al. 2008), may protect the plant against disease through improved nutritional status, direct toxicity toward the pathogen or induced systemic resistance. The potential parameters that affect the efficacy of compost teas are two-fold: the target pathosystem (pathogen and host plant) and the preparation methodologies of the teas (aeration, compost type, nutrient additives, duration of fermentation, etc.) (Scheuerell and Mahaffee 2002).

With reference to the disease suppressive ability of these compost extracts, Edwards et al. (2004) mentioned that the study of Nakasone et al. (1999) reported that aqueous extracts of vermicomposts inhibited the mycelial growth of Botrytis cinerea, Sclerotinia sclerotum, Corticium rolfsii, R. solani and Fusarium oxysporum. Latter, Szczech and Smolinska (2001), Rodríguez-Navarro et al. (2000), and Zaller (2006) demonstrated that the aqueous extracts of vermicomposts reduce the effect of soil-borne or foliar plant pathogens and pests. Singh et al. (2003) conducted a
study in which foliar application of the aqueous vermicompost extracts in pea and balsam infected with *Erysiphe pisi* and *E. cichoracearum*, respectively, was examined. Also, Simsek-Ersahin (2011) reviewed several studies of application of aqueous vermicompost for in vitro inhibition of spore germination of several saprophytic and phytopathogenic fungi (*Alternaria solani*, *A. brassicae*, *A. alternata*, *Curvularia pennisetii*, *Curvularia maculans*, *Curvularia palliscens*, *Curvularia* spp., *Helminthosporium speciferum* and *Helminthosporium penniseti*). It was also observed that under field conditions, post-inoculation treatment offered better protection than pre-inoculation to pea from *E. pisi* (Simsek-Ersahin 2011). Further, the effect of aqueous vermicompost on balsam having powdery mildew was effective in both pre- and post-inoculation treatments where the latter was better. 

In another study, Zaller (2006) examined the effects of foliar sprays of aqueous vermicompost extracts on growth, yields, morphological and chemical fruit quality, and their potential for disease suppression on natural infection with late blight disease (*P. infestans*) on three tomato varieties. It was reported that the decreased susceptibility of tomato leaves, stems, and fruits to natural infection by *P. infestans* in which only half as many vermicompost sprayed plants showed clear signs of *P. infestans* infection as compared to control. It was also stated that no difference between the vermicompost extract or control treatments was found with respect to the severity of the infection. Nevertheless, this variable inconsistent result is difficult to explain.

Manandhar and Yami (2008) conducted a field trial for the comparison of aqueous extracts of aerobic composts and vermicomposts on foot rot disease of rice caused by *F. moniliforme*. They reported that aerated vermicompost tea
presented statistically significant maximum control efficiency. Following the field trial, treatment of the rice seeds with compost tea revealed highest efficiency of aerated vermicompost tea. They concluded that the effectiveness of compost extract seems to depend on several factors including preparation of method (aerated or nonaerated), time of extraction, compost type (compost or vermicompost) and crop applied. It has been pointed out that the need for field evaluations on several crops infected with different types of disease organisms over a long period of time to account for year-to-year variations in disease dynamics before making any recommendation to the farmers for the use of compost tea in plant protection systems.

It was also observed that aerobically produced compost extracts are much more effective than anaerobically produced compost extracts (Hoitink et al. 1997) and aerobic microbes dominate in aerobically produced compost extracts (Hoitink et al. 1997). Edwards et al. (2006) showed that aerobically produced vermicompost teas are much more stable and effective than those produced without aeration. Yami and Shrestha (2005) stated that the vermicompost act as a buffer for plants where soil pH is too high or low, making soil nutrients available to the plant. They also demonstrated greater diversity of beneficial microorganisms in the vermicompost than in the compost. Zaller (2006) suggested that the underlying mechanisms in use of vermicompost extracts for the protection of plant is less understood, but involvement of induced resistance is mostly considered. It can be inferred that the suppressive nature of vermicompost extracts against various kinds of pathogen has a biological nature rather than chemical.
The underlying mechanisms

Literature survey indicated that the involvements of microbial communities are either directly or indirectly accountable for disease suppression (Hoitink et al. 1997; Krause et al. 2001; Scheuerell et al. 2005). These involvements of microbes for various disease suppressive mechanisms can be illustrated by two types: general and specific suppression.

General suppression mechanism

This mechanism includes nutrient competition, antibiosis (in which beneficial organisms secrete antibiotics that directly inhibit the pathogen) hyperparasitism/direct parasitism (one organism feeding on another), and possibly induced systemic plant resistance (Hoitink and Grebus 1994). Further, Serra-Whittling et al. (1996) suggested a concept of fungistatis, which includes the suppression of fungi by high microbial diversity acting as biocontrol agents. Nutrient-dependent suppression of plant pathogens such as *Pythium* and *Phytophthora* was also explained by general suppression mechanism (Krause et al. 2001; Chen et al. 1988). This can be explained as agronomically beneficial microorganisms block the pathogens excess to plant roots by occupying all the available sites.

Another important factor for disease suppression by organic amendments is induced systemic resistance (ISR). ISR develops, in case of soil-borne pathogens, when the rhizosphere is inoculated with a weakly virulent pathogen. After the initiation of systemic resistance by weak pathogen, the plant develops the capacity for future effective response to a more virulent pathogen (Zhang et al. 1998; Pharand et al. 2002). The other factors defined in the context of general disease
suppression are the antibiosis and competition. With respect to antibiosis, some beneficial organisms secrete antibiotics or they become parasite on pathogens (Edwards et al. 2004). Further, on addition of vermicompost to soil, a diversity of competitors, inhibitors, and predators of disease organisms gets developed (Edwards et al. 2004). Competition is the common phenomena for general disease suppression mechanism. Therefore, the level of disease suppressiveness is typically related to the level of active microbial biomass in a soil (Hoitink and Grebus 1994; Edwards et al. 2004). The larger the soil's active microbial biomass, the greater the soil's capacity to use nutrients leading to lowering of the nutrient availability to pathogens. In other words, when most soil nutrients are tied up in microbial bodies, the competition for readily available mineral nutrients gets a higher level (Hoitink and Grebus 1994; Edwards et al. 2004). Therefore, it can be stated that competition for limited nutrients are a key for general suppression.

*Specific suppression mechanism*

This mechanism is includes a narrow range of microorganisms facilitate the suppression or one organism directly suppresses a pathogen (Hoitink et al. 1997) such as *R. solani* and *S. rolfsii*. These pathogens are less reliant on external energy or nutrient sources, make them “nutrient-independent pathogens” and un-susceptible to microbial competition (Krause et al. 2001; Scheuerell et al. 2005). Therefore, the control these two pathogens can be achieved via “specific” beneficial organisms such as *Trichoderma* and *Gliocladium* that colonizes the harmful propagules and causes reduction in the disease potential. Fungal strands of
Trichoderma entangle the pathogen and by releasing enzymes makes Rhizoctonia cells dehydrated eventually kills them (Chung et al. 1988).

Finally, it can be said that vermiwash has the potential for disease suppression which is evident by several above mentioned studies. However, limited application based study has been reported against CLS disease caused by Cercospora spp. with no explanation of their underlying mechanism. As mentioned in the chapter I of this thesis, CLS is a foliar disease, against which, again limited application based studies are available but none of them have reported the mechanism beneath it. In addition, Capsicum assamicum, a chilli crop, which is native to northeastern part of India and has high capsaicin content, was never undertaken as a model plant to study the role of vermiwash against CLS disease caused by C. tezpurensis.

2.3 Role of AM fungi in disease management

Arbuscular mycorrhizal (AM) fungi, forming the order Glomales of the Zygomycota (Morton and Benny 1990), occur on the roots of 80% of vascular flowering plant species (Newman and Reddel 1987), but they are obligate biotrophs and cannot be cultured without the plant. Mycorrhizal fungi facilitate nutrient and water uptake from soil. Fungal hyphae are thinner than plants roots, having roughly a ten times smaller diameter, which allows them to penetrate soil pores inaccessible to plant roots. Mycorrhizal hyphae also grow faster than plant roots into the soil beyond the nutrient depletion zone created by roots (Timonen and Marschner 2005).
They provide an effective alternative method of disease suppression, especially for those pathogens which affect below ground plant organs. Mycorrhizal fungi have an enormous potential for use as biocontrol agents for soil- and root-borne diseases. AM fungi are robust example of both general and specific disease suppression mechanism. These fungi compete for nutrients and release certain antibiotics which suppressed the growth of soil or root borne plant pathogens. They also incite both systemic acquired resistance (SAR) and ISR required for plants against diseases. *Glomus intraradices* was able to incite systemic resistance in banana plants towards *Radopholus similis* and *Pratylenchus coffeae* and the AMF reduced both nematode species by more than 50% (Elsen *et al*. 2008). The mycorrhizal fungi protect plant roots from diseases in several ways. A few examples of physical exclusion have been reported (Ingham 1991). Improved phosphorus uptake in the host plant has commonly been associated with mychorrhizal fungi (Meghvansi *et al*. 2008; Meghvansi and Mahna 2009). When plants are not deprived of nutrients, they are better able to tolerate or resist disease-causing organisms. Protection from the pathogen *Fusarium oxysporum* was shown in a field study using a cool-season annual grass and mycorrhizal fungi. In this study the disease was suppressed in mycorrhizae-colonized grass inoculated with the pathogen (Newsham *et al*. 1995). In field studies with eggplant, fruit numbers went from an average of 3.5 per plant to an average of 5.8 per plant when inoculated with *Gigaspora margarita* mycorrhizal fungi. Average fruit weight per plant increased from 258 grams to 437 grams. A lower incidence of Verticillium wilt was also realized in the mycorrhizal plants (Matsubara *et al*. 1995). In a study conducted by Tabin *et al*. (2009), mycorrhizal inoculation not only reduced the
percentage of damping-off disease of *Aquilaria agallocha* seedlings caused by the pathogenic fungus (*Pythium aphanidermatum*), but also significantly increased host plant height, total biomass and dry matter. The effects of arbuscular mycorrhizal fungi *Glomus mosseae*, *G. fasciculatum* and *Rhizobium leguminosarum* biovar phaseoli were examined on the patho-system of *Sclerotinia sclerotiorum* (L.f.) de Bary (Ss) and common bean by Aysan and Demir (2009). Treatments of single inoculations of AMF and *R. leguminosarum* isolates reduced disease severity by 10.3-24.1%. The mechanism for disease suppression by AM fungi is described below.

**The underlying mechanism**

There are several benefits of AMF colonization in plants, mainly the increase in nutrient uptake (Smith and Read 2008). Despite this, still there is an ambiguity that the AMF has any direct involvement in the host's defence signaling against phytopathogens. Although, there are some indirect functions which contribute to intensify the plant defence responses including augmentation of plant nutrition (Smith and Read 2008) and damage compensation. Moreover, it includes anatomical alterations in the root system (Wehner *et al.* 2010), microbial changes in the rhizosphere and enhancing the attenuated plant defence responses by altering the host's signaling pathways (Pozo and Azcón-Aguilar 2007). This is accomplished primarily through modulation in Jasmonic acid (JA) and salicylic acid (SA) dependent pathways (Pozo and Azcón-Aguilar 2007). Furthermore, the AMF is likely to have role in induction of hydrolytic enzymes (Pozo *et al.* 1999), enhanced levels of Pathogenesis-related (PR) proteins, accrual of phytoalexins (Harrison and Dixon 1994; Morandi 1996; Larose *et al.* 2002), callose deposition
(Cordier et al. 1998) and reactive oxygen species generation (Salzer et al. 1999). Hence, there are several reports exemplifying the potential of AMF in reducing the severity and incidence of phytopathogens for a long. But the knowledge of the mechanism behind it is scarce.

**AM fungal contribution towards suppression of plant pathogens**

During AMF’s colonization, a strong genetic shift occurs which leads to the enhancement of signaling pathways of plant defence response against phytopathogen. After having symbiotic relationship with its host, AMF possibly enhances genes encoded products having antimicrobial activity. For instance, induction of *Medicago truncatula* genes TC104515 (6659-fold), TC101060 and TC98064 was observed in the roots colonized with *Glomus intraradices* (Liu et al. 2007). These genes were predicted to encode cysteine rich proteins that display antifungal activity (Terras et al. 1995). Their function is to elicit the hypersensitivity response with the matching resistance gene (de Wit et al. 1992). This response is mediated by reactive oxygen species (ROS) produced early in the plant-pathogen interaction (Levine et al. 1994). Accumulation of reactive oxygen species (ROS) in the mycorrhized plants has also been observed (Pozo and Azcón-Aguilar 2007). Although its accumulation was not so significant and found to be localized (Pozo and Azcón-Aguilar 2007).

However, TC104515 transcripts were detected only in roots colonized with *G. intraradices* and not in roots colonized with *G. versiforme* or *Gigaspora gigantea*. This gene was also not expressed in *M. truncatula/G. mosseae* roots (Hohnjec et al. 2005). So, there is a considerable variation in the genetic shifts of different plant-
related defence genes colonized with diverse AMF species, which needs further exploration.

In an experiment, a group of genes was identified through differential expression in shoots of AMF colonized plant, showing striking similarities with defence/stress signaling genes and ACRE genes (Liu et al. 2007). The ACRE genes were previously known to respond instantaneously in tomato upon infection of *Cladosporium fulvum* and suggested to be involved in the initial development of defense signaling (Durrant et al. 2000). Based on the split-root analyses, some of ACRE genes including two WRKY-type transcription factors and a TOLL-type protein showed a greater increase in transcripts in the non-colonized roots and shoots of the mycorrhizal plants (Liu et al. 2007).

On the contrary, leaves of mycorrhizal plants infected with the phytopathogens *Botrytis cinerea* or tobacco mosaic virus showed no significant improvement in incidence and severity of necrotic lesions than those of nonmycorrhizal ones (Shaul et al. 1999). Further investigation also revealed the induction of PR-1 and PR-3 expression was observed in the leaves of both non-mycorrhizal and mycorrhizal plants. Although, accretion and mRNA steady-state levels of these proteins were lower, and their appearance were delayed in the leaves of the mycorrhizal plants. They concluded that prior infection of AMF than pathogen attack is required. These evidences are strongly in favour of AMF triggered localized and systemic priming of plants.

The most studied phytohormone in the AMF-plant interactions is Jasmonic acid (JA) which has been exploited for a long. Both, the accommodation of AMF and the nutrient provided by it within the plant root cells are regulated by JA. There are
several studies which show the increase in the endogenous JA levels in arbusculated cells of plant (Hause et al. 2002; Vierheilig and Piché 2002; Stumpe et al. 2005; Meixner et al. 2005) upon phytopathogens attack.

Like other pathogens, SA also recognizes AMF as pathogen and acts against it by delaying its colonization or in some cases suppresses its growth. But, in mycorrhizal defective (myc-) mutants, it has been found that in response to AMF, SA levels are enhanced (García-Garrido and Ocampo 2002).

As discussed, AMF has always been a nutrient provider and defense elicitor for the host plant, but the study of AM fungi and foliar fungal plant pathogen particularly Cercospora sp. are very scarce. Further, limited application based studies on foliar disease such as CLS infected in plants colonized with AM fungi are available, of which the underlying mechanism is less understood. In addition, several AM fungal species have been used to study the colonization in various solanaceae plants including Ca. annuum. However, studies on the role of AM fungi colonizing Ca. assamicum plants against CLS disease caused by C. tezpurensis has never been studied.

2.4 Integrated approach in disease management

The individual effect of AMF and organic amendments as discussed above has shown that they have potential to suppress the phytopathogens but their combined effect can be beneficial or deleterious for plant health. It can be possible that their combined effect shows synergism towards each other and enhances the plant growth even more than their individual effect. Perner et al. (2007) observed that the addition of compost in combination with mycorrhizal inoculation can
improve nutrient status and flower development of plants grown on peat-based substrates. Labidi et al. (2007) also suggested that the effect of compost addition on growth of the AM fungal biomass could be one way to improve survival of planted seedlings in arid regions. In a field experiment undertaken by Caravaca et al. (2002) to evaluate the effect of mycorrhizal inoculation with *Glomus intraradices* and added composted residue on the establishment of *Pistacia lentiscus* L. seedlings in a semiarid area showed that after one year of plantation, the plant height of *P. lentiscus* seedlings increased by 106% with respect to the control. Again, Caravaca et al. (2006) observed that combined treatment involving the addition of a medium dose of amendment (100 mg C kg\(^{-1}\) soil) and the mycorrhizal inoculation with *G. intraradices* or *G. deserticola* produced an additive effect on the plant growth with respect to the treatments applied individually (about 77% greater than plants grown in the amended soil and about 63% greater than inoculated plants).

Maji et al. (2013) conducted an experiment wherein they observed the response of foliar disease of Mulberry variety S-1635 including pseudoCLS disease caused by *Pseudocercospora mori* under organic versus conventional farming system for two years (2007-2009). In this study, they applied following doses: FYM (20t/ha/year) and NPK 336:180:112kg/ha/yr in five split doses (Recommended package), Vermicompost (30t/ha/year) in five split doses, Vermicompost (30t/ha/year in five split doses) + twice foliar spray of vermiwash @600L/ha/crop, Vermicompost (25t/ha/year in five split doses) + green manure (*Crotalaria juncea*), Vermicompost (20t/ha/year) + green manure + recommended dose of bacterial and fungal biofertilizer (*Azotobacter chroococcum* @ 20kg/ha/yr.
and arbuscular mycorrhizal fungi (AMF) @ 80kg/ha/yr), T7 - Vermicompost (15t/ha/yr) + biofertilizers (A. chroococcum 20kg/ha/yr and AMF 80kg/ha/yr) + NPK: 168: 90: 56 in five split doses. Preparation of vermicompost was carried out using sericultural waste (silkworm liters, unused mulberry leaves, weeds of mulberry field). Results in terms of percent of disease index (PDI) presented significant reduction in vermicompost (15t) + biofertilizer + NPK 168:90:56 treatment followed by recommended dose of FYM (20t) + NPK 336:180:112 and then, vermicompost (25t) + green manure (PDI ~5.95); vermicompost (30t). Based on these results, Maji et al. (2013) suggested that the application of balanced organic and inorganic fertilizers helps in enrichment of soil beneficial mycoflora and nutrient supply for a healthy plant growth which may bring forth resistance to diseases.

**Necessity of current study**

As it was clear from the review of literature that the cercospora leaf spot has emerged as a potential disease that can cause considerable economic losses to the crop. The existing control measures suffer from several drawbacks as discussed above. This necessitates the exploration of alternative disease management approaches which are more effective and environmentally-benevolent. Organic amendments (OA) and symbiotic organisms such as arbuscular mycorrhizal fungi (AMF) which are conventionally used as fertilizer supplement have, in recent decades, been considered as potential disease management agents also. Nevertheless, use of OA and AMF has been mainly focussed on managing soil-borne phytopathogens. Role of OA (foliar spray of vermiwash) and AMF in
managing foliar fungal phytopathogens has received little attention of the researchers. Further, still there are many unknowns with regard to the underlying mechanisms of disease suppression by the OA and AMF.

Given the complexity of the plant disease, changes in behaviour of the phytopathogens under the influence of diverse environmental conditions, it is evident that no single component strategy for disease management is effective and sustainable. This has led to the concept of integrating all the disease management strategies such as combined application of AM fungi and organic amendment in a holistic way. During past few decades, a considerable amount of knowledge on various management strategies of CLS disease such as good agronomic practices, use of botanicals, chemical fungicides and biocontrol agents has been generated by researchers working in different parts of the world. However, there is dearth of information in the current scientific literature on combined use of vermiwash foliar spray and AMF for managing cercospora leaf spot disease of *Capsicum*.

Keeping in view the above, the current study entitled “exploring the role of arbuscular mycorrhizal fungi and organic amendments in suppression of the cercospora leaf spot disease of *Capsicum* sp.” was conducted with a particular emphasis on understanding the possible underlying molecular mechanisms involved in the suppression of CLS disease under the influence of AMF and vermiwash application. In addition, physico-chemical edaphic properties and molecular diversity of AM fungi of six agro-climatic zones of Assam were also studied in order to generate the baseline information. Various screening experiments were also conducted for the purpose of selecting appropriate AMF and organic amendment strategy.