REVIEW OF LITERATURE

Plants have the ability to defend themselves against most microbial pathogen with a complex array of physical barriers and antimicrobial compounds, which are either preformed or inducible. The existence of induced resistance, against a broad range of pathogens in host plants previously infected with a pathogen or non-pathogenic microorganisms is well documented (Durant and Dong, 2004). A number of different approaches are being considered by plant pathologists towards enhancing resistance against plant disease through ‘Biotechnology’. Plants have capacity to recognize and reject the pathogens at various stages of their colonization of the plant. Non-specific resistance at the point of recognition is mainly due to the attempt of microbe to break the first line of defense responses. Microbes, which are able to penetrate beyond the barrier of non-host resistance, establish a strong and persuasive relationship with plants (Jones and Takemoto, 2004). The term elicitor was coined for the molecules capable of eliciting production of phytoalexins, but now it is commonly used for compounds capable of stimulating any kind of plant defense response (Ebel and Cosio, 1994; Felbrich et al., 2000).

Elicitors are molecules, produced by the pathogen or generated from the host itself during pathogen attack, which can induce defense responses in the plant (Desender et al., 2007). They act as signal compounds at low concentrations, providing information for the plant to trigger defense, distinguishing elicitors from toxins which may act only at higher concentrations and/or affect the plant determinately without active plant metabolism. Studies on the mode of action of elicitors have revealed that after recognition by membrane bound receptors of the host; they activate Ca$^{2+}$, anionic and K$^+$ channels, phospholipases, NADPH oxides and other proteins that are yet unidentified. Some of the earliest responses following perception of elicitors by the plant cell involve ion fluxes across the plasma membrane, alkalinization of the culture medium and release of hydrogen peroxide (Nurnberger et al., 1994; Lamb and Dixon, 1997). Ultimately, the signal transduction cascades that involve early signaling events regulate the genes involved in defense responses.

Alternatives to fungicides, plant protection have arisen with the discovery of disease resistance inducers of biotic and abiotic origins that induce a localized or
systemic resistance in susceptible plants, which become resistant to subsequent infections. Depending on their efficacy, these compounds can be used in fields either alone or in combination with fungicides (Thakur and Sohal, 2013). Recent studies have indicated remarkable similarities between the defense mechanisms triggered by general elicitors and it is tempting to speculate the recognition of general elicitors which subsequently leads to plant innate immunity (Nurnberger and Brunner, 2002). Eventually, the induction of defense responses may lead to enhanced resistance (Fig. IV). This broader definition of elicitors includes both substances of pathogen origin (exogenous elicitors) and compounds released from plants by the action of the pathogen (endogenous elicitors) (Ebel and Casio, 1994; Boller, 1995; Nurnberger, 1999).

Figure IV: Signal and responses in plant-pathogen interactions to a stress signal resulting from pathogen attack (Source: Benhamou, 1996)

Elicitors may be divided into two groups, general elicitors and race specific elicitors. While general elicitors are able to trigger defense both in host and nonhost plants, race specific elicitors induce defense responses leading to disease resistance only in specific host cultivars. A complementary pair of genes in a particular
pathogen race and a host cultivar determines this cultivar specific (gene-for-gene) resistance (Thakur and Sohal, 2013). Thus, a race specific elicitor produced by the action of an avirulence gene present in a particular race of a pathogen will elicit resistance only in a host plant variety carrying the corresponding resistance gene. The absence of gene product will often result in disease (Hammond Kosak and Jones, 1996; Nurnberger and Scheel, 2001; Tyler, 2002).

Very often chemically complex biological compounds have been used as elicitors. In these cases, the exact composition of the elicitor ingredients is unknown. Examples of such elicitors are yeast extract and microbial cell-wall preparations (Radman et al., 2003). Yeast extract was used as an elicitor in carrot (Daucus carota) hairy-root cell cultures where a three and a half-fold increase were detected in peroxidase levels (Yong and Young, 1996). Hamerski et al. (1990) investigated the elicitors from fungal cell-wall extracts of two pathogenic plant fungi (Phytophthora megasperma and Alternaria carthami) significantly increased the levels of umbelliferone compared to the control cultures. In addition, two novel compounds were also detected. Of the two cell-wall-derived elicitors, the P. megasperma elicitor was the most effective inducer of coumarin production. A relatively large number of chemical communicants (effectors / elicitors) released by the fungi include small proteins, peptides and other metabolites, including volatile ones. These have been recently reviewed, as signal transduction in plants (Harman et al., 2004; Shoresh et al., 2010) conferring increased plant growth under stress (Harman, 2000; Shoresh et al., 2010), systemic resistance to diseases involving both jasmonate and salicylate signalling (Bae et al., 2011), systemic resistance to plant stresses like salt and temperature, enhancement of vigor in poor-quality seeds (Mastouri et al., 2010; Shoresh et al., 2010), improved nitrogen use efficiency (NUE) by plants (Harman and Mastouri, 2010; Shoresh et al., 2010).

Rhizosphere-resident antagonistic microorganisms are ideal biocontrol agents, as the rhizosphere provides the frontline defense for roots against infection by the pathogens (Lumsden et al., 1995). The microorganisms associated with rhizosphere soil of plants may have a neutral, pathogenic or beneficial interaction with the host plant (Raaijmakers et al., 2008) and influence plant growth and development (Rovira, 1991). The natural role of rhizosphere microorganisms is to maintain soil fertility and
improve plant growth or biocontrol effects, which could be of great advantage for the
health of host plant (Hartmann et al., 2009). The dynamic nature of these
microorganisms in the rhizosphere makes a possible venture for disease management
studies. In the rhizosphere, biologically and chemically highly diverse, complex and
dynamic interactions occur between plant roots, soil (micro) biota and the physical
and chemical conditions of the soil. The natural roles of rhizosphere microorganisms
in maintaining soil fertility and improving plant growth and health elicit considerable
research interest (Chandanie et al., 2009). Within this community of competing and
interacting microbes, a whole range of parasitic and beneficial microorganisms can
either cause disease or enhance plant performance, respectively. The definition
of plant growth promoting fungi or PGPF is limited to nonsymbiotic saprotrophic fungi
that live freely in rhizosphere soil or on the plant root surface. Very interestingly,
both PGPR and PGPF are non-pathogenic and occur in large numbers in the
rhizosphere and can stimulate plant growth by suppressing plant diseases (Kloepper et
al., 2004; Hossain et al., 2008; Marina et al., 2011). Not every organism identified as
PGPF will improve plant growth under all conditions or in association with all plant
hosts (Ousley et al., 1993). Beneficial soil-borne microorganisms can induce an
enhanced defensive capacity in above-ground plant parts that provides protection
against a broad spectrum of microbial pathogens and even insect herbivores (van der
Ent et al., 2009).

The local and systemic defense responses that are triggered by beneficial and
parasitic microorganisms are controlled by a signaling network in which the plant
hormones Salicylic acid (SA), Jasmonic acid (JA) and Ethylene (ET) play important
roles (Glazebrook, 2005). There is an ample evidence that SA, JA, ET pathways
cross communicate, allowing the plant to finely tune its defense response depending
on the invader encountered (Koornneef and Pieterse, 2008). Unlike SAR, ISR does
not involve the accumulation of pathogenesis related proteins or SA (Pieterse et al.,
1996), but instead relies on pathways regulated by JA and ET (Knoester et al., 1999;
Yan et al., 2002). During ISR in plants, several well-characterized defense reactions
such as hypersensitive reaction (HR) (Zhang et al., 2004), oxidative burst (Yaeno et
al., 2004), reinforcement of cell wall structures through lignification or callose
deposition (Zhao et al., 2005; Soylu, 2006), accumulation of antimicrobial
Review of Literature

Phytoalexins (McNally et al., 2003; Soylu, 2006) and induction of defense-related proteins with antifungal properties (Andreu et al., 2006) have been extensively reported in many plant species. PGPF may also induce systemic resistance by a number of different mechanisms, but due to limited research (Bent, 2006), this remains unclear. Although, most studies have focused on the interaction between rhizobacteria and plant pathogens, little is known about the molecular mechanisms of response and resistance offered by PGPF. Only a few studies of signaling pathways during PGPF-mediated ISR, all using Trichoderma sp., have been performed (Shoresh et al., 2005), but a mechanistic study with other PGPF has not yet been conducted. The growth promotion ability of PGPF has also been largely attributed to the production of growth-regulating substances as mentioned earlier. According to Shoresh and Harman (2008) increased plant growth and induction of resistance in plants is mediated by different elicitors. Studies using active cellulose from T. longibrachiatum as elicitors have demonstrated the involvement of SA and JA/ET pathways in plant defense (Martinez et al., 2001).

Fungal elicitors may originate from surfaces of germinating zoospores, chlamydospores as well as from cell walls of pathogens, but may also be intracellular. They may be proteins, peptides, glycoproteins, lipids and oligosaccharides act as host recognition molecules, hydrophobins, toxins, signal molecules and lipid transfer proteins (Repka, 2006). Culture filtrate (CF) treated plants expressed resistance to pathogen infection by an alteration of various metabolisms, such as high increase in activities of chitinase, β-1,3-glucanase, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase, indicating that an elicitor substance existed in the CFs (Meera et al., 1994). There are also reports on seed treatment with conidial suspension of Aspergillus niger, T. harzianum and Penicillium citrinum enhanced the growth of Chick pea (Yadav et al., 2011). Djonovic et al. (2007) demonstrated the hydrophobic like elicitor Sml of the beneficial soil borne fungus T. virens induces systemic resistance in maize. The mold A. giganteus, isolated from the soil of a farm in Michigan has been reported to produce a basic low molecular weight protein (51 amino acids) showing antifungal properties, so called antifungal protein (AFP) (Nakaya et al., 1990). The AFP from the soil mold A. giganteus displays a potent antifungal activity against Botrytis cineria, the causal agent of Botrytis blight of
Geranium (Moreno et al., 2003). There are number of reports on Pythium oligandrum a non-pathogenic soil inhabitant has been used as biocontrol agent of damping off and root diseases caused by a number of soil-borne pathogens (Martin and Hancock, 1987; Al-Rawahi and Hancock, 1998). This oomycete has a number of properties as a biocontrol agent that may play an important role in the reduction of disease incidence, such as aggressive mycoparasitism against many fungal and oomycete plant pathogens, antibiosis including hydrolytic enzymes (Benhamou et al., 1999), competition for saprophytic colonization of substrate in the rhizosphere (Martin and Hancock, 1987), plant growth promotion (Al-Rawahi and Hancock, 1998; Le Floch et al., 2003) and induction of plant defense reactions (Benhamou et al., 1997). Among these attributes, the induction of plant defense reactions appears to have the greatest potential as a control measure against a wide range of pathogens.

A disaccharide fractions isolated from Fusarium oxysporum, promotes rapid and transient PAL activity in Rubus fruticosus nano molar concentration (Nita-Lazar et al., 2004). Induction of defense responses by cell wall protein (CWP) of P. oligandrum has also been observed in tomato plants (Hase et al., 2006). An aqueous extract of the mycelium of P. chrysogenum induces resistance independently of known signaling pathways (Thuerig et al., 2006). The aqueous extract of dry mycelium of P. chrysogenum called PEN (an elicitor), conferred resistance not only in cotton plants against wilt disease caused by F. oxysporum f.sp. vasinfectum and by Verticillium dahliae (Dong et al., 2003) but also against the root knot nematode Meloidogyne javanica in cucumber and tomato and protected Arabidopsis thaliana plants against a wide range of pathogens (Thuerig et al., 2006). An elicitin like protein molecule oligandrin secreted by the fungus P. oligandrum induced resistance against P. parasitica in tomato plants (Picard et al., 2000). Benhamou et al. (2001) reported that oligandrin elicits systematic resistance to Fusarium crown and root rot in tomato plants infected with F. oxysporum f.sp. radicis- lycopersici and reduced wilt symptoms. Different studies have shown that the addition of Trichoderma spp. metabolites may act as elicitors of plant resistance, or that their expression in transgenic plants may act also as elicitors and induce the synthesis of phytoalexins, pathogenesis-related (PR) proteins and other compounds (Dana et al., 2001). Secondary metabolites produced by different Trichoderma strains were also isolated
and shown to induce expression of pathogenesis related (PR) proteins as well as reduced disease symptoms systemically (Vinale et al., 2008).

The disease suppressing activity of PGPF is exerted either directly by hampering growth and development of soil-borne pathogens through competition for nutrients or secretion of antibiotics in the rhizosphere (Bakker et al., 2007; De Bruijn et al., 2007; Sultana et al., 2009) or indirectly by eliciting a plant-mediated systemic resistance response (Pozo and Azcon-Aguilar, 2007; van Wees et al., 2008). Thus, rhizosphere competence is the only factor for enhancing the plant growth by the fungal strains. Mechanisms of plant growth promotion include increasing plant nutrient acquisition, modification of plant growth and development, modification of the soil environment to promote plant growth and biocontrol of plant pathogens. Biocontrol can be triggered either directly, where in the pathogen itself is attacked or indirectly through plant defense responses against the pathogen are induced. Several hypothesis have been put forward for the mechanisms of plant growth promotion by PGPF, including hormone production, substrate degradation (mineralization) and suppression of deleterious microorganisms (Hyakumachi and Kuboto, 2004).

PGPF produce plant hormones such as auxins, cytokinins or gibberellins that alter root morphology and stimulate growth (Furukawa et al., 1996; Contreras-Cornejo et al., 2009). The beneficial effects of Trichoderma spp. on plant growth and enhanced resistance to both biotic and abiotic stresses are well documented (Yedidia et al., 1999; Harman et al., 2004; Shoresh et al., 2005). Some Trichoderma spp. have been defined as mutualistic plant symbionts (Harman et al., 2004) which can colonize the root surface and epidermal intercellular spaces of plant roots (Yedidia et al., 1999) and have been shown to have direct effects on plants. The effects include increased growth and yields, increased nutrient uptake as well as increased percentage and rate of seed germination and activation of plant defenses to various diseases (Harman et al., 2004). Shivanna et al. (2005) demonstrated that cucumber plants grew better and produced more marketable fruits due to an increase in soil nutrients caused by PGPF and accumulated more inorganic minerals like Ca, Mg and K in aerial shoots. The main effect of PGPF on plant growth is therefore considered to be the solubilization of minerals in the soil to help the plant to derive necessary nutrients in an easily available form. Hyakumachi (2000) suggest that the mineralization of organic...
substrates by PGPF relates to the plant-growth promoting effect of those PGPF and finally providing the plants with necessary mineral nutrients in an easily assimilating form. During root colonization, *T. harzianum* induces the defense system in cucumber, by increasing chitinase and peroxidase activity in leaves and roots (Yedidia et al., 1999). *Trichoderma* spp. is known to enhance growth promotion which is dependent on either root colonization or colonization of the entire plant (Kleifeld and Chet 1992; Chacon et al., 2007; Shoresh et al., 2010), but in *A. thaliana- Trichoderma* spp. interaction there are only few reports (Korolev et al., 2008; Contreras-Cornejo et al., 2009; Segarra et al., 2009). There are also reports on *T. atroviride* promoting growth in *Arabidopsis* when applied to roots, revealing that growth enhancement might depend on root colonization and the ability of *Trichoderma* spp. to provide nutrients and phytohormones (Harman et al., 2004; Contreras-Cornejo et al., 2009; Shoresh et al., 2010). Studies on using active cellulose from *T. longibrachiatum* as elicitors have demonstrated the involvement of SA and JA/ET pathways in plant defense (Martinez et al., 2001). The selected isolates of *Trichoderma* spp. increased the root length and lateral root numbers of cucumber seedlings.

Pathogen control by PGPF may also occur via niche exclusion, antibiosis, predation, mycoparasitism and ISR induction (Shivanna et al., 1996; Whipps, 2001; Mauchline et al., 2002). The type of plant response induced after challenge with a pathogen resulted in the formation of structural barriers such as thickened cell wall papillae due to the deposition of callose and the accumulation of phenolics compounds at the site of pathogen attack (Benhamou and Belanger, 1998). Fungi may employ more than one control mechanism simultaneously. For example, a nonpathogenic strain of *F. oxysporum* was found to control *P. ultimum* via combination of ISR, antibiosis and mycoparasitism (Benhamou et al., 2002) and *Trichoderma* isolates are known to act directly on pathogens as biocontrol agents, have been also found capable of inducing systemic resistance (de Meyer et al., 1998). Gamliel and Katan (1991) reported that almost all the fungi isolated from the rhizosphere and roots of tomato inhibited the growth of the plant. In contrast other soil-borne fungi such as *Trichoderma* sp., *R. solani* and others can promote significant plant growth. In some cases, significant growth promoting effects of PGPF were
observed as increased yield of plants grown in fields over longer periods of 14 weeks or more (Shivanna et al., 1994).

The induction of plant defense responses mediated by rhizosphere-colonising Trichoderma has been well documented (Harman et al., 2004; Vinale et al., 2008). Some Trichoderma spp. induce growth in addition to ISR (Harman et al., 2004). Induced-systemic resistance has been observed in cucumber plants treated with PGPF from zoysiagrass (Meera et al., 1994; Hyakumachi, 1997; Koike et al., 2001). Almost all of these PGPF could induce resistance against anthracnose in cucumber. In contrast, Ishiba et al. (1981) reported that only 1.9-2.4% of the soil fungi isolated from cucumber rhizosphere, were able to induce systemic resistance against anthracnose in cucumber plants. Different types of PGPF have been isolated from all over the world and it would be interesting to know if any of these have as high performance of induced systemic resistance as PGPF isolated from zoysiagrass. The beneficial effects of certain rhizosphere fungi in terms of plant growth promotion and biological control have been reported by many researchers (Windham et al., 1986; Hall, 1987; Baker, 1991; Narita and Suzuki, 1991). Fungi like Phoma spp., Penicillium spp., F. equisti and Trichoderma spp. as well as other non-sporulating fungi isolated from Zoysiagrass rhizosphere promoted the growth of several plants and suppressed some soil borne and air borne diseases (Meera et al., 1994; Shivanna et al., 1996; Hyakumachi, 1997; Koike et al., 2001). The biological control is exerted either directly through antagonism of soil borne pathogens or indirectly by eliciting a plant mediated resistance response (van Loon et al., 1998; Pozo and Azcon-Aguilar, 2007).

Non-necrotizing mutualistic rhizosphere microorganisms can effectively trigger induced resistance (Tuzun and Kloeper, 1994; Pieterse et al., 1996; Tuzun and Bent, 1999), the best studied are several species of PGPR. Colonization of roots with PGPF also led to systemic resistance in distal parts of the plant (Meera et al., 1995). A non-pathogenic F. oxysporum strain Fo47 induces resistance against P. ultimum infection in cucumber (Benhamou et al., 2002). Dong et al. (2003) reported induced resistance against Verticillium wilt in both upland cotton and sea-island cotton by an aqueous extract of mycelium of P. chrysogenum. Koike et al. (2001) reported that various PGPFs induced systemic defense responses in cucumber plants,
when applied as barley grain inoculum (BGI) and culture filtrate (CF) against several diseases. The PGPF *A. ustus* inoculation on *A. thaliana* and *S. tuberosum* roots induced an increase in shoot and root growth and induces resistance against *B. cinerea* and *Pseudomonas syringae* DC3000 (Salas-Marina *et al.*, 2011). A PGPF, *P. simplicissimum* GP17-2 collected from the rhizosphere of zoysiagrass enhanced the growth of *A. thaliana* (Hossain *et al.*, 2007).

*P. simplicissimum*, a PGPF isolated from the rhizosphere of zoysiagrass has been shown to induce ISR responses in cucumber (Koike *et al.*, 2001) and *A. thaliana* (Hossain *et al.*, 2007). Similar report has been made by Elsharkawy *et al.* (2012) in *P. simplicissimum*, where enhancement of growth in *A. thaliana* and tobacco was recorded due to induced resistance against cucumber mosaic virus. Some strains of *Trichoderma* have been reported to elicit ISR and, moreover, colonized the roots for an intense defense response to subsequent pathogen attack (Hanson and Howell, 2004; Segarra *et al.*, 2007; Tucci *et al.*, 2011; Reglinski *et al.*, 2012). Induced resistance has been reported by *T. harzianum* against downy mildew in grapevine (Perazzolli *et al.*, 2011), while *T. asperellum* against *P. syringae* pv. *tomato* in *Arabidopsis* (Yoshioka *et al.*, 2012). *B. cinerea* infection of tomato plants pre-treated with *Trichoderma* lead to enhanced activation of JA- responsive genes, boosting systemic resistance (Tucci *et al.*, 2011).

Pen protected grapevine from downy mildew and powdery mildew (caused *Plasmopara viticola* and *Uncinula necator*), tomato from early blight (caused by *Phytophthora infestans*), onion from downy mildew (*Peronospora destructor*) and apple trees from apple scab (*Venturia inaequalis*) to a similar extent as fungicides such as copper and sulphur or well-known inducers such as benzothiadiazole or b-aminobutyric acid. Pen had no major direct fungicidal effect and is thus supposed to protect plants by activating their defense mechanisms (Thuerig *et al.*, 2006). The studies have demonstrated that *Penicillium* sp. are used as potential inducers of induced systemic resistance (ISR) in various plants (Madi and Katan, 1998; De Cal *et al.*, 2000; Koike *et al.*, 2001; Thuerig *et al.*, 2006; Chen *et al.*, 2006; Hossain *et al.*, 2007, 2008). Further, some of the PGPF and their elicitors offering resistance against plant diseases have been compiled and tabulated below:
Table showing examples of PGPF and their elicitors/inducers reported to provide induction of resistance against plant diseases

<table>
<thead>
<tr>
<th>PGPF</th>
<th>Crop</th>
<th>Disease/ Pathogen</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile dark mycelial fungus</td>
<td>Wheat</td>
<td>Take all caused by <em>G. graminis</em></td>
<td>Narita and Suzui (1991)</td>
</tr>
<tr>
<td><em>Trichoderma</em> sp., <em>Penicillium</em> sp. and <em>Phoma</em> sp.</td>
<td>Cucumber</td>
<td>Anthracnose caused by <em>Colletotrichum orbiculare</em></td>
<td>Meera <em>et al.</em> (1994)</td>
</tr>
<tr>
<td><em>Phoma</em> sp. and Non-sporulating fungus</td>
<td>Wheat</td>
<td>Take all and common root rot <em>G. graminis</em> and <em>C. sativus</em></td>
<td>Shivanna <em>et al.</em> (1996)</td>
</tr>
<tr>
<td><em>Trichoderma</em> sp., <em>Fusarium</em> sp., <em>Penicillium</em> sp., <em>Phoma</em> sp. and Sterile fungus</td>
<td>Cucumber</td>
<td>Anthracnose</td>
<td>Koike <em>et al.</em> (2001)</td>
</tr>
<tr>
<td><em>P. chrysogenum</em></td>
<td>Melon</td>
<td><em>Fusarium</em> wilt</td>
<td>Dong and Cohen (2002 a)</td>
</tr>
<tr>
<td><em>P. chrysogenum</em></td>
<td>Cotton</td>
<td><em>Fusarium oxysporum</em></td>
<td>Dong and Cohen (2002 b)</td>
</tr>
<tr>
<td><em>P. chrysogenum</em></td>
<td>Cotton</td>
<td><em>Fusarium</em> wilt and <em>Verticillium</em> wilt</td>
<td>Dong <em>et al.</em> (2003)</td>
</tr>
<tr>
<td><em>Trichoderma virens</em></td>
<td>Cotton</td>
<td><em>Colletotrichum</em> sp.</td>
<td>Djonovic <em>et al.</em> (2006)</td>
</tr>
<tr>
<td><em>P. chrysogenum</em></td>
<td><em>Arabidopsis thaliana</em></td>
<td><em>Hyaloperonospora parasitica,</em> B. <em>cineria,</em> <em>P. syringae</em></td>
<td>Thuerig <em>et al.</em> (2006)</td>
</tr>
<tr>
<td><em>Pythium oligandrum</em></td>
<td>Tomato</td>
<td>Bacterial wilt <em>R. solanacearum</em></td>
<td>Hase <em>et al.</em> (2006)</td>
</tr>
<tr>
<td><em>T. virens</em></td>
<td>Maize</td>
<td><em>C. graminicola</em></td>
<td>Djonovic <em>et al.</em> (2007)</td>
</tr>
<tr>
<td><em>P. simplicissimum</em></td>
<td><em>A. thaliana</em></td>
<td>Bacterial speck <em>P. syringae</em></td>
<td>Hossain <em>et al.</em> (2007)</td>
</tr>
<tr>
<td><em>Phoma</em> sp.</td>
<td><em>A. thaliana</em></td>
<td>Bacterial speck <em>P. syringae</em></td>
<td>Hossain <em>et al.</em> (2008)</td>
</tr>
<tr>
<td><em>Trichoderma</em> spp.</td>
<td>Tomato and canola</td>
<td><em>B. cineria</em> and <em>Leptosphaeria maculans</em></td>
<td>Vinale <em>et al.</em> (2008)</td>
</tr>
<tr>
<td><em>Fusarium equiseti</em></td>
<td>Tomato</td>
<td><em>Fusarium</em> crown and root rot</td>
<td>Horinouchi <em>et al.</em> (2008)</td>
</tr>
<tr>
<td><em>Phoma</em> sp.</td>
<td><em>A. thaliana</em></td>
<td>Bacterial speck <em>P. syringae</em></td>
<td>Sultana <em>et al.</em> (2009)</td>
</tr>
<tr>
<td><em>P. simplicissimum</em> and <em>T. harzianum</em></td>
<td>Cucumber</td>
<td>Damping off</td>
<td>Chandanie <em>et al.</em> (2009)</td>
</tr>
</tbody>
</table>
The interaction between the pathogen and host plant induces some changes in cell metabolism, primarily in the enzyme activities, including that of phenylalanine ammonia lyase (PAL), peroxidase (POX), lipoxygenase (LOX), superoxide dismutase (SOD) and β-1,3 glucanase (Hammerschmidt et al., 1982; Ohta et al., 1991; Fukasawa-Akada et al., 1996; Wojtaszek, 1997). PAL is one of the most intensively studied enzymes in plant secondary metabolism because of its key role in phenylpropanoid biosynthesis (Whetten and Sederoff, 1995). PAL has been demonstrated in metabolic activity of many higher plants and is the key enzyme in the synthesis of several defense-related secondary compounds like phenols and lignins (Hemm et al., 2004). Geetha et al. (2005) suggested the involvement of PAL in resistance mechanism of pearl millet to S. graminicola. Similarly, there was an early increase in PAL activity in rice upon infection with the blast pathogen Pyricularia oryzae (Wang et al., 2004) and in barley in response to fungal pathogens and elicitor treatments (Kervinen et al., 1998). Howell et al. (2000) found that cotton radicals
treated with *T. virens* or *T. virens* protein fractions exhibited consistent pattern of up-regulated PAL expression. Expression of various defense related enzymes was found involved in the induction of systemic resistance against pathogen infection. Tomato plants treated with talc based formulation of *T. virens* (Tv1) isolated from rhizosphere soil with challenge inoculation of *Fusarium* enhance the maximum induction of defense enzyme such as PAL, POX and PPO rather than the other isolates of *T. virens*. The enzyme activity increased from 7th day of sampling and the maximum was observed on 14th day of sampling and then it slightly decreased (Christopher *et al.*, 2010). Cucumber plants treated with *B. pumilus* strain SE49 showed an increased activity of total peroxidase and lignification in response to ingress of *C. orbicularae* (Jetiyanon *et al.*, 1997) and inoculation of *Trichoderma* spp. increased peroxidase and chitinase activities in roots and leaves of cucumber (Yedida *et al.*, 1999). Kauffmann *et al.* (1987) first reported the involvement of β-1-3-glucanase in disease resistance. This enzyme has a direct role in defense by acting on the glucan of fungal cell walls and the release of oligosaccharide fragments that elicit phytolexin production. β-1-3-Glucanase is a host-encoded PR protein induced in response to pathogen attack (van Loon, 1997). The β-1-3-glucanase (PR2) encoding gene is highly induced in leaves in response to inoculation with *T. atroviride*. Several studies have indicated that root colonization by *Trichoderma* strains results in increased levels of defense related enzymes in plants, including peroxidases, chitinases, β-1-3-glucanase (Howell *et al.*, 2000; Yedidia *et al.*, 1999, 2003; Harman *et al.*, 2004).

Transcript accumulation of PAL, chalcone synthase (CHS) (Arfaoui *et al.*, 2007), POX (Fossdal *et al.*, 2001), β-1,3-glucanase, chitinase (Derckel *et al.*, 1996; Renault *et al.*, 1996) and LOX (Kolomiets *et al.*, 2000; Alkharouf *et al.*, 2006) has been described in induced resistance against various pathogens in crop plants. Systemic resistance responses can also be activated by the colonization of roots with certain nonpathogenic microorganisms such as selected strains of PGPR and PGPF, a phenomenon known as ISR (van Loon *et al.*, 1998; van der Ent *et al.*, 2009). Although the majority of studies on beneficial microbe-induced resistance point to a role for JA and ET in the ISR response, several examples of PGPR and PGPF that trigger SA-dependent SAR responses have been documented (van der Ent *et al.*, 2009). During local and systemic responses a large group of defense enzymes, the PR
proteins and signal molecules are synthesized to provide a broad spectrum of antimicrobial activity (Bowles, 1990). Numerous genes involved in these processes have been identified (Hammond-Kosack and Jones, 1996), but the transcript accumulation patterns of defense enzymes during PGPF- mediated induced resistance have received less attention. Some studies reported that ISR by Trichoderma agents involve JA and ET signaling (Shoresh et al., 2005; Djonovic et al., 2007; Korolev et al., 2008; Moreno et al., 2009; Segarra et al., 2009; Bae et al., 2011), whereas in other cases it seemed SA- dependent (Alfano et al., 2007; Shoresh and Harman, 2008). Moreover activation of both the SA and JA pathway by some strains of Trichoderma has been reported (Segarra et al., 2007; Harman, 2011; Salas-Marina et al., 2011; Yoshioka et al., 2012).

A significant elevation in the mRNA levels of Chit1, β-1,3-glucanase gene and peroxidase gene was observed in leaves of Trichoderma- induced plants, 48 h post challenge with P. syringae pv. lachrymans, relative to plants treated exclusively with T. asperellum or P. syringae pv. lachrymans (Shoresh et al., 2005). The local and systemic defense responses that are triggered by beneficial and parasitic microorganisms are controlled by a signaling network in which the plant hormones SA, JA, and ET play important roles (Glazebrook, 2005). There is ample evidence that SA, JA, ET pathways cross communicate, allowing the plant to finely tune its defense response depending on the invader encountered (Koornneef and Pieterse, 2008). Cucumber plants pre-inoculated with beneficial fungus T. asperellum T203 developed a JA/ ET- dependence systemic resistance that was associated with potential PR gene expression in response to pathogen challenge (Shoresh et al., 2005). A similar observation was noticed in Arabidopsis following colonization of the roots by a beneficial Penicillium sp. (Hossain et al., 2008). A priming mechanism appeared to be activated, with B. cinerea infection of plants pre-treated with Trichoderma leading to enhanced activation of JA-responsive genes, boosting systemic resistance in a plant genotype- dependent manner (Tucci et al., 2011).

Shoresh et al. (2005) demonstrated that T. asperellum induces resistance against P. syringae pv. lachrymans in cucumber through a pathway mediated by JA and ET. The role of Trichoderma induced resistance was further established by Korolev et al. (2008) when they demonstrated that Arabidopsis mutants with a defect
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on JA or ET signalling failed to put up basal resistance against grey mould pathogen *B. cinerea* after *T. harzianum* treatment. Proteinaceous nonenzymatic elicitor from *T. virens* named as Sm1, efficiently elicited plant defense responses and systemic resistance against a foliar pathogen of cotton (Djonovic *et al*., 2006). The protective activity of Sm1 was associated with the accumulation of reactive oxygen species and phenolic compounds and increased levels of transcription of the defense genes regulated by SA and JA/ ET as well as genes involved in the biosynthesis of sesquiterpenoid phytoalexins (Djonovic *et al*., 2006). The important component of local and systemic resistance induced by *T. asperellum* in cucumber plants (Yedidia *et al*., 2000, 2003) was shown to be mediated by the ability of *Trichoderma* hyphae to penetrate several epidermal layers and colonize the intercellular spaces (Yedidia *et al*., 1999) accompanied by increased levels of plant defense transcripts. *T. harzianum* Tr6 isolated from the rhizosphere of cucumber were tested as a single application and in combination for their abilities to elicit induced resistance in cucumber against *F. oxysporum* f.sp. *radicis cucumerinum*. The Tr6 treatment induced a significantly higher level of resistance in cucumber, which was associated with the primed expression of a set of defense-related genes like *CHIT1*, β-1,3-Glucanase, *PAL1* and *LOX1* upon challenge with *Fusarium* (Alizadeh *et al*., 2013).

Pearl millet accounts for 50% of the total millets in the world and India produces more than half of world’s pearl millet. A major biotic constraint for the production of pearl millet is downy mildew disease caused by the oomycetous, biotrophic fungus *Sclerospora graminicola* (Sacc.) Schroet., causing 40-60% crop loss (Safeeulla, 1976; Shetty, 1987; Thakur *et al*., 1999; Amruthesh, 2000; Khairwal, 2008). Among the strategies employed to manage downy mildew disease, use of resistant cultivars and chemicals are primary, but these methods have their own limitations and disadvantages (Shetty, 1987; Thakur *et al*., 1999; Amruthesh, 2000; Amruthesh *et al*., 2005). The maximum production of pearl millet in India relies on hybrids and it seems to survive anything except downy mildew disease.

The downy mildew disease of pearl millet is being managed by seed treatment with fungicides and by exploiting host resistance (Singh and Shetty, 1990; Sudisha *et al*., 2010). However, there are instances wherein the pathogen develops resistance to fungicides as well as residue problems and breakdown of host resistance
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(Thakur et al., 1999; Amruthesh, 2000). Thus, an effective downy mildew disease management is important to prevent yield loss. Henceforth, the current situation is to develop an alternative mean that supplements the use of resistance breeding and chemical management with novel strategies that may replace or complement traditional methods and one of the important options is inducing resistance in the host to exploit its innate immunity.

Elicitors of microbial origin induce defense responses in host by mimicking the infection processes of the pathogen and sensitizing the host against actual pathogen invasion. Hence, the present study PGPF from rhizosphere soil and their identification, partial purification and subsequent seed treatment to pearl millet against downy mildew disease was targeted. Studies were also perceived the morphological, histological, biochemical and molecular aspects to understand the role of PGPF elicitors in resistance mechanism involved during pearl millet downy mildew host-pathogen interaction. The potent PGPF were also used for development of bioformulation for their field applications.

From the literature it is clear that many PGPF isolated from different rhizosphere soils have been used to induce disease resistance and to promote plant growth in various agricultural crops. However, the use of PGPF elicitors for the management of pearl millet downy mildew disease has not been investigated and its defense mechanism at biochemical and molecular level. Hence, the present study was undertaken with the following objectives:

Objectives

- Isolation and identification of plant growth promoting fungi (PGPFs) from rhizosphere soil for pearl millet downy mildew disease management and development of Bioformulations for field applications.
- Isolation of crude protein extracts from selected PGPFs to study defense responses in pearl millet against downy mildew disease.
- Elicitation of defense responses by crude elicitors of PGPFs in pearl millet against downy mildew disease.
- Elicitation of defense related genes by elicitors of PGPF in pearl millet downy mildew host-pathogen interaction.