Chapter 2

Review of Literature
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2.1 Fusarium wilt

Tomato, a well known household vegetable, is prone to fungal, bacterial, nematode, viral and mycoplasmal diseases. Lukyaneko (1991) stated that tomato is susceptible to more than 200 diseases resulting in 75 to 95 percent yield loss. Among them, Fusarium wilt is the most important disease caused by *Fusarium oxysporum* f.sp. *lycopersici*. Walker (1952) reported this disease for the first time in the Channel Islands (U.K.) as early as 1895.

The disease, a typical vascular wilt, is studied all over the world (Beckman, 1987). The pathogen being both soil and seed-borne in nature persists for longer periods in soil and causes systemic infection. In the field, infected plants produce typical symptoms viz., yellowing of leaves, browning of vascular system and finally the wilting and death of the plant. Further, most of the popular varieties grown in India were reported to be susceptible to fusarium wilt (Chauhan, 1988; Kapoor, 1988). The disease was more severe when tomato crop grown as monoculture was rotated with cereal crops (Williams, 1979). Kapoor (1988), noticed that the pathogen was more prevalent during the main cropping season i.e., March and April, while in off-season i.e., September and October, the root-rots (*Fusarium solani* and *Rhizoctonia bataticola*) were more predominant. El-healy *et al.*, (1962) reported that high incidence of disease was due to *F. oxysporum* f. sp. *lycopersici* in the hot season in Egypt with 30ºC and soil moisture of 40 per cent water holding capacity.

Pathogenic fungi in general and *Fusarium* spp. in particular are highly destructive pathogens of both greenhouse and field-grown major crops under favourable conditions for disease development. The disease caused by this fungus is characterized by yellowing of the older leaves, browning of the vascular system, wilting in a later stage and finally death of the whole plant. Chlamydospores of the pathogen remain in infested soils for several years and invasion occurs through wounds on the root surface (Kiran Kumar, 2008). At present, emerging fungal wilt diseases are one of the biggest challenges confronting African coffee growers, with noticeable yield losses (Adugna *et al*., 2001; Geiser *et al*., 2005; Serani *et al*., 2007). Coffee wilt disease or Tracheomycosis caused by *Fusarium xylarioides* (teleomorph: *Gibberella xylarioides* Heim and Saccas) is becoming an important major coffee disease of both Robusta and Arabica coffee in coffee growing regions of Africa (Adugna *et al*., 2001; Geiser *et al*., 2005; Silva *et al*., 2006). The incidence of coffee vascular disease (Tracheomycosis) in Ethiopia is reported to be
60%, with significant yield losses due to very severe damage and ultimate death of millions of coffee bushes (Adugna et al., 2001). However, studies reveal that *F. xylarioides* causes more deaths of young coffee plants than any other *Fusarium* spp. (Serani et al., 2007).

*Fusarium oxysporum* f. sp. *lycopersici* (FOL) is a highly destructive pathogen of both greenhouse and field grown tomatoes in warm vegetable production areas. The disease caused by this fungus is characterized by wilted plants, yellowed leaves and minimal or absent crop yield. There may be a 30 to 40% yield (Kirkampmar et al., 2008).

### 2.2 Disease survey

From the survey made at North Dakota Province, De Done and Venette (1993) reported that out of 30 serially affected root-rot soil samples, about 24 percent of them were affected by *F.oxysporum*. Padmodaya (1994) surveyed 13 districts in Karnataka, India and found that out of 65 infected tomato plant samples 3 gave *Fusarium* spp. in isolation from roots and collar regions.

### 2.3 Pathogen

Several species of *Fusarium* are responsible for causing wilt disease in tomato. Fusarium wilt of tomato was first described in England during 1895. In India, Varma (1954) observed that wilt in tomato was caused by several species of Fusaria. Among various species, *F.oxysporum* f.sp. *lycopersici* was highly specific to tomato and produced typical vascular wilt. On solid culture media, the growth of the pathogen appeared as cottony white producing pinkish or reddish pigments, as age advanced.

The fungus produces three types of asexual spores in culture. Macroconidia are hyaline, long, boat or sickle shaped, containing up to six cells in each conidia. Micrconidia are colourless (hyaline), small and ellipsoidal, produced freely on the aerial mycelium and of 1-2 septal segments in each. Chlamydospores are either intercalary or terminal (Booth, 1971).

### 2.4 Inoculum density

The percentage of wilted tomato plants due to *Fusarium oxysporum* f. sp. *lycopersici* varied in proportion to the quantum of inoculum added in the soil. It appeared that 20 percent
inoculum and 80 percent sterilized soil was required to cause the death of the plant. The inoculum levels at which 50 percent of the plants were infected was found to be 300 Chlamydospores per gram of fumigated soil (Marios and Mitchell, 1981).

2.5 Pathogenecity

Pathogenecity of the *Fusarium* isolates was distinguished on the basis of the relative percentage of plants killed. Variability in pathogenecity of strains of *F. oxysporum* f. sp. *lycopersici* was reported by several investigators (Varma, 1954). The isolates within *Fusarium* spp. differed in their potentiality to induce pre and post-emergence death of seedlings and wilt incidence in mature plants. However, the isolates within a species differ in their ability to cause pre and post-emergence death and induce wilt in main crop (Kapoor, 1987). Both wilt and root-rot are attributed to *F. oxysporum* f. sp. *lycopersici*. Race1 and 2 are commonly found causing the incidence (Abdullah and Ismail, 1976).

2.6 Methods of inoculation

Wellman (1939) followed dipping of injured roots of tomato in the mycelial suspension for pathogenecity test. Schroeder et al. (1953) followed inoculation of fungal culture to the soil and transplanting of the seedlings for pathogenecity test. Henderson and Winsted (1961) reported that pouring fungal suspension around the root zone helps in the establishment of the disease.

2.7 Survival

Abdullah and Ismail (1976) reported that the fungus normally survives on various non-host weeds and cultivated plants. The pathogen penetrates into the broken roots when the seedlings are transplanted in the infested areas and slowly move to the other parts of the plant through the conducting tissues. The pathogen is both seed and soil borne. Seeds collected from infected plants can contain about three percent infected seeds, sometimes the fungus is present beneath the seed coat. The fungus survives in the soil for a longer period and the warm climate was favorable for the survival of the fungus. Since the fungus produces chlamydospores, it could survive and persist indefinitely in fields (Beckman, 1987).
2.8 Symptomology

*F. oxysporum* f. sp. *lycopersici* is a vascular pathogen invades the plants mostly through roots. Two of the earliest symptoms on young plants are clearing of the veins and veinlets and drooping of the petioles and later leading to the wilting of entire plant. The earliest symptoms appear within 48 hours after the entry of the pathogen when veinlets become translucent followed by epinasty of older leaves. Symptoms may appear on the entire plant or on only on new branches, leaflets on one side of a petiole may be affected, while those on other side may remain symptomless. At advanced stage, browning of the vascular system can be seen and the oldest leaves turn yellow followed by younger petioles and stem, later the whole plant slowly wilts. In green house, wilting might occur at midday when sunlight is bright (Shref and Macnab, 1988).

2.9 Disease assay

Assessment of disease severity enables to find out the effectiveness of any practice employed. The pathogen *F. oxysporum* f. sp. *lycopersici* causes continuing grade of symptoms with epinasty and ending with wilting and withering. Foster and Walker (1947) gave detailed disease index which was accepted widely. Their system was composed of numbers ranging from 0-4, indicating plants in the range of healthy to dead. This method was being widely adopted by several investigators (Kapoor, 1987). The other method employed was to calculate the percentage of plants diseased or wilted.

2.10 Screening of tomato cultivars against Fusarium wilt

A resistant variety is one of the best answers to sustain the attack of a soil inhabitant pathogen like *F. oxysporum* f. sp. *lycopersici* which defies other means of control. In India, host resistance to fusarium wilt of tomato was studied by evaluating a collection of 134 cultivars, of these eight possessed a high degree of resistance to races of a soil inhabitant pathogen like *F. oxysporum* f. sp. *lycopersici*. Chauhan (1988) studied the reaction of varieties/lines of tomato of fusarium wilt and found that none of them were immune or resistant. Banerjee *et al.* (1990) investigated fusarium wilt resistance in *Lycopersicon*. Out of 48 entries tested, two entries were near immune while the other two were highly resistance.
2.2 Management

2.2.1 Biocontrol of fungal plant diseases

Phytopathogenic microbes have an immense impact on agricultural productivity, greatly reducing crop yields and sometimes causing total crop loss (Antoun & Prévost, 2006). Major pathogens induce well-known root or vascular diseases with obvious symptoms (Weller, 1988).

2.2.2 Biofertilizers for sustainable agriculture

Sustainable farming systems strive to minimize the use of costly and environmentally unfriendly synthetic pesticides/agrochemicals and to optimize the use of alternative management strategies to improve soil fertility and control soil-borne pathogens (Harrier & Watson, 2004). A more sustainable agriculture that is ‘ecologically sound, economically viable, socially just and humane should aim to recycle minerals in the soil with no or few external inputs, maintain a high biodiversity in agro ecosystems, favour mechanical and biological weed control, and better exploit soil-plant-microbe interactions for plant nutrition and protection against pests (Edwards et al., 1990). An answer to this is the biofertilizer, an environmentally friendly fertilizer now used in many countries. During the last couple of decades, the use of biofertilizers-PGPR for sustainable agriculture has increased tremendously in various parts of the world. Vessey (2003) defined biofertilizer as a substance that contains living microorganisms which, when applied to seed, plant surfaces or soil, colonize the rhizosphere or the interior of the plant and promote growth by increasing the supply or availability of primary nutrients to the host plant. The term is not synonymous with organic/biological fertilizer or biopesticide. The main sources of biofertilizers are PGPR, beneficial rhizospheric fungi such as arbuscular mycorrhizae, Penicillium and cyanobacteria (blue-green algae) that are long known to have plant growth promoting effects via increasing the nutrient status of host plants (Vessey, 2003). Various studies have demonstrated a positive influence of biofertilization on horticultural plant growth, development and yield (Rodríguez Sr., 2006). Significant increases in growth and yield of agronomically important crops in response to inoculation with biofertilizers have been reported (Asghar et al., 2002). Moreover, AM products are now
commercially available as biofertilizers in Europe, Asia and the U.S.A (Narutaki & Miyamoto, 1996; Talavera et al., 2001). In addition, some PGPR appear to promote growth by acting as both biofertilizer and biopesticide. For instance, strains of *Burkholderia cepacia* have been shown to have biocontrol characteristics to *Fusarium* spp. but also to stimulate growth of maize under iron-poor conditions via siderophore production (Beivivio et al., 1998).

The mode of action by which biofertilizers enhance the nutrient status of host plants can be categorized into some important areas (Vessey, 2003): (1) Biological N\(_2\) fixation; (2) Increasing the availability of nutrients in the rhizosphere (e.g. solubilization of phosphorus); (3) Inducing increase in root surface area; (4) Enhancing other beneficial symbioses of the host such as arbuscular mycorrhizae and phytohormone production; 5) Production of enzymes that decrease phytohormone production by the host, induction of the host to produce signal substances to other symbionts (e.g. flavonoids); and (6) Combination of modes of action. Recorded important benefits from biofertilizers include: 1) Increasing crop yield by 20-30%; 2) Replacing chemical nitrogen and phosphorus by 25%; 3) Activating the soil biologically; 4) Restoring natural soil fertility; and 5) Providing protection against drought and some soil-borne diseases (http://www.vasat.org/learning_resources/OrganicFAQs/biofertilizer.htm 21-Aug 2007).

### 2.2.3 Influence of microorganism in bio-control of Fusarium wilt

Biological control agents have been reported to be an effective method to control plant pathogens. Among the plant pathogens, *F.oxysporum* was found to be the cause for the most serious disease of commercial plants in the world. The plant pathogens produce enzymes and toxin that degrade the plant cell wall components (Omokolo et al., 2003).

The rhizosphere is a reservoir of genetically diverse populations of bacteria, the composition of which is determined by the selective influence of plant and soil type. Rhizodeposition through plant root exudates plays a major role in defining resident microflora, which differs from that in bulk soil (Lynch, 1990). Rhizobacterial diversity is influenced by plant species, soil type and other agricultural practices. Since root exudation is species-specific, it is a major factor that determines community composition within the rhizosphere (Chiarini
et al., 1994; Mahaffee and Kloepper, 1997; Griffiths et al., 1998; Germida and Siciliano, 2001; Mittal and Johri, 2007; Monterio et al., 2009; Micallef et al., 2009).

2.2.4 Mechanisms of biocontrol

Pathogen suppression by antagonistic microorganisms can result from one or more mechanisms depending on the particular antagonist involved (Barea et al., 2005). An effective biocontrol agent often acts through a combination of several different mechanisms (Whipps, 2001).

An understanding of the mechanisms by which biological control of plant diseases occur is critical to the eventual improvement and wider use of biocontrol methods. These mechanisms are generally classified as competition, parasitism/predation, and antibiosis (Baker, 1968).

2.2.4.1 Competition

Competition for nutrients and suitable niches is another key mechanism among pathogens and biocontrol PGPR in biocontrol of some plant diseases (Bashan and de-Bashan, 2005). Members of Pseudomonads are highly efficient in competition for root resources among rhizobacterial communities (Barea et al., 2005). On plant surfaces, host-supplied nutrients include exudates, leachates, and waste products of other organisms or senesced tissue (Pal and Gardener, 2006). To successfully colonize the phytosphere, a microbe must effectively compete for the available nutrients. Biocontrol rhizosphere bacteria have the ability to multiply and spread in the rhizosphere environment, to colonize potential infection sites on the root and to act by direct contact with the pathogens (Insunza et al., 2002). Although difficult to prove directly, much indirect evidence suggests that competition between pathogens and non-pathogens for nutrient resources is important for limiting disease incidence and severity (Bashan & de-Bashan, 2005; Pal & Gardener, 2006). The degree of the susceptibility of soil-borne pathogens to the prevailing competition remarkably varies among microbes. In general, soil-borne phytopathogens such as species of *Fusarium* and *Pythium* that infect through mycelial contact are more susceptible to competition from other soil and plant-associated
microbes than those pathogens that germinate directly on plant surfaces and infect through appressoria and infection pegs (Pal and Gardener, 2006).

Studies have often revealed multiple modes of action of the population of putative PGPR inhabiting the rhizosphere (Weller, 1988; Haas and Keel, 2003). It is important to remember that in a given biological agent more than one mechanism may operate to suppress a pathogen, and the relative importance of a particular mechanism may vary with the physical or chemical conditions in the rhizosphere (Weller, 1988). In addition, *Pseudomonas* spp. produce several metabolites with antimicrobial activity towards other bacteria, fungi and even nematodes (Haas and Keel, 2003). Several reports also show the potential of combining different Biocontrol agents with different disease-suppressive mechanisms in the field (de Boer *et al*., 2003) and the combined inoculation of selected rhizosphere microorganisms has been recommended for maximizing plant growth and nutrition (Probanza *et al*., 2001).

### 2.2.4.2 Antibiosis in biocontrol

Antibiosis is the antagonism mediated by specific or nonspecific metabolites of microbial origin, by lytic enzymes, volatile compounds or other toxic substances (Jackson, 1965). The role of antibiotics in biocontrol has been studied by the generation of mutants that do not produce antibiotics. Panteleev (1975) reported that a stain of *Pseudomonas* reduced wilt incidence by producing antibiotics. Production of an antibiotic, Phenazine has been implicated in biocontrol (Gurusiddaiah *et al*., 1986 and Thomashow *et al*., 1986).

### 2.2.4.3 Siderophore production

Living organisms require iron as a component of proteins involved in important life processes such as respiration, photosynthesis and nitrogen fixation. Iron is one of the major elements in the earth’s crust but soil organisms such as plants and microbes have difficulty in obtaining sufficient iron to support their growth because of formation under aerobic conditions of ferric oxides, which cannot be readily transported into cells. Under such iron starvation, bacteria, fungi and plants secrete small, specialized efficient iron (III) chelator molecules commonly known as siderophores (Drechsel & Jung 1998). After the iron-siderophore complexes have formed, these now soluble complexes are internalized via active transport into
the cells by specific membrane receptors (Glick et al., 1999). Following either cleavage or reduction to the ferrous state, the iron is released from the siderophore and used by a cell (Glick et al., 1999). Lankford (1973) coined the term siderophore to describe low molecular weight (approximately 600 to 1500 daltons) molecules that bind ferric iron with an extremely high affinity. Siderophore was derived from a Greek term meaning iron carrier (Ishimaru, 1993). The dominant iron-binding ligands of siderophores are hydroxamates and catecholates (phenolates), but carboxylate, oxazoline, α-hydroxy carboxylate and keto hydroxyl bidentate siderophores have also been found (Essén et al., 2006). In addition, hybrid siderophores with more than one type of ligand group exist (Neilands, 1981). Each functional group presents two atoms of oxygen, or less commonly, nitrogen, that bind to iron (III). While bacterial siderophores are structurally diverse, fungal siderophores are dominated by hydroxamate siderophores (Drechsel and Jung, 1998). On the other hand, plant siderophores are linear hydroxyl and amino-substituted iminocarboxylic acids, such as mugineic and avenic acids (Sugiura et al., 1981). Many bacteria are capable of producing more than one type of siderophore or have more than one iron-uptake system to take up multiple siderophores (Neilands, 1981). A considerable number of wild Arabica coffee-associated rhizobacteria (67%) produce siderophores. Wide arrays of beneficial plant-associated bacterial genera, e.g. Pseudomonas, Azotobacter, Bacillus, Enterobacter, Serratia, Azospirillum and Rhizobium secrete various types of siderophores (Glick et al., 1999; Loper and Henkels 1999). Siderophores function mainly in the solubilization, transport and storage of iron (Stephan et al., 1993). Some other important mechanisms by which siderophore-producing bacteria contribute to the promotion of plant growth are described briefly below.

Siderophores produced by certain strains of fluorescent Pseudomonas spp. have been linked to suppression of soil-borne plant diseases. It has been suggested that siderophores act antagonistically by sequestering iron from the environment, restricting growth of the pathogen (Bashan & de-Bashan, 2005). Convincing evidence for the involvement of siderophores in disease suppression is readily available (Bashan & de-Bashan, 2005). For example, a mutant strain of P. putida that overproduces siderophores has been shown to be more effective than the wild bacterium in controlling the pathogenic fungus F. oxysporum in tomato. Many wild strains that lose their siderophore trait also lose biological control activity. The extent of disease
suppression as a consequence of bacterial siderophore production is affected by several factors (Bashan & de-Bashan, 2005), including the specific pathogen, the species of biocontrol PGPR, the soil type, the crop and the affinity of the siderophore for iron. For instance, siderophore-mediated suppression should be greater in neutral and alkaline soils than in acid soils (Bakker et al., 1986). Thus, disease suppression under controlled laboratory conditions is only an indication of the efficacy of the biocontrol agent in the field.

Pathogens are thought to be sensitive to suppression by siderophores for several reasons: (a) they produce no siderophores of their own; (b) they are unable to use siderophores produced by the antagonists or by other microorganisms in their immediate environment; (c) they produce too few siderophores or biocontrol PGPR produce siderophores that have a higher affinity for iron than those produced by fungal pathogens, allowing the former microbes to scavenge most of the available iron, and thereby prevent proliferation of fungal pathogens; or (d) they produce siderophores that can be used by the antagonist, but they are unable to use the antagonist’s siderophores (Weller, 1988; Bashan & de-Bashan, 2005).

Bashan & de-Bashan (2005) have reported that depletion of iron from the rhizosphere normally does not affect plant growth, as plants can thrive on less iron than can microorganisms. However, some plants can bind and release iron from bacterial iron-siderophore complexes, and use the iron for growth. Thus, these plants benefit in two ways: from the suppression of pathogens and from enhanced iron nutrition, resulting in increased plant growth. *Pseudomonas* siderophores have also been implicated in inducing systemic resistance (ISR) in plants (Leeman et al., 1996), i.e. enhancement of the defense capacity of the plant against a broad spectrum of pathogens. Exposure to pathogens, non-pathogens, PGPR and microbial metabolites stimulates the plant’s natural self-defense mechanisms before a pathogenic infection can be established, effectively ‘immunizing’ the plant against fungal, viral and bacterial infections (Bashan & de-Bashan, 2005). Protection occurs by accumulation of compounds such as salicylic acid, which plays a central protective role in acquired systemic resistance, or by enhancement of the oxidative enzymes of the plant. While acquired systemic resistance is induced upon pathogen infection, induced systemic resistance can be stimulated by other agents, such as PGPR inoculants. The feasibility of protecting plants by induced systemic resistance has been demonstrated for several plant diseases. For instance, plants inoculated with
the Biocontrol PGPR *P. putida* and *Serratia marcescens* were protected against the cucumber pathogen *P. syringae* pv.*lachrymans* (Bashan & de-Bashan, 2005).

### 2.2.4.4 Hydrogen cyanide (HCN) production

Considerable number of free-living rhizospheric bacterial communities, mainly *Pseudomonas* spp. (Faramarzi *et al*., 2004; Ahmad *et al*., 2006; Faramarzi & Brand, 2006), are capable of generating HCN by oxidative decarboxylation from direct precursors such as glycine, glutamate, or methionine (Castric, 1977). Other rhizobacterial genera reported to produce HCN include *Bacillus* (Ahmad *et al*., 2006; Faramarzi & Brand, 2006) and *Chromobacterium* (Faramarzi & Brand, 2006). However, hydrogen cyanide has not been detected in cultures of *Pseudomonas aeruginosa*, *Serratia marcescens*, *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* (Michaels and Corpe, 1965).

In general, cyanide is formed during the early stationary growth phase (Knowles and Bunch, 1986). Cyanide occurs in solution as free cyanide, which includes the cyanide anion (CN-) and the non-dissociated HCN. It does not take part in growth, energy storage or primary metabolism, but is generally considered to be a secondary metabolite that has an ecological role and confers a selective advantage on the producer strains (Vining, 1990). Cyanide is a phytotoxic agent capable of inhibiting enzymes involved in major metabolic processes and is considered as one of the typical features of deleterious rhizobacterial isolates (Bakker & Schippers, 1987). Nevertheless, at present its applications in areas of biocontrol methods (see below) are increasing (Voisard *et al*. 1989; Devi *et al*., 2007). Cyanogenesis in bacteria accounts in part for the biocontrol capacity of the strains that suppress fungal diseases of some economically important plants (Voisard *et al*., 1989). For instance, for many pseudomonads, production of metabolites such as hydrogen cyanide is the primary mechanism in the suppression of root fungal pathogens. Cyanogenic bacterial species have also been found to be effective in killing the subterranean termite Odontotermesobesus, an important pest of major agricultural crops and forest plantation trees, under *in vitro* conditions (Devi *et al*., 2007), in addition to suppression of plant parasitic nematodes (Siddiqui *et al*., 2006). Hydrogen cyanide effectively blocks the cytochrome oxidase pathway and is highly toxic to all aerobic microorganisms at picomolar concentrations. However,
producer microbes, mainly pseudomonads, are reported to be resistant (Bashan & de-Bashan, 2005).

### 2.2.4.5 Production of lytic enzymes

A large array of other microbial substances are involved in the suppression of phytopathogenic growth and subsequent reduction in damage to plants. These substances include lytic enzymes such as chitinase, β-1,3-glucanase, protease and lipase (Bashan & de-Bashan, 2005). Many *Pseudomonas* and *Bacillus* species are capable of producing some of these hydrolytic enzymes. For example, *Pseudomonas stutzeri* produces extracellular chitinase and β-1,3-glucanase, which lyse the pathogen *Fusarium* sp. (Bashan & de-Bashan, 2005). *Cladosporium werneckii* and *Bacillus cepacia* can hydrolyze fusaric acid (produced by *Fusarium*), which causes severe damage to plants (Bashan & de-Bashan, 2005). Direct evidence for the role of cell-wall degrading enzymes in biocontrol *in vivo* comes from studies utilizing mutant strains over expressing or lacking a particular enzyme, or transgenic plants expressing these enzymes (Pozo *et al*., 2004).

### 2.2.4.6 Antibiotics

Many organisms operative in pathogen suppression also act via antibiosis (Mazzola, 2002). Antibiotic production by biocontrol PGPR is perhaps the most powerful mechanism against phytopathogens (Bashan & de-Bashan, 2005). Indeed, the first clear-cut experimental demonstration that a bacteria produced antibiotic could suppress plant disease in an ecosystem was made by Tomashow & Weller (1988). Fluorescent pseudomonads have been shown to produce a range of antibiotics, *e.g.* 2,4 diacetylphloroglucinol, which suppress the growth of various soil-borne fungal phytopathogens (Mazzola, 2002).

### 2.2.5 Use of Plant growth promoting rhizobacteria (PGPR) as antagonists in biocontrol

Microorganisms that can grow in the rhizosphere are ideal for use as a biocontrol agent. The rhizosphere provides the front line defense for roots against attack by pathogens. Pathogens encounter organism from rhizosphere microorganisms before and during primary infection and also during secondary spread on the root. A comprehensive review on the use of
rhizosphere bacteria is biocontrol agents against soil-borne diseases has been made by Weller (1988). Weller (1988) in his review has stressed importance of establishment and maintenance of a threshold population of bacteria on planting material or in soil and any drop in viability below that level may reduce the possibility of biological control. Many soil edaphic factors like soil temperature, soil moisture, pH and clay content influence the survival and establishment of bacteria and their interaction with the pathogens. The way in which the bacteria are cultured and then processed will affect their viability and tolerance to adverse conditions once applied. Root colonization by the introduced bacteria is essential for biocontrol of root pathogens and that increasing the population of an introduced bacterium on the root should enhance disease control (Suslow, 1982). The ability of different rhizobacteria to colonize roots offering control of several soil-borne pathogens has been reviewed (Weller, 1988).

Direct growth promotion by PGPR was first reported by Lifshitz et al. (1987). They studied the growth promotion activity of *Pseudomonas putida* strain GR 12-2 in gnotobiotic growth pouch assay and reported that inoculation of rapeseed with GR12-2 significantly increased root length, shoot length and phosphorus uptake compared with an inoculated control. Kleopper et al. (1988) collected 4,000 bacterial strains from the root zone of rape seed and screened them at 4-14°C on the basis of growth promotion, metabolism of seed exudates and root colonization. They further tested 887 strains for growth promotion of rapeseed in greenhouse trials and reported that 35 strains increased yield by 6-13% during the 2-year trial. In another study, Kleopper et al. (1991) found that inoculation with PGPR increased the emergence and dry weight of 2-day-old rapeseed seedlings by 23% and 79% respectively, over an uninoculated control. Similarly, a significant increase in emergence rate was also observed due to inoculation with certain root colonizing bacteria, including *Pseudomonas* (Kleopper et al., 1986).

Chen et al. (1994) isolated PGPR strains from the roots and rhizosphere soil and used these to inoculate sterile peat. For many years (1979-1990) they grew rapeseed inoculated with five strains and observed a significant increase in yield (11.5% over the uninoculated control). Significant yield increases in many other crops such as wheat, maize and potato have also been reported in response to inoculation with PGPR (Iswandi et al., 1987; Javed et al., 1996; Khalid et al., 1997; Zahir et al., 1998).
Gracia et al., (2003) have studied the effect of three plant–growth promoting rhizobacteria on the growth of seedlings of tomato and pepper in two different sterilized and non-sterilized media. They isolated two bacteria from the rhizosphere of Alnus glutinosa and identified them as Bacillus pumilus and Bacillus licheniformis. The third strain was isolated from the rhizosphere of Lupinus albus and was identified by FAMEs and confirmed as Pseudomonas fluorescens. All the three isolates had the capacity to modify the plant growth. They had correlated the better growth in tomato over pepper with root colonization. They have reported that growth promotion activity of selected rhizobacteria was not affected by sterilized and non-sterilized peat. Therefore, factors other than competition with other microorganisms must be more important in the performance of the bacterial inoculants. Root growth was only modified in nonsterilized black peat with the two bacteria, in pepper it increased, and in tomato it decreased. According to them, these different effects could be due to a hormonal effect of the auxins which influence root growth.

The effect of plant growth promoting rhizobacteria on Nicotiana tabacum L. plant growth promotion against blue mold disease caused by Peronospora tabacina under greenhouse conditions was studied by Zhang et al. (2004). They have tested five bacterial strains viz., Serratia marscenes 90-166, P. fluorescens 89B-61, Bacillus plumilus SE34, and Bacillus pasteurii C-9 with reported PGPR activity. PGPR strains were applied as seed treatments and root drenches and tested at two different concentrations (10^7 and 10^9 cfu mL^{-1}). Strain 90-166 at 10^9 cfu mL^{-1} increased plant growth in several parameters and reduced the disease severity of blue mold severity. Treatments with PGPR may cause a series of physiological and biochemical changes which lead to increased resistance against pathogens or stimulation of plant growth. The selected strain 90-166 can stimulate plant growth and result in disease reduction systematically. The growth promotion and disease reduction is important because visible growth promotion can serve as an indication or prediction of systemic disease protection.

Currently, Pseudomonas spp. are receiving much attention as bicontrol agents in the studies initiated at the University of California, Berkeley during 1970s. There is evidence that Pseudomonas have a role in the suppressiveness of certain soils to Fusarium wilt of flax, radish and cucumber (Scheer and Bakker, 1982). Many of the fluorescent Pseudomonads, like P.
putida, P. aurignosa, P. aureofaciens predominantly P. fluorescens suppress the soil-borne pathogens through rhizosphere colonization (Mills et al., 1989; Ongena et al., 1999; Stephens et al., 1993; Weller and Cook, 1986). Aspirus and De fa Cruz (1986) reported the suppression of bacterial wilt pathogen R. solanacearum by using P. fluorescens reduced root rot of black gram caused by Macrophomina phaseolina (Jayashree et al., 2000; Shanmugam et al., 2001). Seed and foliar application of P. fluorescens reduced the sheath blight of rice caused by Xanthomonas oryzae pv. oryzae (Vidyasekaran et al., 2001; Nanda kumar et al., 2001). Application of P. fluorescens reduced the incidence of fusarium wilt in tomato, damping-off in tomato and hot pepper both under green house and field conditions (Ramamoorthy et al., 2002a; Ramamoorthy et al., 2002b). Fluorescent pseudomonads are known to inhibit the growth of plant pathogens by diverse mechanisms besides, they also act as plant growth-promoting rhizobacteria by producing growth-promoting substances and enhancing the crop yield (Lifshitz et al., 1987). Ramamoorthy et al. (2002b) have isolated twenty isolates of fluorescent pseudomonads from rhizosphere soil of different crops grown in different parts of South India and identified based on biochemical characterization. 18 isolates belong to P. fluorescens and two identified as P. putida. These isolates were further tested for their ability to promote growth and for the management of damping-off disease caused by Pythium aphanidermatum under greenhouse conditions. Among these isolates, P. fluorescens isolate Pf1 biovar I showed maximum inhibition of mycelial growth in vitro and increased plant growth promotion of tomato and hot pepper. All the isolates were effective in managing the damping-off disease in tomato and most of them in pepper. P. fluorescens isolate Pf1 biovar I showed the lowest disease incidence in tomato and hot pepper respectively, and increased the plant growth under greenhouse conditions. Powder formulations of Pf1 biovar I was effective in controlling the disease and promoted the plant growth under field conditions.

Anith et al. (2004) reported that PGPR can be used to reduce bacterial wilt incidence in tomato. There was suppression in bacterial wilt of Eucalyptus urophylla, crown gall disease of grapevine; and black pepper against foot rot by P. fluorescens (Ran et al., 2005; East well et al., 2006; Paul and Sarma, 2006). P. fluorescens reduced the disease incidence of grey mould in tomato plants inoculated with Botrytis cinerea in greenhouse trials and potato bacterial wilt caused by R. solanacearum (Yildiz et al., 2007; Kuwarbachew et al., 2007). Wet seed treatment
with *P. fluorescens* Pfl significantly increased the seed germination and seedling vigour of cotton. Seed treatment followed by foliar application of Pfl significantly reduced the incidence of bacterial blight of cotton caused by *Xanthomonas axonopodis* pv. *malvacearum* (Salah Eddin et al., 2007). Reduction in *Alternaria triticina* infected leaf area and also improved growth of wheat to a greater extent followed by *P. fluorescens* treatment was observed by Siddiqui (2006). *P. fluorescens* isolated from rice were used as biocontrol agents against *R. solanacearum* (Lawongsa et al., 2008). Biswas and Singh (2008) attempted to manage bacterial wilt of tomato using *P. fluorescens*. They have reported that the efficacy of the bioagent might be attributed to the production of siderophores, which have strong inhibitory effect on the pathogen. Sharma et al. (2008) reported that the lowest disease incidence of core rot of kinnow fruits treated with *Bacillus subtilis* and *P. fluorescens*. Nikum et al. (2008) used *P. fluorescens* as seed dressing and succeeded to manage seedling diseases of cotton. Patro et al. (2008) designated *P. fluorescens* as a potential bioagent for management of blast in ragi. *P. fluourescens* showed fungal growth inhibition in plate assay in the laboratory and reduced ragi blast severity in the field. *P. fluorescens* was effective in reducing the disease incidence of *Alternaria alternata* in watermelon (Umamaheshwari et al., 2008). Fluorescent Pseudomonads was compared for its efficacy along the chitosan for the control of Fusarium head blight disease of cereals and associated mycotoxin contamination of grain (Khan and Doohan, 2009).

### 2.2.6 Use of bioformulations in biocontrol

A powder formulation of *P. fluorescens* has been developed by Vidyasekaran et al., 1997a, tested against root and foliar diseases (Vidyasekaran et al., 1997b; Meena et al., 2002) and is currently being used by the farmers in India. A successful biocontrol agent must survive formulation and storage, and must be a competitive and aggressive colonizer after inoculation (Beatty and Jensen, 2002; Selim et al., 2005). The talc-based formulation has been reported for the management of several crop diseases in India (Samiyappan, 1998).

Successful formulations of *B. subtilis* and *P. fluorescens* are commercially available. Those of *Bacillus* are very stable due to the ability of this bacterium to form spores (Emmert and Handelsman, 1999) that are long lived, and resist heat and desiccation (Kloepper, 1991). *B. subtilis* and *P. fluorescens* are being marketed as dry formulations with talc or peat as carriers, and are also used for mixing with potting soil or with compost for incorporation into nursery
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beds or field soil (Sridhar et al., 1993; Kannan and Jayaraj, 1998). Reddy et al. (2008) tested the *P. fluorescens* isolates for antifungal activity against the different fungi that are known to attack rice plants. According to the results of previous studies (Shah-Smith Burns, 1997; Bharathi et al., 2004) when PGPR are formulated using inorganic or organic carriers, their stability and durability are increased (Mathivanan et al., 2005). The application of talc-formulation of *P. fluorescens* through seed, seed treatment plus foliar spray and foliar spray alone significantly reduced the leaf blight of groundnut incidence both under greenhouse and field conditions (Chitra et al., 2006).

Bioformulation of fluorescent *Pseudomonas* spp. induces systemic resistance against red rot disease caused by *Colletotrichum falcatum* and enhances commercial sugar yield in sugarcane (Vishwanathan and Samiyappan, 2008). Formulations with gram negative biocontrol agents were tried with peat and other carriers. Entrapment of bacteria in polymer gels such as anthum gum or alginate also proved potential (Bashan, 1986). Mixing mineral or organic carriers with the PGPR has also been found to increase the biocontrol efficacy (Radja Commare, 2000; Viswanathan and Samiyappan, 2001). Saravanakumar et al. (2007) tested the efficacy of PGPR formulations against *Exobasidium vexans* in *Camellia sinensis*, finding that foliar applications of *P. fluorescens* pf1 at 7-day intervals reduced the incidence of blister blight, almost as much as fungicide treatments, and increased the yield significantly. Moreover, Bharathi et al. (2004) in evaluating the efficacy of 13 PGPR strains against chilli fruit rot and dieback incited by *Colletotrichum capsici* observed that *P. fluorescens* pf1 and *B.subtilis* were effective in increasing seed germination and seedling vigour, and that a mixed bioformulation (pf1+B.subtilis+neem+chitin) was the best for reducing fruit rot incidence, while increasing plant growth and yield. In field experiments, the talc-based bioformulation mixture containing Pf1 along with chitin showed significantly less disease incidence and correspondingly resulted in enhanced yield (Nandakumar et al., 2001a; Bharathi et al., 2004). Talc-based formulations of plant growth promoting rhizobacteria *P. fluorescens* (Pf1) and *Bacillus subtilis* either single or mixed along with or without chitin and neem amendments were developed and tested under greenhouse and field conditions was studied by Bharathi et al., (2004). *P. fluorescens* Pf1 along with chitin amendment was effective for survival and colonization of bacteria under field conditions. Sabuquillo et al., (2006) reported the eight formulations of *Penicillium oxalicum* (FOR1 to FOR8) obtained by the addition of various ingredients. These formulations
significantly reduced tomato wilt caused by *Fusarium* sp. under greenhouse conditions and, in a preliminary trial, by *Verticillium* spp. in a field assay. Formulations included a talc-based powder and bentonite-based powder as inorganic carriers and peat and rice bran as organic carriers for increasing stability in interaction between associated PGPR and cotton plants, promoted seedling height, root length, seedling dry weight and root dry weight more effective than the control treatment under greenhouse conditions (Ardakani, 2010).

### 2.3 Plant resistance to pathogens

Plant resistance can be generally defined as the plant’s ability to suppress or slow down the damaging activity of the pathogen. Resistance in plants is manifested by the inability of the pathogen to grow or multiply, and spread and often takes the form of hypersensitive reaction (Agrios, 1988). Defense responses include the reaction of plants to disease-causing organisms (Hammond-Kosack and Jones, 1996). Plants are continually exposed to a vast number of potential pathogens and as a result they have evolved intricate defense mechanisms to recognize and defend themselves against a wide array of these disease causing agents by inducing a set of defense responses that can defeat the invading pathogens. Plants are resistant to most pathogens in their environment, as they are not host plants for particular pathogen or host plants, but resistant genes, allowing them to recognize specifically distinct pathogen races (Scheel, 1998).

#### 2.3.1 Induced systemic resistance (ISR)

The induced systemic resistance develops systemically in response to colonization of plant roots by certain plant growth promoting rhizobacteria (PGPR). This type of resistance, known as rhizobacteria mediated induced systemic resistance, is transferred by a jasmonate/ethylene sensitive pathway (Van Loon *et al.*, 1998).

Several strains of PGPR applied to seeds or roots of field crops have been used as elicitors of induced systemic resistance, leading to reduction in disease severity (Hammerschmidt and Kuc, 1995). Systemic resistance triggered in the plant rhizobacteria is referred to as rhizobacterial mediated induced systemic resistance (Van Loon *et al*, 1998). Induced systemic resistance is brought about by PGPRs through fortification of physical and mechanical strength of the cell wall as well as changing the physiological and biochemical reactions of the host leading to synthesis of defense chemical against the pathogen challenge. It is well known that PGPRs induce cell wall structural modification in response to pathogen
attack (Benhamou *et al.*, 1996; M’Piga *et al.*, 1997). Activation of defense gene production prior application with PGPR against a challenging pathogen is a novel strategy in plant protection. The increased activity of the above substances in the PGPR treated plants may play either a direct or an indirect role in the suppression of pathogen development in the host ultimately protecting the plants from pathogenic microorganisms. Plant growth promoting rhizobacteria mediated stimulation of defense related biochemical chemical compounds have been reported earlier (Zdor and Anderson, 1992; Maurhofer *et al.*, 1994; M’Piga *et al.*, 1997; Chen *et al.*, 1998; 2000). Induced systemic resistance mediated through biocontrol agents results in lignification and with increased activities of defense gene products that are synthesized via phenyl propanoid pathway (Boller and Mauch, 1988; Hammerschmidt and Kuc, 1995). In cucumber, seed treatment with PGPR has resulted in ISR against several pathogens in greenhouse and field experiments (Wei *et al.*, 1996).

Research over the past years has demonstrated that ISR can be a potential mechanism by which PGPR demonstrate biological disease control (Kleopper *et al.*, 1996). Fluorescent *Pseudomonas* are non-pathogenic rhizobacteria among the most effective rhizosphere bacteria used to suppress disease caused by soil-borne plant pathogens. Induced resistance by fluorescent pseudomonads has broad spectrum activity against several fungal, bacterial and viral diseases (Hofland *et al.*, 1996; 1997; Maurhofer *et al.*, 1994; Wei *et al.*, 1991; Zehnder *et al.*, 2001).

Increased PAL and PPO activity in groundnut plants and PPO in rice against *Rhizoctonia solani* when prior sprayed with *P. fluorescens* was observed (Meena *et al.*, 2000). Induction of defense related enzymes involved in phenylpropanoid pathway and accumulation of PR-proteins in tomato root tissue and hot pepper collectively contributed to enhance resistance against invasion of *F. oxysporum* f. sp. *lycopersici* and *Pythium* (Ramamoorthy and Samiyappan, 2001). Phenylalanine ammonia lyase and peroxidase activity also increased in green gram plants pretreated with mixture of biocontrol agents (Thilagavathi *et al.*, 2007). Induction of defense gene products such as POX, PPO and chitinase was observed in the plants challenged with Pfl against powdery mildew disease of grapevine (Sendhivel *et al.*, 2007). The activity of defense enzymes PAL, POX, PPO, β-1, 3-glucanase, chitinase, catalase and total phenols were found to be significantly higher in the application of biological
agents. *P. fluorescens* has contributed to restriction of invasion of *Macrophomina phaseolina* in mulberry roots and sunflower downy mildew pathogen (Nandeesh kumar et al., 2008).

### 2.3.2 Native page

Peroxidase isozyme analysis indicated that application of *P. fluorescens* and inoculation with *Xanthomonas axonopodis pv. ralvacearum* resulted in induction of a new peroxidase (PO5) (Salah Eddin et al., 2007). Expression of PO2, PPO1 and PPO2 isoforms were found in all the plants treated with Pf1 while additional PO1, PPO3, PPO4, and PPO5 were observed in Pf1-treated plants followed by challenge inoculation with the pathogen (Chitra et al., 2006). Plants prior treated with Pf1 against *Uncinulanecator* expressed three isoforms of peroxidase (PO1-PO3) while only one isoform of PO was expressed in control in less intensity. The expression pattern of PPO showed that there was no extra isoform except the level of induction in Pf1-treatment against the powdery mildew pathogen. Native gel electrophoresis detected four isoforms of chitinase in plants challenged with Pf1 (Sendhilvel et al., 2007). *Pseudomonas fluorescens*-treated plants after challenge inoculation with *Alternaria solani* and *Septoria lycopersici* showed higher induction of different isoforms of POX and PPO compared to control plants (Anand et al., 2007).

### 2.3.3 Phenylalanine Ammonia Lyase (PAL)

Phenylalanine Ammonia Lyase (PAL EC.4.1.3.5) is the first enzyme in phenyl proponoid metabolism in higher plants which plays a significant role in regulation of phenol biosynthesis as a response in pathogen infection (Lawton et al., 1980). The product of the PAL enzyme reaction, trans cinnamic acid, provides phenyl proponoid skeletons which serve as building blocks for lignins or is utilized in the biosynthesis of plant secondary metabolites such as cinnamic, coumaric, ferulic and caffeic acids, flavonoids and tannins associated with resistance (Hemm et al., 2004). Products of phenylproponoid pathway are structurally and functionally diverse and are synthesized in response to developmental and environmental cues (Sgarbi et al., 2003; La Camera et al., 2004).

Several workers have indicated that the activation of PAL, and subsequent increase in phenolics content is the general response associated with plant disease resistance (Friend et al.,
1973; Ride, 1975). Increase in PAL activity is associated with incompatible interactions in several host pathosystems (Henderson and Friend, 1979 and Moerschbavher et al., 1989). PAL genes have been found to be rapidly induced upon various biotic and abiotic stimuli both in whole plants and cell cultures (Dixon and Palva, 1995).

The activation of phenylproponoid metabolism is one of the initial disease resistance reactions in many plants. Increase in PAL activity is associated with induced defense reactions such as hypersensitive response, systemic acquired resistance and wounding in several host pathosystems. The observations of Moerschbacher et al. (1989) revealed a strong increase in the activity of PAL in incompatible interactions of wheat and stem rust fungus *Puccinia graminis* f.sp. *tritici*. The PAL activity was also observed in many plant-pathogen interactions like barley in response to fungal and elicitor treatments (Ride et al., 1989). Cucumber/*Pythium aphanidermatum* (Chen et al., 2000), Rice/*Pyricularia oryzae* (Wang et al., 2004), Cotton/*Xanthomonas axonopodis* pv. *malvacearum* (Rajendra et al., 2006), Pearl millet/*Sclerospora graminicola* (Geetha et al., 2005), Tomato/*Clavibacter michiganensis* (Umesha, 2006), Tomato/*Clavibacter pv. vesicatoria* (Kavitha and Umesha 2008).

### 2.3.4 Lignifications

Induced resistance by inducing agents in several crops is associated with enhancement of lignifications and also increased activities of enzymes involved in phenylpropanoid pathway and PR-protein synthesis (Hammerschmidt and Kuc, 1995). Induced lignifications have been proposed as a mechanism of disease resistance in plants against the invasion of fungal pathogens (Vance et al., 1980; Tiburzy and Reisener, 1990). Many studies have shown that PAL induction from pathogen infection leads in alternative process. Such as significant lignifications and production of phenolics compounds, this in turn offers protection against diseases. Rapid increase in the activity of PAL followed by the deposition of lignin-like materials has been reported by the incompatible interactions between potato and *Phytophthora infestans* (Friend et al., 1973; Henderson and Friend, 1979). Lignin and other phenolic polymers can serve as physical barriers and therefore are thought to retard fungal penetration of host cells (Ride, 1983; Tiburzy and Reisens, 1990). However bacterial pathogens such as *Xanthomonas oryzae* pv. *oryzae* do not penetrate host cells. Consequently cell wall lignification
probably would not be an effective defense against these organisms. Unless lignified materials prevent the bacterial spread by blocking movement between the epithelium and xylem cells. The lignin biosynthetic process itself could also be an important component of the defense response against bacteria, because bacteria may be inhibited by toxic phenolic compounds (Venere, 1980), phenolics free radicles activated oxygen (Elstner, 1982) all at which are associated with lignification.

2.3.5 Peroxidase (PO)

Plant peroxidases (POX EC. 1.11.1.7) are widespread enzymes that play an integral role in plant physiology. Plant peroxidases are a group of haem containing glycosylated proteins that catalyze the oxidoreduction of various substrates using peroxide ($H_2O_2$). Peroxidases have been implicated in the phenol oxidation. Oxidation of hydroxyl-cinnamyl alcohol into free radical intermediates (Gross, 1980), wound healing (Espelie et al., 1986), regulation of plant cell elongation (Goldberg et al., 1986), Polysaccharide cross linking, cross-linking of extension monomers (Everdeen et al., 1986).

Plants peroxidases can be directly involved in defense mechanisms acting as catalysts for the polymerization of phenolic compounds to form lignin and suberin in the cell wall, which can act as barriers to block the spread of the pathogen in the plant (Gasper et al., 1985; Fritig et al., 1987). An increase in POX activity in plants is common during wounding, disease resistance and physiological stress such as salinity, radiation and pollution (Smith et al., 1994). Peroxidase plays a role in resistance; the isoenzyme would be expected to accumulate in the xylem vessels. Peroxidases have been found in xylem sap collected from stem exudates and guttation fluids of healthy Helianthus, strawberry, tomato, cucumber, pear and apple (Magwa et al., 1993). Secretion of pathogenesis-induced peroxidases into intercellular spaces is thought to be involved in controlling the invasion of different pathogens (Nicholson and Hammerschmidt, 1992). Smith et al. (1994) reported that after wounding of French bean hypocotyls tissue, a cationic peroxidase accumulated in the xylem at site of secondary thickening and in the middle lamella.
Peroxidase enzymes are important in oxidizing phenolics and lipid components such as lignin monomers, toxin precursors and fatty acids into signaling elements or physical and chemical defensive barriers (Nicholson and Hammerschmidt, 1992). Peroxidase is involved in lignin synthesis and degradation of phytotoxic levels of hydrogen peroxide generated in plant tissues as a result of pathogen attack (Van Loon et al., 1998).

Plant peroxidase activity has been shown to be affected by light quality, intensity or duration. In some cases the longer plants are exposed to light, the higher the total peroxidase activity observed increased POX activity and appearance of two to five new isoenzymes were observed in cotton balls inoculated with Rhizoctonia solani (Reddy et al., 1985). Higher plants possess a number of different POX isozymes (Hiraga et al., 2000).

### 2.3.6 Polyphenol oxidase (PPO)

Polyphenol oxidase (PPO, EC.1.14.18.1 or EC.1.10.3.2) are nuclear encoded copper containing enzymes of almost ubiquitous distribution in plants (Mayer and Harel, 1979; Mayer, 1987). Polyphenol oxidase is localized in the plastids, while its phenolic substrates are mainly present in the vacuole (Vaughn et al., 1988). In healthy leaves, PPO is localized in the chloroplast, where it is bound to the thylakoid membrane (Robinson and Dry, 1992). Polyphenol oxidases catalyze the oxygen dependent oxidation of phenols to quinones. The quinonoid products of PPO are highly reactive molecules which can covalently modify and cross-link a variety of cellular nucleophiles via 1,4 addition mechanism, resulting in formation of melanin-like black or brown condensation polymers. The occurrence of this black or brown polymer is responsible for diminished quality and losses of many fresh and processed fruits and vegetables (Vamos-Vigyazo, 1981; Bachem et al., 1994). Polyphenol oxidase is also known as polyphenolase, phenolase, catechol-oxidase, catecholase, mostly found in higher plants especially mushroom, apple, tobacco, and tea leaves (Whitaker and Lee, 1995).

The exact physiological role of PPO on plant cells is not well established. Polyphenol oxidase has been implicated to function in buffering of plastid oxygen levels, biosynthesis of phenolics, wound healing and in the formation of pigment (Mayer and Harel, 1979; Mayer and Haree, 1981; Vaughn and Duke, 1984; Vaughn et al., 1988). Polyphenol oxidase has been
proposed to be involved in photosynthesis due to its localization in the thylakoid lumen (Vaughn et al., 1988), oxygen scavenging and Pseudocyclic photophosphorylation in chloroplasts (Vaughn and Duke, 1984; Trebst and Depka, 1995). Because of its conspicuous reaction products and its wound inducibility, PPO has frequently been suggested to play a defensive role in plant pathogen interactions (Mayer and Harel, 1979; Constabel et al., 1995; Thipyapong et al., 1995). For example: (1) the active quinines produced by PPOs may possess direct antibiotic and cytotoxic activities to pathogens (Mayer and Harel, 1979; Peter, 1989), (2) systemic induction of PPO expression in response to wounding the pathogens might provide an additional line of defense to protect plants against further attack by pathogen and insects (Bashan et al., 1987; Constabel et al., 1995; Thipyapong et al., 1995; Stout et al., 1999), (3) the ability of PPO-derived quinines to covalently bind plant proteins, thereby decreasing the nutritive availability of nucleophilic aminoacids thought to exert an anti nutritive defense against insects and pathogens. Ramamoorthy et al., (2002a), Ramamoorthy et al., (2002b), Saravanakumar (2002) observed the increased activity of PPO in greengram plants treated with P. fluorescens. The groundnut plants treated with biocontrol agent P. fluorescens and challenge inoculated with Alternaria alternata recorded significantly increased activity of POX, and PPO (Chitra et al., 2006). There are few reports of the role of PPO in plant defense against different pathogens such as Cabbage/Fusarium oxysporum f.sp. conglutinans, Onion/Botrytis, Sunflower/Sclerotinia sclerotiorum, Soyabean/Phytopthora megasperma f. sp. glycinea and Bean/Rhizoctonia (Goodman et al., 1986).

2.3.7 Phenolics

Phenolic compounds enhance the mechanical strength of host cell wall and also inhibit the invading pathogenic organisms. Seed treatment with P. fluorescens 63 induced the accumulation of phenolics in tomato root tissue (M’Piga et al., 1997). Many studies indicate that greater accumulation of phenolics due to increased PAL activity offered protection against diseases and herbivory (Grey et al., 1997; Goujan et al., 2003; Zeier et al., 2004; Singh et al., 2002, 2003). Increased POX activities and accumulation of phenol have been correlated with disease resistance in plants. These include Potato/Phytophthora, Sweet potato/Ceratostomella fimbriata and Cotton/Verticillium (Goodman et al., 1986; Leina et al., 1996). Chen et al., (2000) reported that various rhizobacteria induced PAL, POX, and PPO activity and also increased
phenolics in cucumber root tissues against *Pythium aphanidermatum*. Higher levels of total phenol content in plants pretreated with biocontrol agents and challenge inoculated with various pathogens were reported by M’Piga et al. (1997), Benhamou et al. (2000), Chen et al. (2000), Howell et al. (2000) and Ramamoorthy (2002a). Levels of phenols were highest in plants with *Xanthomonas axonopodis pv. malvacearum* (Rajendran et al., 2006).