REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The review of literature pertaining to the present study **"Induction of Defense Related Enzymes and Plant Growth Promoting Traits in Tomato Plant (*Lycopersicon esculentum*)"** is discussed under the following headings:

2.1. Tomato plant (*Lycopersicon esculentum*)

2.2. Damping-off disease

2.3. *Pythium aphanidermatum*

2.4. *Pseudomonas fluorescens* as potential biocontrol agents

2.5. Development of Bioformulation

2.6. Effect of *Pseudomonas fluorescens* bioformulation on the Damping-off incidence under greenhouse conditions

2.7. Induction of Defense - related enzymes and Phenolics

2.7.1. Phenylalanine ammonia lyase

2.7.2. Peroxidase

2.7.3. Polyphenol oxidase

2.7.4. Total phenols

2.8. Growth hormones

2.8.1. Indole acetic acid

2.8.2. Gibberellic acid

2.9. Biometric parameters

2.1. Tomato Plant (*Lycopersicon esculantum*)

Tomato (*Lycopersicon esculentum* Mill.) belongs to the family Solanaceae and is a major summer vegetable crop (Noureen *et al.,* 2010).

Tomato is of great nutritional importance because it is an excellent source of vitamins such as vitamins A, C, thiamine, niacin, riboflavin and minerals like iron and calcium (Suleiman, 2011).

Many diseases and disorders can affect tomatoes during the growing season (Anitha and Rabeeth, 2009). One such disease is root rot (Morsy *et al.* 2009).

2.2. Damping – Off Disease

One of the most common problems encountered in vegetable production is "damping-off". Damping-off is a disease that results in the rotting, collapse and finally death of seedlings just before (pre-emergence) or soon after they emerge (post-emergence). It may occur in transplants growing in the greenhouse or it may occur in direct seed or transplanted crops out in the field. In the greenhouse or the field, it may infect a few plants and spreads quickly into neighbouring plants.

Soil-borne pathogens such as *Pythium* and *Phytophthora*, often called 'water molds', can be particularly destructive if the soil is kept too wet for a long period of time. Although temperature is important, different species of these so-called water molds can infect at different temperatures. *Pythium* is the most common water mold pathogen found on diseased vegetable seedlings and is often associated with excessive nutrition or ammonium toxicity. Damping-off caused by the water molds is less likely to occur during warm dry springs such as the spring experienced so far during 2012 (Celetti, 2012).

2.3. *Pythium aphanidermatum*

Pythium aphanidermatum, one of the causes of root rot disease in tomato is a destructive soil-borne pathogen which attacks several other plants including monocotyledons and dicotyledons. With hot and humid weather being conducive for growth, the fungus is more devastating in tropical and sub-tropical regions of the world (Suleiman, 2011).

Damping-off and root rot caused by *Pythium* sp. are considered to be among the most devastating diseases of greenhouse-grown crops. This pathogen affects nearly every crop grown in every part of the world. The main causal agent of the damping-off and root rot is *P.aphanidermatum*. In spite of its devastating effect, chemical fight and physical control of this fungal pathogen are very difficult to realize. Some authors studied the fight by organic amendment. Biological control of *Pythium* is a promising approach, seeing that it is comparatively benign towards the environment (Jenana *et al.*, 2009).

Pythium species are fungal-like organisms (Oomycetes), commonly referred to as water molds, which naturally exist in soil and water as saprophytes, feeding on organic matter. Some *Pythium* species can cause serious diseases on greenhouse vegetable crops resulting in significant crop losses. *Pythium* infection leads to damping off in seedlings and crown and root rot in older plants. Several *Pythium* species, including *P. aphanidermatum, P.irregulare and P.ultimum,* are known to cause 'damping-off' and 'crown and root rot' in greenhouse cucumber, pepper and tomato crops.

Pythium can be introduced into a greenhouse in plug transplants, soil, debris, pond and stream water, growing media and roots or plant refuse of previous crops. Greenhouse insects such as fungus gnats (*Bradysia impatiens)* and shore flies (*Scatella stagnalis*) can also carry *Pythium*. *Pythium* spreads by forming sporangia, sack-like structures, each releasing hundreds of swimming zoospores (Figure 1). Zoospores that reach the plant root surface encyst, germinate and colonize the root tissue by producing fine thread-like structures of hyphae, forming masses of mycelium. These hyphae release hydrolytic enzymes to destroy the root tissue and absorb nutrients as a food source. *Pythium* forms oospores and chlamydospores on decaying plant roots which can survive prolonged adverse conditions in soil, greenhouse growing media and water, leading to subsequent infection.

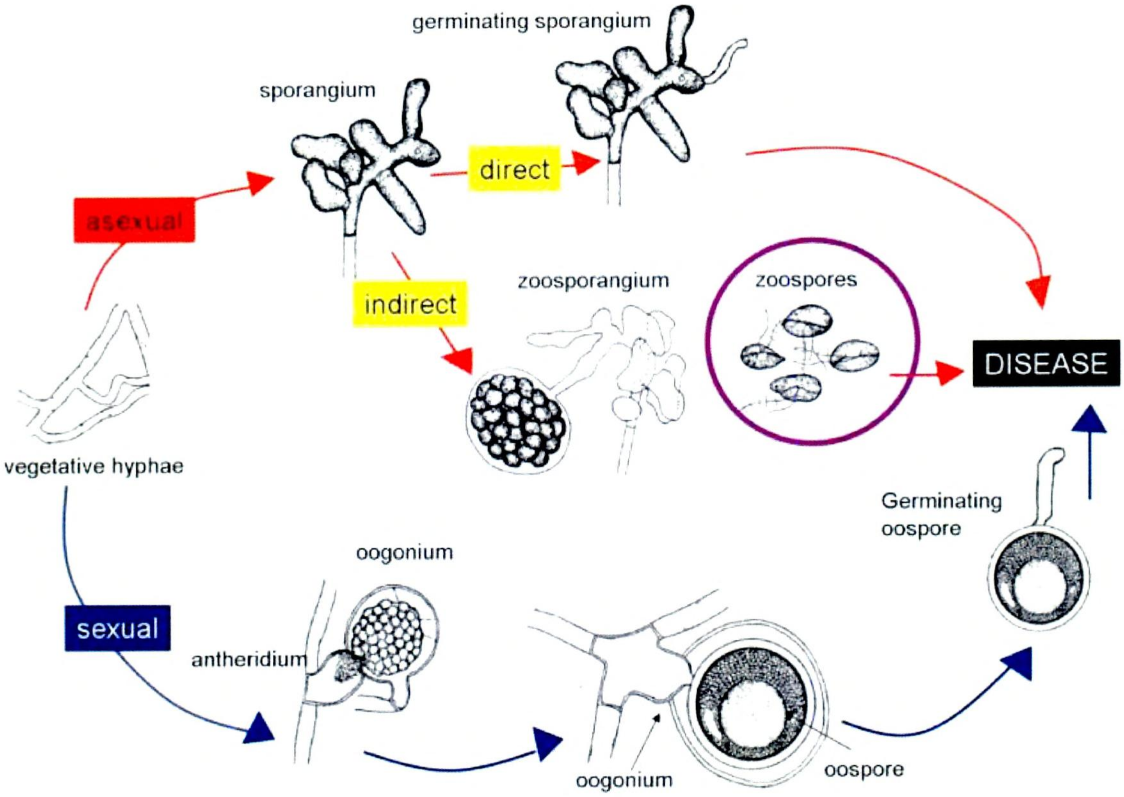


Figure1: Life Cycle of *Pythiumaphanidermatum*(Matthews, 1931)

2.4. *Pseudomonas fluorescens* as Potential Biocontrol Agents

Microorganisms play a vital role in agriculture in order to promote the exchange of plant nutrients and reduce application of chemical fertilizers as much as possible. Plant Growth-Promoting Rhizobacteria (PGPR) is able to exert a positive effect on plant growth. Beneficial plant–microbe interactions in the rhizosphere can influence plant vigor and soil fertility. These beneficial effects of PGPR have direct or indirect performance on plants. Direct promotion of growth by PGPR includes production of metabolites that enhance plant growth such as auxins,cytokinins, gibberellins and through the solubilization of phosphate minerals. *Pseudomonas* sp. is a widespread bacterium in agricultural soils and has many traits that make it well-matched as PGPR. The most effective strains of *Pseudomonas* are gram negative, motile, rod shaped bacteria and have various phytobeneficial traits.*Pseudomonas* *fluorescens* bacteria, a major constituent of Rhizobacteria, encourages plant growth through diverse mechanisms (Noori and Saud 2012).

P.fluorescens can survive under dry conditions and hyperosmolarity. *Pseudomonas* spp. commonly inhabits soil and has been applied for biocontrol, promoting plant growth and bioremediation. These strains are major groups in biocontrol microorganisms, because of their easy colonization, good competition and broad antimicrobial spectrum (Gao *et al.,* 2012).

Bacterial strains of *Pseudomonas fluorescens* and other species are the most commonly reported fluorescent pseudomonads used to suppress damping-off disease is reported to be efficient to control of *Pythium* damping off using *Bacillus marinus*, *P. fluorescens* and *P.aeruginosa*. *Pseudomonas* antagonists were found to be superior to *Bacillus* antagonists in the control of damping-off of cucumber and sugar beet (Al-Hinai *et al*., 2010).

2.5. Development of Bioformulation

The properties of the formulation used to deliver these bio-control agents can influence the success of the inoculation. Formulation of bio-control agents have been designed to promote their survival in soil and colonization of the rhizosphere and effective disease suppression (Ashnaei *et al.,* 2008).

Formulation of biocontrol agents may greatly affect the success of biocontrol. Formulation can also influence the length of time. The biocontrol agent can be stored. And the survival and proliferation of the biocontrol agent takes place in soil. Sodium alginate, commonly used in many food products any residue in plant or soil should not be toxic (Anis *et al*., 2012).

In some formulations, enrichment materials comprising of nutrient-rich medium such as, molasses, trehalose, maltose and sucrose are incorporated to further enhance the viability of core (active) materials. Granular formulations, powder or dust formulations, microcapsules, or oil-emulsion formulations are the various types of bioformulations. Most often, dry formulations are generally preferred over wet formulations because they provide extended shelf life and are easier to store and transport (Omer, 2010).

In the search for efficient alternative biofungicides, eight new bioformulations were developed and prepared using two strains of *Pseudomonas fluorescens* (B1) and *Bacillus coagulans* (B2) isolated from different rhizospheric soils and plant roots of Iranian sugar beet fields. The bioformulation included a talc-based powder and bentonite-based powder as inorganic carriers and peat and rice bran as organic carriers (Jorjani *et al.,* 2012).

2.6. Effect of *Pseudomonas fluorescens B*ioformulation on the Damping-off Incidence under Greenhouse Conditions

Damping-off of papaya caused by *Pythium debaryanum* was found to be reduced by soil and seed application of talc-based formulations of *Pseudomonas fluorescens* in pot experiments. *Pseudomonas fluorescens* significantly reduced the population of *P. debaryanum* in soil (Dar *et al.,*2012).

Talc-based powder formulations of isolates of *P. fluorescens* (Pf32, Pf93) and *B. subtilis* (B49) were developed and evaluated individually and in combination for their efficacy in the management of bacterial blight of cotton under greenhouse and field conditions. The *P. fluorescens* isolates Pf32 and Pf93 and *Bacillus subtilis* isolate B49 survived well in the talc-based formulation for more than 90 days. The application of a mixture of Pf32, Pf93 and B49 to seed, soil and foliage significantly reduced the bacterial blight incidence and increased the plant height, number of branches and number of bolls under field conditions (Salaheddin *et al.,* 2010).

The cell suspensions of *P.fluorescens* should be immobilized in certain carriers and should be prepared as formulations for easy application, storage, commercialization and field use. The potential *P. fluorescens* are formulated using different organic and inorganic carriers either through solid or liquid fermentation technologies. Thus, for field application of *P. fluorescens* towards the management of the bacterial wilt disease of brinjal, development of commercial formulations with suitable carriers that support survival of the bacteria for a considerable length of time is necessary (Chakravarty and Kalita, 2011).

2.7. Induction of Defense-related Enzymes and Phenolics

When plants are attacked by pathogens, they defend themselves with an arsenal of defense mechanisms, both passive and active. The active defense responses, which require *de novo* protein synthesis, are regulated through a complex and interconnected network of signaling pathways that mainly involve three molecules, salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) and which results in the synthesis of pathogenesis-related (PR) proteins (Almagro *et al.*, 2009).

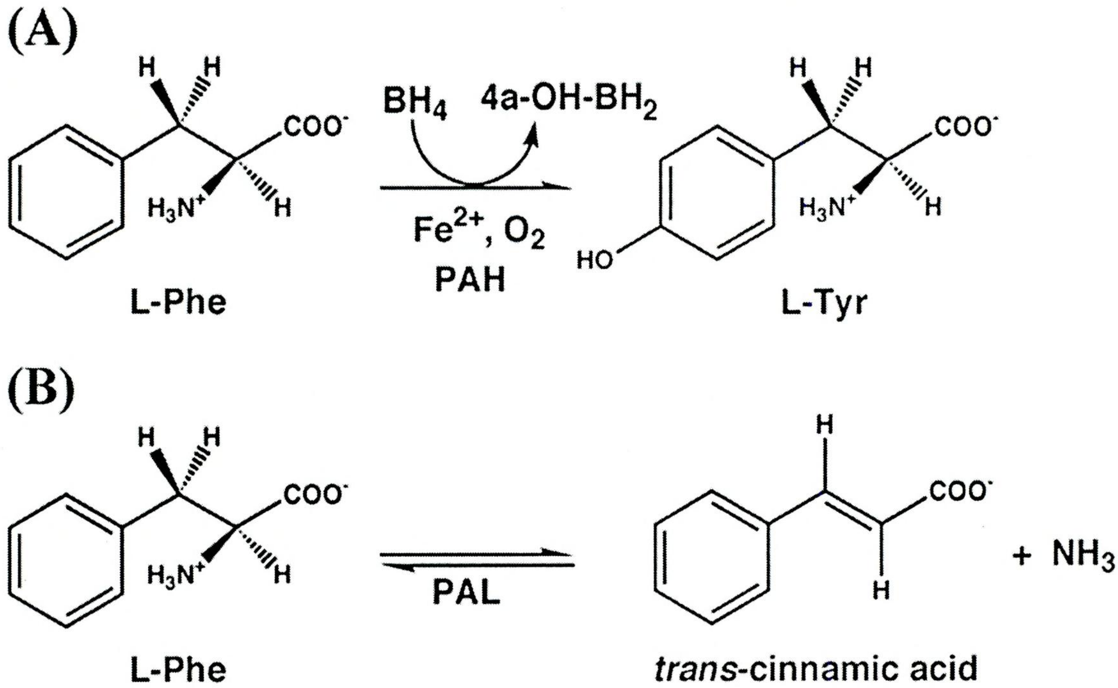
The biocontrol agents bring about induced systemic resistance (ISR) fortifying the physical and mechanical strength of cell walls and changing the physiological and biochemical reactions of the host leading to synthesis of defense chemicals against challenge inoculation of pathogens. Defense reaction occurs due to accumulation of PR proteins such as chitinase, â-13-glucanes, phenylalanine ammonia lyase, peroxidase, phenolics and phytoalexins (Christopher *et al.*, 2010).

Higher levels of polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), β-1, 3 glucanase (PR-2) and phenolics were observed in roots and shoots of resistant cultivars than that of susceptible cultivars on treatment with elicitors and pathogen. The defense gene products include polyphenol oxidase (PPO), peroxidase (PO) that catalyzes the formation of lignin, and phenylalanine ammonia-lyase (PAL) that are involved in phytoalexins and phenolics synthesis (Raju *et al.*, 2008).

Resistance in plants towards pathogens depends on various factors. Several authors have demonstrated a distinct correlation between the degree of plant resistance and phenolics present in plant tissues Phenylalanine ammonia lyase (PAL) and peroxidase (PO) are two oxidative enzymes which are frequently involved in biosynthetic processes associated with infection by phytopathogens (Bhagat and Chakraborty, 2010).

2.7.1. Phenylalanine ammonia lyase

L-phenylalanine is one of the essential amino acids that cannot be synthesized in mammals in adequate amounts to meet the requirements for protein synthesis. Fungi and plants are able to synthesize phenylalanine via the shikimic acid pathway. L-phenylalanine, derived from the shikimic acid pathway is used directly for protein synthesis in plants or metabolized through the phenylpropanoid pathway. This phenylpropanoid metabolism leads to the biosynthesis of a wide array of phenylpropanoid secondary products. The first step in this metabolic sequence involves the action of phenylalanine ammonialyase (PAL). The discovery of PAL enzyme in fungi and the detection of $^{14}CO_2$ production from ^{14}C-ring-labeled phenylalanine and cinnamic acid demonstrated that certain fungi can degrade phenylalnine by a pathway involving an initial deamination to cinnamic acid, as in plants.



http://www.nature.com/mt/journal/v10/n2/fig_tab/mt20041219f1.html

Figure 2: Deamination of L-phenylalanine by L-phenylalanine ammonia-lyase (PAL)

Phenylalanine ammonia-lyase (PAL; E.C. 4.3.1.5) catalyzes the nonoxidative deamination of L-phenylalanine to form *trans*-cinnamic acid and a free ammonium ion. The conversion of the amino acid phenylalanine to *trans*-cinnamic acid is the entry step for the channeling of carbon from primary metabolism into phenylpropanoid secondary metabolism in plants. The importance of this enzyme in plant metabolism is demonstrated by the huge diversity and large quantities of phenylpropanoid products found in plant materials. In plants, PAL activity has been detected in many species, representing monocots, dicots, gymnosperms, ferns, and lycopods (Hyun *et al.*, 2011).

Phenylalanine ammonia-lyase (PAL) is an important enzyme in both plant development and pathogen defense. Phenylalanine ammonia-lyase catalyzes the conversion of L-phenylalanine into *trans*-cinnamate, the initial committed step of the multi-branched phenylpropanoid pathway in higher plants. As the first step in phenolic metabolism, this is a key biochemical reaction in both plant development and defense (Chang *et al.*, 2008).

Phenylalanine ammonia-lyase (PAL) is involved in phytoalexins and phenolics synthesis (Raju *et al.*, 2008).

2.7.2. Peroxidase

Among the proteins induced during plant defense and playing a key role in several metabolic responses, class III plant peroxidases (EC 1.11.1.7) are well known. Peroxidases are involved in a broad range of physiological processes throughout a plant's life cycle, probably due to the high number of enzymatic isoforms (isoenzymes) and the versatility of their enzyme-catalysed reactions. Thus, plant peroxidases are involved in auxin metabolism, lignin and suberin formation, cross-linking of cell wall components and phytoalexin synthesis (Almagro *et al.*, 2009).

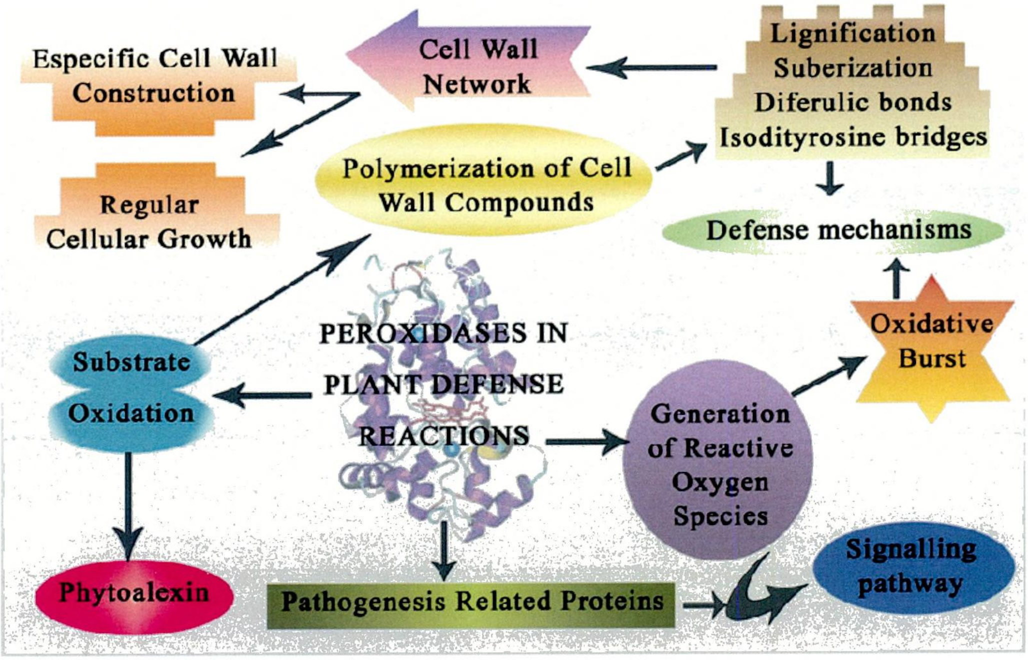


Figure 3:Overview of the Specific Roles of Plant Peroxidase in DefenseReactions (Almagro*et al.*,2009)

Peroxidase enzyme is a key enzyme of the phenyl propanoid pathway, activated in response to pathogen infection. Changes in the activity of phenol-oxidizing enzymes including peroxidase, may play a role in the regulation of metabolic pathways in diseased or injured tissues. The activities of both peroxidase and polyphenol oxidase reached their maximum on the 28th day, when the plants were treated with *F. oxysporum*and SA (1.5 mM) (Ojha and Chatterjee, 2012).

Peroxidase enzymes participate in hormone catabolism, phenol oxidation, polysaccharides and cell wall proteins intercrossing, lignin polymerization, fruit ripening, and defense against pathogens. During fruit ripening, and particularly during climacterium, peroxidase activity is increased alongwith the polygalacturonase and cellulase enzymes (Ortiz *et al.*, 2007).

2.7.3. Polyphenol oxidase

Polyphenol oxidases (PPO) catalyze the O_2-dependent oxidation of mono- and *o*- diphenols to *o*-diquinones, highly reactive intermediates, the secondary reactions of which are believed to be responsible for the oxidative browning that occurs as a consequence of plant senescence, wounding and pathogen infection. PPO have been known to biochemistry for a century and several hypotheses regarding the function of PPO have been proposed, including roles in the phenylpropanoid pathway, the Mehler reaction, electron cycling, oxygen regulation, flower petal coloration and plant defense. A defensive role for PPO has frequently been suggested due to the conspicuous appearance of PPO reaction products upon wounding, pathogen infection or insect infestation and due to the inducibility of PPO in response to various abiotic and biotic injuries or signaling molecules (Thipyapong *et al.*, 2007).

Polyphenol oxidases (PPO) are a group of copper-proteins, widely distributed phylogenetically from bacteria to mammals that catalyze the oxidation of phenolics to quinines which produce brown pigments in wounded tissues. PPO has been implicated in the formation of pigments, oxygen scavenging and defense mechanism against plant pathogens and herbivory insects. Phenolic compounds serve as precursors in the formation of physical polyphenolic barriers limiting pathogen translocation. PPO has been regarded to be a critical enzyme in food technology and it has been intensively studied in several plants. It is known that plant PPO are synthesized as preproteins and contain putative plastid transit peptides at the N-terminal region which target the enzyme into chloroplast and thylakoid lumen.

Plants PPO have broad substrates specificities and are able to oxidize a variety of mono, di or polyphenols. Phenolic compounds are natural substances that contribute to the sensorial properties (color, taste, aroma and texture) associated with fruit quality. Structurally they contain an aromatic ring bearing one or more hydroxyl groups together with a number of other substituents. Some of PPO substrates occur naturally in fruits and vegetables, e.g., apples, very suitable to enzymatic browning, are rich in chlorogenic acid, catechin and epicatechin (Queiroz *et al.*, 2008).

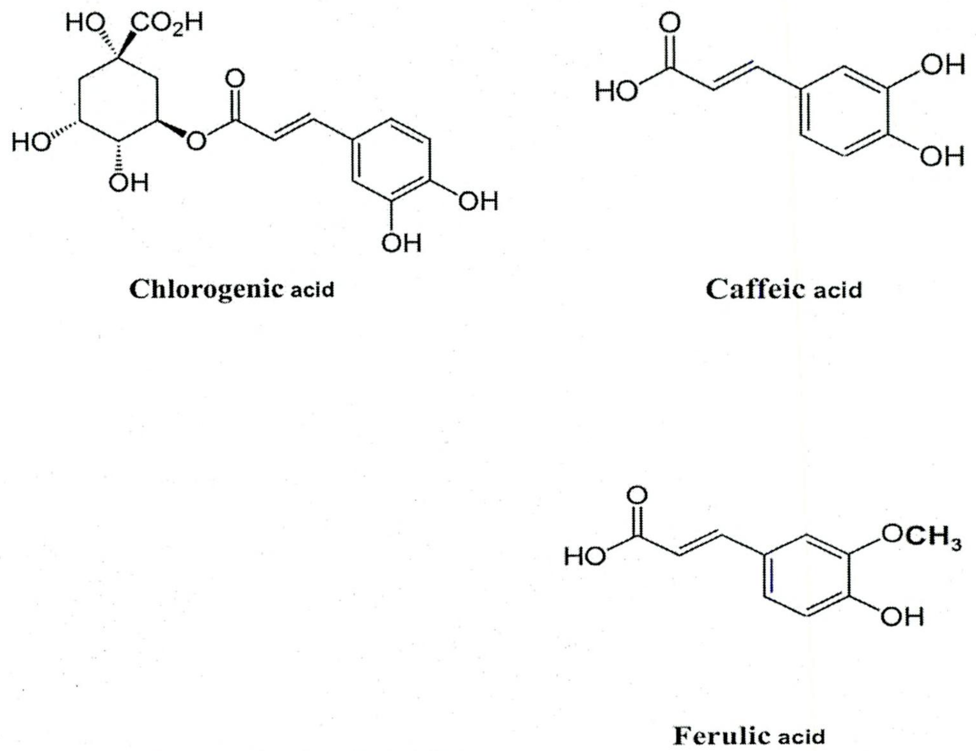
2.7.4. Total phenols

Phenolic compounds and related oxidative enzymes are mostly considered as one of the important biochemical parameters for disease resistance (Ojha and Chatterjee, 2012).

They could be an important part of the plants defense system against pests and diseases including root parasitic nematodes. Elevated ozone (mean 32.4ppb) increased the total phenolic content of leaves and had minor effects on the concentration of individual compounds (Mazid *et al.,* 2011).

Plant phenolics are generally involved in defense against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as contributing to plant colors. They are ubiquitous in all plant organs and are therefore an integral part of the human diet. Phenolics are widespread constituents of plant foods (fruits, vegetables, cereals, olive, legumes, chocolate, *etc.*) and beverages (tea, coffee, beer, wine, *etc.*), and partially responsible for the overall organoleptic properties of plant foods. For example, phenolics contribute to the bitterness and astringency of fruit and fruit juices, because of the interaction between phenolics, mainly procyanidin and the glycoprotein in saliva (Dai and Mumper, 2010).

Phenolic acids (PAs) are metabolites with a diverse structure characterized by hydroxylated aromatic rings. Despite some constitutive expression, their relative location and concentration may vary in response to cell invasion by microorganisms. Phenol acids can fulfil different functions, as constituents of plant cell walls regulating cell wall plasticity as antimicrobial compounds and they may act as signaling molecules modulating plant-microbe interactions. In tomato, the phenol acids vanillic, caffeic, chlorogenic and ferulic acids have been shown to display biocidal activity and/or associated with resistance to pathogens. Interestingly, ferulic acid has been also related with the induction of plant resistance by biocontrol agents (López-Ráez *et al.*, 2010).



(López-Ráez *et al.*, 2010)

Figure 4: Chemical Structure

2.8. Growth Hormones

Phytohormones, mainly including auxins, cytokinins, abscisic acid, gibberellins, and ethylene, induce some important physiological responses at different stages of plant development at low concentrations. Plant growth regulators as well as fertilizers should be used to maximize production efficiency (Bilkay *et al.*, 2010).

2.8.1. Indole acetic acid

Indole acetic acid (IAA) is one of the most physiologically active auxins. IAA is a common product of L-tryptophan metabolism by several microorganisms including PGPR. Organisms such as bacteria, fungi and algae are able to make physiologically active IAA that may have pronounced effects on plant growth and development. Microbial isolates from the rhizosphere of different crops appear to have a greater potential to synthesize and release IAA as secondary metabolites because of the relatively rich supply of substrates. There is firm evidence that indole-3-acetic acid (IAA), gibberellins and cytokinins all produced by plants and essential to their growth and development are produced also by various bacteria which live in association with plants. There is also evidence that the growth hormones produced by the bacteria can in some instances increase growth rates and improve yields of the host plants (Shahab *et al.*, 2009).

2.8.2. Gibberellic acid

Gibberellic Acid (GA), which comes from a naturally occurring growth hormone, is a member of a type of plant hormone called gibberellins which regulate the growth and development of plants. The GA are associated with various plant growth and development processes such as seed germination, stem and hypocotyl elongation, leaf expansion, floral initiation, uniform flowering, floral organ development, reduced time to flowering, increased flower number and size and induction of some hydrolytic enzymes in the aleurone of cereal grains. In plants, certain secondary metabolite pathways are induced by infection with microorganisms. It was reported that, arbuscular mycorrhizal symbiosis maintained more normal water relation in plants (Kavina *et al.*, 2011).

2.9. Biometric Parameters

Biometric parameters such as measurement of root length, shoot length and chlorophyll contents were studied. Photosynthesis is known as being the ultimate source of all plant growth (Gladden *et al.*, 2012).

With the literature mentioned above the following experimental procedure is carried out and explained in the forthcoming chapter.