4. RESULTS AND DISCUSSION

Biological control of plant diseases opened an era of new technology to manage crop diseases and received the attention of researchers throughout the world, which will enhance the sustainability of agricultural production systems and to reduce the use of chemical pesticides. Biological control of plant diseases may be achieved by the use of microorganisms, application of naturally derived compounds and enhancing natural disease resistance of plants.

Biological suppression is proved to be a reliable component of integrated management of phytopathogenic fungi following greenhouse and field demonstration of several biocontrol agents. However, combined use of different bio-control agents or integration of biocontrol agents with other disease management options has improved disease protection and the activity spectrum of biocontrol agents (Matar et al., 2009). A few of the available biocontrol agents mostly belonging to *Pseudomonas spp.*, show broad-spectrum antifungal activity by virtue of volatile and diffusible antibiotics (Krishna and Pande, 2005).

4.1. Isolation and characterization of phytopathogen

In Czapek dox agar (CDA) and Sabouraud dextrose agar (SDA) surface of the colony was first greyish white, wooly and later became greenish black or brown with a light border. It eventually became covered by short, greyish, aerial hyphae. The reverse of the Petri plate appeared as black colour (Plate-1a&1b). In the microscopic appearance, the hyphae were septate and dark. The conidiophores were septate and variable in length. Individual conidiophores were formed with bushy heads and conidia were having short conical beak at the tip (Plate-1c&1d). Thus with all these macroscopic and microscopic evidences, it was confirmed as *Alternaria alternata* (Mehrotra and Aneja, 2003).

4.1.1. Qualitative assay of Cell wall degrading enzymes

The plant cell wall contains complex polysaccharides that act as a suitable source of carbon for certain phytopathogenic fungi, which results in releasing hydrolytic
enzymes that display highly complex molecular architectures and functions (DeBoy et al., 2008). In the present study, the phytopathogenic isolate from leaf spot and stem canker of tomato, *Alternaria alternata* produced maximum cellulase of 3.01U/ml and pectinase of 3.70 U/ml, when tomato stem powder was substituted as carbon source in the production medium (Fig-1). Pectinase has the ability to elicit defense reaction in host plant, resulting in necrosis along with plasmolysis and cell wall swelling (Collmer and Keen, 1986). Accumulation of cell wall degrading enzymes with the infected tissues reveals its role in establishment of the fungus on local tissues. The fungus with higher growth rate has the ability to produce large quantities of cell wall degrading enzymes are considered highly virulent strains (Cleveland and Coty, 1991).

4.1.2. Anti-fungal assay of fungicides

The fungicides thiram, carbendazim, hexaconazole, kitazone, and trimedorph showed antifungal activity against *Alternaria alternata* with a minimum inhibitory concentration (MIC) of 30, 15, 75, 45,and 30 µg/ml respectively, the most effective being carbendazim (Fig-2). Csinos and Stephenson (1999) have reported 50% inhibition of fungal growth by carbendazim at the concentration of 15µg/ml; contrarily our pathogen has been inhibited by 90%. The dry weight of *alternata* and total protein gradually decreased in relevance to increase in concentration of fungicides. The concentrations of fungicides are inversely proportional to fungal growth, indicating the interference of fungicides in protein machinery of fungus (Fig-3&4).

The activities of cell wall degrading enzymes produced by phytopathogens were reduced remarkably in the presence of the fungicides namely Carbendazim and Thiram (Srinivasulu and Rangaswamy, 2006). Application of fungicides in combination with local detergents or alteration in fungicides, results in good crop yield. Botanicals can be supplied along the fungicides for the effective control of *alternata* (Harlapur et al., 2007)
4.2. Isolation and Characterization of Antagonist

In recent times, microbes have been the cynosure as plant growth promoters or biocontrol agents against various phytopathogens. In commercial greenhouse trials; *Pseudomonas fluorescens* strain WCS 374 suppressed *Fusarium* wilt and increased radish yield. Induction of systemic resistance by selected plant growth- promoting rhizobacteria (PGPR) strains of *Pseudomonas fluorescens* was involved in the suppression of *Fusarium* wilt of radish. PGPR strains of *fluorescens* and their crude cell wall extracts, which contain Lipopolysaccharides or purified LPS, induced systemic resistance, whereas *putida* did not (Vann Peer et al., 1991; Leeman et al., 1996). From the 25 different groundnut rhizosphere soil, 10 strains of *fluorescens* were isolated (Plate-2a), and they were named as AP1, AP2, AP3…..AP10. On *Pseudomonas* isolation agar (PIA), King’s medium B and Nutrient agar, the colonies appeared as bluish green (Plate-2b&2c), indicating the growth of *fluorescens*. Further by the results of conventional biochemical tests the cultures were confirmed as *Pseudomonas fluorescens* (Table-1).

Induced resistance of plants against fungal disease often has been observed after pre-inoculation with weakly aggressive strains or avirulent or incompatible forms of the diseases causing fungus. A few reports also have mentioned the possible involvement of induced resistance in the biological control of soil borne pathogens with plant growth promoting rhizosphere pseudomonads. In the later cases, however, sufficient experimental evidence for the occurrence for induced resistance was not obtained because the competition for nutrients could not be entirely excluded (De wilt, 2007).

4.2.1. Optimization of Antagonist based on Growth Parameters

Understanding the environmental factors that regulate the growth of *Pseudomonas fluorescens* is an essential step towards inferring the level and reliability of their biocontrol activity, under various parameters such as temperature, pH, sugar, nitrogen source and amino acid (Brion et al., 1999). Different temperature like 25°, 30°, 35°, 40° and 45°C were opted for incubation of *fluorescens* inoculated nutrient broth, comparatively 35°C was more conducive for the growth of *fluorescens*. pH plays an
important role in the solubility of iron and thereby resulting in its availability to the growing organism in the medium.

Based on the growth of *fluorescens* the optimum pH was assessed, sequentially resulting in scrutinizing pH 7.0 as optimum. Among the 9 sugars tested for their influence on growth, glucose was found to have a satisfactory effect. Thus in the presence of Glucose, the *fluorescens* showed a maximum growth. Sorting of nitrogen source was also a vital phase of assessing various parameters, among the four nitrogen sources, yeast extract produced maximum growth. Finding out the role of amino acids in the growth of isolates, the best result was observed in the broth with the amino acid threonine for AP₃ and AP₆ (Fig-5 to 9).

According to De Meyer and Hofte (1997), the optimal temperature assessed was 30°C but the test isolates had maximum growth at 35°C. The test isolates were indigenous population of tropical country soil that favors the optimum to 35°C. Other than temperature, pH at 7.0; glucose as best carbon source; threonine as an enhancing amino acid; yielding maximum growth by opting yeast extract as best nitrogen source, were the results obtained that made to hold with the study of Sayyed *et al.*, 2005. Among the 10 isolates AP₃ and AP₆ showed maximum growth than other isolates.

### 4.2.2. Assay for Secondary Metabolite Production

The term rhizobacteria was coined to describe rhizosphere bacteria that exhibit root colonization and exerting a beneficial effect on plants are termed PGPR (Plant growth promoting rhizobacteria). They also increased growth indirectly, by changing the microbial balance in the rhizosphere. Iron chelating siderophore antibiotics, and HCN are produced by some PGPR and have been implicated in reductions of plant pathogens and deleterious rhizobacteria with a corresponding improvement in plant growth (Wei *et al.*, 1991)

Salicylic acid is an important signal molecule that plays a critical role in plant defense against pathogen invasion; exogenous application of SA would activate systemic acquired resistance (SAR) significantly resulting in the increase of...
endogenous SA level in leaves. Salicylic acid has been observed for several bacterial strains and exogenously applied SA can induce resistance in many plant species. Its ability of ISR has been linked to production of SA (De Meyer and Hofte, 1997). However, recent evidence strongly suggests that SA is not the inducing compound from 7NSK2, but the compounds pyochelin and pyocyanin produced by this strain are a prerequisite for the induction of resistance (Audenaert et al., 2002).

While adding FeCl₃, a purple layer was developed in the aqueous phase indicating the production of salicylic acid, but there was no purple layer in the controlled tubes (Plate-3a). Production of salicylic acid was found to be higher in glucose, beef extract and histidine (Fig-10 to 12), to a maximum level of 5.4, 5.1 & 5.3 mg/ml respectively. Highest expressions of SA levels in fluorescens were recorded when they were grown on glucose, succinic acid, and citric acid (Bakker et al., 2003). Cystine is one of the amino acids secreted by tomato roots. So it is likely that salicylic acid is efficiently aggregated to pyochelin in the rhizosphere of tomato in the presence of cystine. Similar phenomenon has been described for fluorescens, which produces salicylic acid, by influence of cystine at iron limiting conditions which result in effective colonization of Pseudomonas fluorescens and increased SA in plant roots that act as elicitor of systemic resistance (Nandi et al., 2002).

Hydrogen cyanide, a volatile metabolite is playing a major role in biological control of some soil borne and air borne diseases by significantly increasing the peroxidase activity, that enhances the plants disease resistance potential (Siddiqui and Shoukat, 2006). Hydrogen cyanide production by certain fluorescent pseudomonads was found to influence the plant root pathogens. Suppression of black root rot of tobacco (T. basicola) by Pseudomonas fluorescens CHAO was mainly due to the production of hydrogen cyanide (Stutz et al., 1986). Voisard et al., (1989) reported that mutants of CHAO deficient in HCN production were less suppressive than the parental strain to T. basicola in Tobacco. Defago et al., (1990) highlighted that cyanide secreted by Pseudomonas fluorescens strain CHAO played a role in the suppression of take-all (G. graminis var tritiae) and root rot (R. solani) of wheat.
In the present study, the maximum absorption for HCN production at 625 nm (Plate-3b) was by AP, followed by AP in lactose, valine and peptone (Fig-13 to15) in contrary to isoleucine and D-alanine (Walters et al., 2005). Functional analog of SA, such as 2, 6-dichloro isonicotinic acid (NIA) or benzothiadiazole-7-carbothinic acid or 8-methyl ester were developed, which activates the resistance mechanisms downstream of SA. Since the observation of Chester, little attention was given to the role of induced resistance in general, and of amino acids in particular, in plant defense (Yigal, 2002).

The influence of iron availability over the induction of systemic resistance in radish against Fusarium wilt mediated by fluorescens was studied. The Fe³⁺-chelating SA, produced by fluorescens strains at low iron availability was involved in the induction of systemic resistance to Fusarium wilt of radish. The pseudobactins produced by them may be involved in the induction process (Leeman et al., 1996).

Iron-chelating molecule that was well studied with respect to induced plant defense is salicylic acid. Although the siderophore capacity of SA is rather poor, it appears to play an important molecule in induced resistance by the rhizobacteria aeruginosa. Pseudomonas fluorescens and Pseudomonas putida are known to produce a variety of antibiotics and siderophores, some of which are active in soil. They have been implicated in many cases of natural biological control and plant growth promotion (Kloepper et al., 1988)

The wine red colour upon the addition of 2% FeCl₃ indicates the production of siderophores (Plate- 3c), there was no color change in the control tubes. Upon the addition of various carbon, nitrogen and amino sources for enhanced production of siderophore, it was found to be maximum of 5.6, 5.7 & 5.7µM/ml in maltose, tyrosine, and beef extract respectively (Fig-16 to18). Carbon and nitrogen sources greatly influence the siderophore production. Barbeau et al., (2002) reported that among the
different carbon and nitrogen sources, mannitol (2%) and glutamine (0.1%) were found to increase the siderophore production.

4.2.3. Assay of plant growth promoters

Strains of *Pseudomonas fluorescens* increased the growth of tomato plants in solarized soil but, not in non-solarized soil. The minor pathogen *Penicillium pinophilum* caused growth retardation in tomato and cotton plants. Inoculation of tomato roots with fluorescent pseudomonads or transplanting tomato roots to solarized soil suppressed colonization of *Penicillium pinophilum* and nullified the plant-growth retardation. The results suggest that *Pseudomonas fluorescens* are effective agents in suppressing both major and minor pathogens in soil (Gamliel and Katan, 1993).

AP6 showed maximum Auxin production of 7.67, 7.92 and 7.33mg/l with mannitol, tryptophan and yeast extract respectively; similarly 78, 73 and 71 µg/ml phosphatase is secreted in same carbon, amino and nitrogen sources respectively (Table-2). Secretion of growth promoting substances in the form of auxins and phosphatase along with other secondary metabolites will surely elevate the growth of the plant (Ponmurugan and Gopi, 2006). Synonymous findings were reported by Gupta *et al.*, 2006.

4.2.4. Assay for plant defense enzyme production by isolates

In *Pseudomonas fluorescens* CHAO, mutation of *aprA* gene encoding protease resulted in reduced biocontrol, which supports the role of protease in plant protection and antagonistic effect (Siddiqui and Shoukat, 2005). The maximum protease enzyme production was found in galactose, beef extract and isoleucine by AP6 of 45.2, 47.7 and 47.9 U/ml respectively (Fig-19 to 21). In contrary, addition of inorganic nitrogen sources in the production medium resulted in low enzyme yield, but among the ten carbon sources studied, starch, sucrose, and lactose were proved to be appreciably good for the protease production. Lactose, starch, soy meal and sucrose were reported to be good for industrial protease production (Haggag and Wafaa, 2002). Thus some of the earlier results were compatible to the present investigation.
Pseudomonas fluorescens can induce physiological changes throughout entire plants making them more resistant to pathogens by inducing some plant protecting enzymes like lipases. The maximum lipase enzyme production was found in dextrose, peptone and lysine by AP₆ i.e., 2.8, 4.9 and 5.1 U/ml respectively (Fig-22 to 24). According to Van Lon and Bakker, 2003; the highest lipase activity was observed in plant supplemented with yeast extract and protease- peptone (5.58 U ml⁻¹), while the lowest lipase activity was obtained with tryptone + lactose (2.81 U ml⁻¹) in *fluorescens*. It has been reported that some micro-organisms showed higher activities when grown in medium containing glucose. It creates strong support to the present study that glucose and yeast extract are increasing lipase activity in plants.

Cell wall degrading enzymes, such as chitinase and glucanase are important features of antagonists against colonizing mycoparasite on their own host and they exhibit considerable antifungal activity (Hermosa *et al*., 2000). One of the main mechanism involved in antibiosis, depend on recognition, binding and enzymatic disruption of the host-fungus cell wall (Haggag and Wafaa, 2002). Chitinase and glucanase induction in plants by PGPR’s could help plants to digest the fungal cell wall, extending to the release of saccharides that result in the production of Phytoalexins (Akiyama *et al*., 2004).

The maximum production of β-1,3-Glucanase and Chitinase was found in the dextrose, beef extract and serine as 1.4 & 2.8 µMol/ml, 1.2 & 2.5 µMol/ml, and 1.2 & 2.4 µMol/ml respectively by AP₆ (Fig-25 to 30). Production of glucanase in medium containing glucose, sucrose and rhamnose increased up to 5.5 CHU after 3 days of growth, after which it decreased slightly. A higher activity of glucanase was detected when *Pseudomonas fluorescens* was grown on SSM. β-1,3-Glucanase secretion in medium containing either ammonium tartrate or serine was significantly higher than that in medium containing ammonium nitrate (Anand and Kulothungan, 2009).
4.2.5. Antifungal activity by Antagonist

Antifungal activity of different strains of *Pseudomonas fluorescens* were tested against some plant pathogens such as *Alternaria cajani*, *Curvularia lunata*, *Fusarium* spp., *Bipolaris* spp., and *Helminthosporium* spp., *in vitro*. Different concentrations of *Pseudomonas fluorescens* were used and maximum spore germination of fungus was inhibited at 4000 and 5000µg/ml. The results indicated that all the strains of *fluorescens* presented a most significant value against *Alternaria cajani* and *Curvularia lunata* (Rachana and Shalini, 2008). According to Whipps (2001), the plants inoculated with *fluorescens* will induce the plants to grow fast in the initial phase that is more sensitive for the pronounced pathogen attack.

Antagonistic activity of *Pseudomonas fluorescens* against *Alternaria alternata* was tested with the dual-culture methods (Plate-4). Among the 10 isolates of *fluorescens*, AP₆ showed higher inhibition against growth of *Alternaria alternata* (85%) in both the methods, when compared to other isolates (Fig-31). Similar reports where made when, *fluorescens* was screened for its antimicrobial activity against various fungi including *Alternaria cajani* by Srivastava and Shalini, 2008. The *fluorescens* was found to show highest inhibition percentage (81% to 100%) against *Alternaria cajani* than other fungi.

Likewise, Yogesh kumar *et al.*, (2005) also observed that *Pseudomonas fluorescens* successfully inhibited the growth of *Fusarium solani*, causal agent of root rot in pea. He also observed that inhibition of the fungal growth by culture filtrate of *Pseudomonas fluorescens* was significant (60-100%) compared to control. Mycelial growth was completely inhibited, when the volume of the culture filtrate increased to 50% in the broth. In the similar observations made by Krishna and Pande, (2005). *Pseudomonas spp.*, were highly inhibitory against 8 fungal pathogens of groundnut. *Pseudomonas spp.*, quite often emerged as potent antagonists in several screening programs. Broad-spectrum activity of *Pseudomonas spp.*, contributes to their *in-vitro* antifungal activity and *in-vivo* disease control. These results fit with the results obtained by Rosales *et al.*, 1995, which shows that *Pseudomonas spp.*, including *Pseudomonas*
fluorescens were clearly inhibitory to the fungus Rhizobacteria solani, where wide zones devoid of mycelial growth were observed around bacterial colonies.

4.3. Synergistic study of plant extract as an alternative for carrier formulation

Parthenium hysterophorus is an exotic weed that was accidentally introduced in India by 1956, through imported food grains. It has become a common weed causing dermatitis of epidemic proportions. It is found as an ecological niche without natural enemies and spread rapidly along the canal banks. Both rural and urban areas have been invaded by this weed. Purple nut sedge (Cyperus rotundus) was identified as the world’s worst weed based on the number of countries where it was reported as the serious principal or the common weed competing with crops was the major factor in determining weediness (Lonkar et al., 2004). The aqueous extracts of Cyperus rotundus and Parthenium hysterophorus obtained by Awuah(1989) method (Plate-5&6) were more compatible with the antagonist fluorescens (AP$_6$) at 20% among all other concentrations, by yielding maximum level of salicylic acid, siderophores and hydrogen cyanide (Fig-32&33).

Similarly maximum level of growth promoting molecules, IAA and phosphatase were produced of 8.0 µg/l and 81µg/ml respectively (Fig-34&35). Compare to the production by antagonist alone, along with plant extract yielded high, which gives positive direction for opting the weeds as a substrate for biocontrol agent. Synchronously the identical results were obtained for protease, lipase, β-1,3 glucanase and chitinase of 51 U/ml, 5.7 U/ml, 1.7µMol/ml and 1.7µMol/ml, remarkably a drastic increase in the level of defense enzymes that goes parallel to secondary metabolites and growth parameters but above the level of isolates alone (Fig-36 to 39). Biologically active plant derived pesticides are expected to play an increasingly important role in plant protection strategies (Verma and Dubey, 1999)

Parthenium can be utilized to nourish the crops. Parthenium along with Azotobacter chroococcum was found to be beneficial for better growth and yield of wheat. This suggests that Parthenium before flowering may reduce its spreading as well
as menace of human health hazards worldwide (Grija Ganeshan, 2000). Allelopathic potential of *Parthenium hysterophorus* L., against three pathogenic fungal species viz. *Drechslera hawaiiensis*, *Alternaria alternata* (Fr.) Keissl and *Fusarium moniliforme* was studied. These species were subjected to various concentrations of aqueous extracts of aerial parts of *P. hysterophorus* in liquid malt extract medium. The dry biomass productivity assays were carried out periodically with 5 days intervals up to fifteen days. The growth of all the three test pathogenic species was generally inhibited by lower concentrations viz. 10, 20, 30 and 50% of the *Parthenium* extracts while aqueous extracts of higher concentrations (60 and 70%) stimulated biomass production of test fungal species which supports the present investigation in the requirement of detecting specific level of concentration of plant extract (Rukhsana Bajwa *et al*., 2004).

The *Cyperus rotundus* rhizome meal affected the mycelial growth and sporulation of all the tested fungi, viz. *Alternaria solani*, *Aspergillus* spp., *Colletotrichum* spp., *Curvularia pallescens* [*Pseudochliobolus pallescens*], *Fusarium udum* [*Gibberella indica*], *Helminthosporium spiciferum* [*Cochliobolus spicifer*], *Heterosporium* [*Cladosporium*] spp., *Leptoxypium axillatum*, *Mucor* spp. and *Penicillium* spp. (Blakeman and Fokkema, 1982). The *in vitro* antifungal activity of *Pseudomonas fluorescens* (AP6) with extracts showed better zone of inhibition (Fig-40) of 90% than the antagonist alone.

4.4. *In vivo* study for host response and effective delivery system

The *in vivo* study plant pots were prepared according to the formulations; based on delivery, it was segmented in to seven groups with replica of eight and each having seven types of formulations along with control (Plate 7- 9). The tomato seeds were sown and after 7 days, the percentage of seed germination was calculated. From the results it is evident that, Morphometric parameters like, the maximum average seed germination, root length, shoot length, root biomass, shoot biomass, number of primary roots, and leaf surface area were 100%, 24cm, 15cm, 0.35 g/plant, 0.25 g/plant, 13/plant and 11.03 sq.cm respectively (Table- 3). All the best outcomes were obtained from both seed dressing and root drenching in the combination of *Pseudomonas*...
fluorescens (AP6) and *Parthenium hysterophorus* extract (20%) (Dp1+Dp2). Among the various biocontrol agents, fluorescent pseudomonads are known to survive both in rhizosphere and phyllosphere (Wilson *et al.*, 1992). In contrast, the lack of colonization of tomato leaves by strain 89b61 suggests that the observed induced systemic resistance (ISR) was due to systemic protection by strain 89B61 and not attributable to a direct interaction between pathogen and biological control agent (Heller and Gessler, 1986). It correlates with the best outcomes of the present study in seed treatment only.

According to Singh and Mehrotra (1980), the most effective treatment for controlling all tested pathogens was seed-coating of *Pseudomonas fluorescens* against early blight of tomato. The most effective treatment for the efficacy of chilly juice and/or chilly (capsicum) extract oleoresins as antiseptic agents was evaluated against two common wood-discoloring fungi, *Sphaeropsis sapinea* and *Leptographium procerum*. Possible synergy between chilly juice and *Lactobacillus casei* as antiseptic agents was also assessed. Both the chilly juice and the *Lactobacillus casei* showed moderate antifungal activity. No growth of the test fungi was observed on plates amended with 50% chilly juice after 3 weeks of incubation. In the presence of 0.1% oleoresins, fungal biomass was reduced by more than half when compared with controls (Nandi *et al*., 2002). Bacterization of tomato seeds with *Pseudomonas fluorescens* was found to promote seed germination, vigor, biomass, and proliferation of root and shoot system (Kishore *et al*., 2005)

### 4.4.1. Total Protein, Carbohydrate and Chlorophyll

Recent studies indicate that prior application of fluorescent pseudomonads strengthen host cell wall structures resulting in restriction of pathogen invasion in plant tissue (Benhamou *et al*., 2000). Estimation of biochemical components in test plants, treated especially by the combination of seed dressing and root drenching was, supporting evidence to the morphometric results; with maximum level of total protein, carbohydrate and chlorophyll production by the SET(Dp1+Dp2) with values of 0.051, 0.042 and 0.13 mg/gm of leaf respectively (Fig-41). Generally the growth promoting
effect of antagonistic bacteria and plant extract could be correlated with induction of defense enzymes and secretion of growth factors (Kamalakannan et al., 2001).

The phenolic compounds contribute to enhance the mechanical strength of host cell wall and inhibit the fungal growth, as phenols are fungi toxic (M’Piga et al., 1997). The total phenol produced by Lycopersicon esculentum was found to accumulate more in shoots and also the concentration increased with age of seedling after transplantation to 243mg/gm on 15th day. The increase in phenolic contents of the host plant could be due to defense mechanism triggered by the interaction with an incompatible necrotizing pathogen (Durrant and Dong, 2004). Similarly in the present investigation, the host plants were treated with antagonist, their total phenolic content increased. Generally concentrations of phenolics are higher in resistant varieties, which is a positive note of the present study (Arora and Wagle, 1985). Significant level of defense enzymes like peroxidase (37 mg/gm), poly phenol oxidase (35 mg/gm), PAL (35 mg/gm), β-1, 3-Glucanase (0.40 mg/gm) and chitinase (0.49 mg/gm) were induced and recorded with combined treatment of fluorescens (AP6) and Parthenium hysterophorus (Dp1+Dp2).

Soil pseudomonads are reported to control a wide range of phytopathogens and induce the plants to over express plant defense enzymes (Silvia et al., 2004). Peroxidase is a key enzyme in the biosynthesis of lignin (Bruce and West, 1989). Increased activity of cell wall bound peroxidases has been elicited in different plants such as cucumber, rice, tomato (Chen et al., 2000; Mohan et al., 1993). Similar activity was observed when our host plant was colonized by fluorescens. Chen et al., (2000) reported about PPO activity induced by rhizobacteria in young leaves of tomato when matured leaflets are injured.

4.4.2. GCMS study

Fresh plant treated produced many compounds (Fig-42) like cyclobutanol (RT-12.954), glycine (RT-16.972), etanamine (RT-23.441), N-ethyl-N-methyl (RT-30.659), diethylmethylamine (RT-32.527), methyldiethylamine (RT-35.428), benzeneethanamine (RT-36.527), and N-octadecane (RT-37.533). Where the most
important and well-known compound was cyclobutanol that induces resistance, followed by benzeneethanamine that increases the potential of the plant collectively and N-octadecane that give anti-oxidant property to plants (Burrow et al., 2000).

The plant treated with *Pseudomonas fluorescens* (AP6) produced compounds like Piperidine (RT-2.150), 2- aminohexamine (RT-2.867), butylcycloheptanone (RT-27.408), tetradecone (RT-27.958), 2,6,10-trimethyltetradecane (RT-32.476), butanamine (RT-32.483), 3-methylL-1,2-benzenedicarboxylic acid(RT-33.531), (Fig-43). Among which piperdine is having fungicidal activity on a wide range of phytopathogenic fungi. This piperdine is a most convincing and concluding compounds that will play a key role when *Pseudomonas fluorescens* is adopted as a biocontrol agent (Tsitsigiannis et al., 2005).

Pesticide treated alone produced compounds like acetic acid (RT-4.60), cyano-acetic acid (RT-6.52), melonic ketone (RT-14.19), propanamine (RT-17.71), isopropylamine (RT-21.51), 2- amino propane(RT-24.58) (Fig-44). acetic acid produced is an inducer of resistance, propanamine is a pesticide product and decan-1-ol is a factor that prevents tumor and necrosis.

*Cyperus rotundus* and *Pseudomonas fluorescens* (Dc1+Dc2) treated plants produced acetic acid(RT-10.453), cyano-acetic acid(RT-16.972), malonic mononitrile(RT-32.527), benzeneethanamine(RT-35.445), alpha-methyl phenethylamine(RT-36.662), heptanal heptaldehyde(RT-36.772), octadecane(RT-37.221), N-octadecane(RT-37.338) (Fig-45). acetic acid is produced similar to salicylic acid that plays key role in inducing resistance (Vyas and Gulati, 2009).

*Parthenium hysterophorus* and *Pseudomonas fluorescens* (Dp1+Dp2) has produced 12 compounds (Fig-46) which are dodecanal (RT-2.11), N-dodecanal (RT-2.134), 1-dodecanal (RT-2.991), lauraldehyde (RT-6.50), aldehyde C-12 (RT-17.71), hexamine (RT-32.459), 4 methyl-, heptanamine decen-1-ol., heptin (RT-36.772), tuamine (RT-
Pesticide and *Pseudomonas fluorescens* (E<sub>1</sub>+E<sub>2</sub>) produced 5 different compounds (Fig-47) like decen-1-ol (RT-6.50), Melonic ketone (RT-12.67), propanamine(CAS) (RT-21.48), isopropylamine (RT-31.15), 2- amino propane (RT-24.55), acetic acid produced is a inducer of resistance, propanamine (CAS) is a pesticide product, decan-1-ol is a factor that prevents tumor and necrosis. Piperdine is a most convincing and concluding compounds that will play a key role when *Pseudomonas fluorescens* is adopted as a Biocontrol agent. From GCMS study 61 compounds were identified among which most of them are adopted for production of commercial pesticides and fungicides which is an emulating aspect based on SAR. Among 61, property of them is known only for 10 compounds that reveals the need for IR and NMR studies. It is puzzling where property of so many compounds needs to be defined in a refined mode of study through obtaining their fractions.

In supporting our view on biocontrol agents as more suitable one than commercial fungicides, a study by Pablo *et al.*, (2001) has revealed that application of Carbendazim alone has inhibited the induction of pathogens related protein (PRP) and defense enzymes like polyphenol oxidase (PPO) and peroxidase (PO). This effect of fungicides could be harmful to the resistance of tobacco plants to pathogen. According to the study of Vann peer *et al.*, 1991 there was increased accumulation of phytoalexins in stems of artificially bacterized plants compared to non-bacterial fungal-infected plants. The context of the preent study reveals that increased accumulation of defense enzymes and phytoalexins will enhance the plant defense mechanism.

Subbarao *et al.*, (1990) have described that the phytoalexin production may be an important mechanism by which the plant resist pathogen. One way to exploit this phytoalexin production is to bring natural variations within the species with regard to chemical structures of the compounds elicited. Elicitation by salicylic acid was faster than infection with fungi but it needs comparative study to that of *fluorescens*. This
suggests that it would be worth extending study to identify a source by analysis to make swift the elicitations in *Lycopersicon esculentum* by AP6 and *Parthenium hysterophorus*.

Plants have inbuilt immune mechanism in terms of physical and chemical means to defend against phytopathogens, environmental stress *etc.*. In spite, phytopathogens could infect and cause several diseases by conquering the natural barriers with their lytic enzymes and toxins. An attack by potential pathogens elicits profound changes in the metabolism of a plant, particularly altered protein synthesis. These proteins are called pathogenesis related proteins (PRP) or resistance related proteins (RRP) (Somassich *et al.*, 1986).

4.5. **Induction of systemic resistance using SA, Challenge inoculation and disease symptoms**

It is widely known that plants can defend themselves against pathogen infection through a variety of mechanisms that can be local, constitutive or inducible. Inducible resistance mechanisms such as systemic acquired resistance are broad spectrum plant defense responses that can be induced biologically by challenging a plant with a weaker strain of a specific pathogen or exposing a plant to natural and/or synthetic chemical compounds. SAR has been studied by plant biologist for the past hundred years as a means to increase resistance to fungal, bacterial and viral pathogens in crop plants (Glyn, 2001).

Many plant-associated microbes are pathogens that impair plant growth and reproduction. The plants respond to infection using the two-branch innate immune system. The first branch recognizes and responds to molecules common to many classes of microbes, including non-pathogens. The second responds to pathogen virulence factors, either directly or through their effects on host targets. These plant immune systems, and the pathogen molecules, to which they respond, they provide extraordinary insights into molecular recognition, cell biology and evolution across
biological kingdoms. A detailed understanding of plant immune function will underpin crop improvement for food, fiber and bio-fuel production (Jonathan et al., 2006).

The evocation of SAR response causes the rapid accumulation of several pathogenesis-related protein families in the intracellular and extra cellular regions of the leaf. Some of the PR proteins that accumulate during SAR have been demonstrated to have enzymatic activities that coincide with defense mechanism against fungal, viral and bacterial pathogens. Although the most abundant and tightly correlated molecular markers identified for SAR appear to be the family of PR-1 proteins, the precise biochemical function of these proteins remains to be elucidated.

The involvement of mechanisms other than competition for iron in biological control of *Fusarium oxysporum* of carnation by *Pseudomonas spp.* was investigated. Along with induced resistance there was an accumulation of phytoalexins in stems of bacterized and inoculated plants compared with non-bacterized, fungus-infected plants. No accumulations of these compounds were found in the non-infected bacterized plants (Vann Peer et al., 1991). De Meyer et al., (1999) have reported that the rhizosphere colonization by *Pseudomonas aeruginosa* 7NSK2 activated PAL in bean roots and increased the salicylic acid in leaves.

Efforts devoted to discovering the signal translocation during SAR revealed the central role of salicylic acid (SA) in activating the defense mechanism leading to SAR. GCMS studies of plant methanolic extract treated with salicylic acid shows various compounds like 9-octa decanoic acid (RT-21.48), methyl esters (RT-34.07), butanedioles (RT-4.63) and 1,3 dioonales(RT-13.51)(Fig-48) which act as precursor for hydroproxy octadeca trienoic acid, a phenomenal compound associated with plant defense as reported by Burow et al., (2000).

Combination of *Pseudomonas fluorescens* (AP6) and salicylic acid has produced compounds (Fig-49) like dodecanal (RT-4.60), N-dodecanal (RT-14.19), 1-dodecanal (RT-21.49), aldehyde C-12 (RT-24.58), hexamine (RT-32.42), heptin (RT-36.52),
tuamin (RT-17.69), rineptil (RT-6.50). Where Lipoxygenase pathway leads to synthesis of various compounds like heptine similar to that of 9S- hydroperoxy linoleic acid and 13S- hydroperoxy linoleic acid (Shah, 2005). The earlier mentioned compounds are also precursors of traumatin, jasmonic acid and methyl jasmonate, having hormone regulatory and defense related role in plants (Blee, 2002).

Salicylic acid is an important signal molecule that plays a critical role in plant defense, against pathogen invasion; it was investigated, if the exogenous application of SA would activate systemic acquired resistance (SAR) to the root system significantly increased the endogenous SA content of leaves. Thus the result indicated that root feeding 200μM SA to tomato plant can (1) induce PR-IB gene expression, (2) significantly elevate the foliar SA levels and (3) activates SAR that is effective against Alternaria solani (Bakker et al., 2003). In our study the maximum production of SA was 6.33mg/ml (Fig-50) by (Dp1+Dp2). Foliar application of salicylic acid at the concentration of 1mM significantly reduced the leaf blight disease intensity and increased the pod yield under greenhouse conditions. In earlier study salicylic acid treated leaves, had increase in phenolic content after five days of challenge inoculation with Alternaria alternata in groundnut plants pretreated with salicylic acid (Chitra et al., 2006).

4.5.1. Challenge inoculation of Alternaria alternata and disease scoring

The rating was observed as 7 in control, where the spots were present all over the plant, lower and middle leaves defoliating (Plate-10). Thus the severity of disease by Alternaria alternata was high with more than 70%, so it should be considered as a serious disease and there was an essential need to control it. But the plants treated with the combination of Pseudomonas fluorescens (AP6) and Parthenium hysterophorus extract (20%) (Dp1+Dp2) showed less disease scoring symptoms (Fig-51).

In tomato leaves, the 1-9 rating of disease score was analyzed by Krishna and Pande, (2005) with the pathogenic fungi Phaeoisariopsis personata. They showed the disease severity of 4.2, compared to which our pathogen had more severity. Soil
inoculation of tomato seedlings with *Alternaria alternata* has increased the accumulation of chitinase and glucanase initially after inoculation, later decreased after 15 days of inoculation (Kalaiarasan *et al.*, 2006).

### 3.5.2. SEM analysis

Accumulation of cell wall degrading enzymes within the infected tissues reveals its role in establishment of the fungus on lobular tissue (Cleveland and Cotty, 1991). Fungus with rapid growth rate, capable of producing large quantities of oxalic acid and cell wall degrading enzymes. This can be applied to our phytopathogen, since it is evident from the SEM analysis (Fig- 52 to 57). Extracellular enzymes produced by plant parasitic fungi not only digest plant cell wall polymers to obtain nutrients but also degrade the cell wall to aid in penetrating cells and spreading through plant tissue.

In our samples, fungal hyphae have penetrated through cells of plant leaves and have degraded them to put apart for hyphae proliferation and establishment resulting in leaf spot. The densities of the cells were found to be variable according to the mode of treatment in accordance to *in vitro* studies. Intercellular space between the cells found to be less in plants treated with *Pseudomonas fluorescens* (AP6) and *Parthenium hysterophorus* extract (20%) (Dp1+Dp2). If the cell density is high, automatically it results in increase of protein, carbohydrate and chlorophyll that adds inner strength of plants defense mechanism, the ultimate motto of our study.

Wei *et al.*, (1991) demonstrated the ability of different PGPR strains in reducing the incidence of anthracnose (*Colletotrichum orbiculare*) of cucumber. Two fluorescent pseudomonad isolates LEC1 and LEC2 were antagonistic to *Septoria tritici* and *Puccinia recondite in vitro*. The pyonidial coverage of *S. tritici* and symptoms of blotch were significantly suppressed by pseudomonads (Levy *et al.*, 1992). Bacteria which are shown to have potential for biocontrol of destructive diseases are distributed in many genera. Among them, fluorescent pseudomonads are currently considered as the most effective bacteria for biological control of soil and foliar diseases.
Plants are under continuous threat of infection by pathogens endowed with diverse strategies to colonize their host. Comprehensive biochemical and genetic approaches are now starting to reveal the complex signaling pathways that mediate plant disease resistance. Initiation of defense signaling often involves specific recognition of invading pathogens by the products of specialized host resistance (R) genes. Potential resistance signaling components have been identified by mutational analyses to be required for specific resistance in the model *Arabidopsis* and some crop species (Toyoda *et al*., 2002). Strikingly, many of the components share similarity to that of innate immune systems in animals. Bio-prospecting to find novel approaches in this scenario can help the farming community to increase their production in a biological way.

Majority of the small-scale farmers are not willing to leave chemical fertilizers, because of the unsure crop returns owing to the high incidence of fungal diseases and unpredictable terminal drought. In this context, there is greater scope for development and popularization of bio-inoculants in agriculture production system. Earlier attempts for selection of PGPR for tomato growth promotion in Indian soils identified an increase in tomato yield following seed treatment with *Pseudomonas spp.*, (Kishore *et al*., 2005). Even though various strategies and analytical methods were adopted in our research still it is a nascent one that needs renovations, which will end in lucrative destiny for farmers.
Table-1: Biochemical characteristics of Antagonist

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Test</th>
<th>P. fluorescens</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gram staining</td>
<td>Gram Negative</td>
</tr>
<tr>
<td>2</td>
<td>Hanging drop</td>
<td>Motile</td>
</tr>
<tr>
<td>3</td>
<td>Indole</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>Methyl Red</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>Voges –Proskeur</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>Citrate</td>
<td>Positive</td>
</tr>
<tr>
<td>7</td>
<td>Oxidase</td>
<td>Positive</td>
</tr>
<tr>
<td>8</td>
<td>Catalase</td>
<td>Positive</td>
</tr>
<tr>
<td>9</td>
<td>Urease</td>
<td>Negative</td>
</tr>
<tr>
<td>10</td>
<td>Starch hydrolysis</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Table-2: Production of Auxin and Phosphatase by Antagonists

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Isolates</th>
<th>Mannitol µg/ml</th>
<th>Tryptophan µg/ml</th>
<th>Yeast extract µg/ml</th>
<th>Mannitol µg/ml</th>
<th>Tryptophan µg/ml</th>
<th>Yeast extract µg/ml</th>
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<td>70</td>
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<td>AP₂</td>
<td>6.33</td>
<td>7.45</td>
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<td>73</td>
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<td>AP₃</td>
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<td>69</td>
<td>60</td>
<td>65</td>
</tr>
<tr>
<td>4</td>
<td>AP₄</td>
<td>7.01</td>
<td>6.92</td>
<td>6.47</td>
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<td>59</td>
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<td>AP₅</td>
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<td>7.56</td>
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<td>72</td>
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<td>AP₆</td>
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<td>7.92</td>
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<td>78</td>
<td>73</td>
<td>71</td>
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<td>AP₇</td>
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<td>6.98</td>
<td>6.76</td>
<td>72</td>
<td>63</td>
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Table-3: Effect of Antagonistic Bacteria and Hazardous Weed extracts on Morphological Parameters of *Lycopersicon esculentum* (Tomato)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatments</th>
<th>Seed Germination %</th>
<th>Root Length (cm)</th>
<th>Shoot Length (cm)</th>
<th>Root Biomass (g/plant)</th>
<th>Shoot Biomass (g/plant)</th>
<th>No. of Primary Roots/plant</th>
<th>Leaf Surface Area/plant Sq.cm</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>C</td>
<td>80</td>
<td>19</td>
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<td>10.32</td>
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<td>2</td>
<td>A1+A2</td>
<td>90</td>
<td>20</td>
<td>13</td>
<td>0.22</td>
<td>0.31</td>
<td>11</td>
<td>10.55</td>
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<td>3</td>
<td>B1+B2</td>
<td>85</td>
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<td>12</td>
<td>0.21</td>
<td>0.31</td>
<td>12</td>
<td>10.22</td>
</tr>
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<td>4</td>
<td>Cc1+Cc2</td>
<td>80</td>
<td>20</td>
<td>11</td>
<td>0.21</td>
<td>0.31</td>
<td>11</td>
<td>10.33</td>
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<tr>
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<td>Cp1+Cp2</td>
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<td>0.34</td>
<td>13</td>
<td>10.36</td>
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<tr>
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<td>Dc1+Dc2</td>
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<td>14</td>
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<td>12</td>
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<td>Dp1+Dp2</td>
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<td>0.25</td>
<td>0.35</td>
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<td>11.03</td>
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<td>0.33</td>
<td>13</td>
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<td>Gc1+Gc2</td>
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<td>0.30</td>
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<td>10.55</td>
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NOTE: C- Control, A1+A2- Pseudomonas fluorescens alone, B1+B2- Carbendazim alone, Cc1+Cc2- Cyperus rotundus, Cp1+Cp2 – *Parthenium hysterophorus*, Dc1+Dc2- Pseudomonas fluorescens and Cyperus rotundus, Dp1+Dp2- Pseudomonas fluorescens and *Parthenium hysterophorus*, E1+E2- Pseudomonas fluorescens and Carbendazim, Fc1+Fc2- Cyperus rotundus and Carbendazim, Fp1+Fp2- *Parthenium hysterophorus* and Carbendazim, Gc1+Gc2- Pseudomonas fluorescens, Cyperus rotundus and carbendazim, Gp1+Gp2- Pseudomonas fluorescens, *Parthenium hysterophorus* and carbendazim
Table-4: Effect of Antagonistic Bacteria and Hazardous Weed extracts on Phenolics and plant defense enzymes of *Lycopersicon esculentum* (Tomato) on 15th day

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatments</th>
<th>Total Phenol mg g⁻¹ FW</th>
<th>PAL mg g⁻¹FW</th>
<th>Peroxidase mg g⁻¹FW</th>
<th>Poly Phenol Oxidase mg g⁻¹ FW</th>
<th>β-1, 3- Glucanase mg g⁻¹FW</th>
<th>Chitinase mg g⁻¹FW</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>26</td>
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<td>0.31</td>
<td>0.41</td>
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<tr>
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<td>29</td>
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<td>0.41</td>
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<td>Cp₁+Cp₂</td>
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<tr>
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<td>29</td>
<td>30</td>
<td>0.30</td>
<td>0.40</td>
</tr>
<tr>
<td>10</td>
<td>Fp₁+Fp₂</td>
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<td>28</td>
<td>33</td>
<td>0.29</td>
<td>0.39</td>
</tr>
<tr>
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<td>34</td>
<td>35</td>
<td>33</td>
<td>0.37</td>
<td>0.47</td>
</tr>
</tbody>
</table>

NOTE: C- Control, A₁+A₂- *Pseudomonas fluorescens* alone, B₁+B₂- Carbendazim alone, Cc₁+Cc₂- *Cyperus rotundus*, Cp₁+Cp₂– *Parthenium hysterophorus*, Dc₁+Dc₂- *Pseudomonas fluorescens* and *Cyperus rotundus*, Dp₁+Dp₂- *Pseudomonas fluorescens* and *Parthenium hysterophorus*, E₁+E₂- *Pseudomonas fluorescens* and Carbendazim, Fc₁+Fc₂- *Cyperus rotundus* and Carbendazim, Fp₁+Fp₂- *Parthenium hysterophorus* and Carbendazim, Gc₁+Gc₂- *Pseudomonas fluorescens*, *Cyperus rotundus* and carbendazim, Gp₁+Gp₂- *Pseudomonas fluorescens*, *Parthenium hysterophorus* and carbendazim

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Fig-1 Production of cell wall degrading enzymes by *Alternaria alternata*

Fig-2: *In vitro* assay of fungicides against *A. alternata*

Fig-3: Mycelial dry weight of *A. alternata* with Fungicides
Fig-4: Total protein estimation with fungicides by *A. alternata*

Fig-5: Effect of temperature on growth of antagonists

Fig-6: Effect of pH on growth of antagonists
Fig-7: Effect of carbon source on growth of Antagonists

Fig-8: Effect of nitrogen source on growth of antagonists

Fig-9: Effect of amino acids on growth of Pseudomonas fluorescens
Fig-10: Production of salicylic acid by Antagonists in carbon source

Fig-11: Production of SA by antagonists in nitrogen sources of SSM

Fig-12: Production of SA by antagonists in amino acids of SSM
Fig-13: Production of HCN by antagonists in carbon sources of SSM

Fig-14: Production of HCN by antagonists in nitrogen sources of SSM

Fig-15: Production of HCN by antagonists in amino acids of SSM
Fig-16: Production of siderophores by antagonists in carbon sources of KMB

Fig-17: Production of siderophores by antagonists in nitrogen sources of KMB

Fig-18: Production of siderophores by antagonists in amino acids of KMB
Fig-19: Production of protease by antagonists in carbon sources of YECA

Fig-20: Production of protease by antagonists in nitrogen sources of YECA

Fig-21: Production of protease by antagonists in amino acids of YECA
Fig-22: Production of lipase by antagonists in carbon sources in minimal media

Fig-23: Production of lipase by antagonists in nitrogen sources in minimal media

Fig-24: Production of lipase by antagonists in amino acids in minimal media
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Fig-25: Production of chitinase by antagonists in carbon sources of KMB

Fig-26: Production of chitinase by antagonists in nitrogen sources of KMB

Fig-27: Production of chitinase by antagonists in amino acids of KMB
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Fig-28: Production of glucanase by antagonists in carbon sources of KMB

Fig-29: Production of glucanase by antagonists in nitrogen sources of KMB

Fig-30: Production of glucanase by antagonists in amino acids of KMB
Fig-31 Antagonistic activity of *P. fluorescens* (AP₆) against *A. alternata*

Fig-32: Production of secondary metabolites by AP₆ with *C. rotundus* extract

Fig-33: Production of secondary metabolites by AP₆ with *P. hysterophorus* extract
Fig-34: Production of IAA (Auxin) by AP₆ with plant extracts

Fig-35: Production of phosphatase by AP₆ with plant extracts

Fig-36: Production of protease by AP₆ with plant extracts
Fig-37: Production of lipase by AP6 with plant extracts

Fig-38: Production of β-1, 3 glucanase by AP6 with plant extracts

Fig-39: Production of chitinase by AP6 with plant extracts
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Fig-40: Percentage of inhibition by AP6 along with plant extracts

Fig-41: Estimation of total protein, carbohydrate and chlorophyll under *in vivo* study

Fig-42: GC-MS study on control plant leaves
Fig-43: GC-MS study on plant leaves treated with *Pseudomonas fluorescens* (AP$_6$) (A1+A2)

Fig-44: GC-MS study on plant leaf sample treated with *Carbendazim* (B1+B2)

Fig-45: GC-MS study on plant leaves treated with *Cyperus rotundus* and *Pseudomonas fluorescens* (Dc$_1$+Dc$_2$)

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Fig-46: GC-MS study on plant leaves treated with *Parthenium hysterophorus* and *Pseudomonas fluorescens* (Dp1+Dp2)

Fig-47: GC-MS study on plant leaves treated with Pesticide and *Pseudomonas fluorescens* (E1+E2)

Fig-48: GC-MS study on plant leaves treated with Salicylic acid

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Fig-49: GC-MS study on plant leaves treated with Pseudomonas *fluorescens* (AP_6) and SA

Fig-50: Production of salicylic acid by treated host plants

Fig-51: Disease scoring symptoms by *Alternaria alternata* in host plant
Fig-52: SEM study of control plant leaves

Fig-53: SEM study of *Pseudomonas fluorescens* (AP₆) treated leaves

Fig-54: SEM study of *Alternaria alternata* infected leaves
Fig-55: SEM study of (AP₆) and *Parthenium hysterophorus* treated leaves

Fig-56: SEM study of *Pseudomonas fluorescens* and *Cyperus rotundus* treated leaves

Fig-57: SEM study of pesticide treated leaves
Plate-1a: Infected Part of Tomato Leaves in CD Agar

Plate-1b: Mixed Fungal growth on PDA

Plate-1c: Alternaria alternata, fungal growth in CD Agar

Plate-1d: Lacto Phenol Cotton Blue Mount of Alternaria alternata

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A Study on Biological Control of leaf spot of Lycopersicon esculentum.................
Plat-2c: *Pseudomonas fluorescens* on NA

Plat-3a: SA production by *Pseudomonas fluorescens*

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Plat-3b: HCN production by *Pseudomonas fluorescens*

Plat-3c: Siderophore production by *Pseudomonas fluorescens*
**Plat-4:** *In vitro* Antifungal activity by *Pseudomonas fluorescens*

**Plate-5a:** Aqueous Extract of *Parthenium hysterophorus*  
**Plate-5b:** *Parthenium hysterophorus* plant morphology

*A Study on Biological Control of leaf spot of Lycopersicon esculentum* .................
Plate-6a: Aqueous Extract of *Cyperus rotundus*

Plate-6b: *Cyperus rotundus* plant morphology

Plat-7: A view of potted plants for Green House studies

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Plat-8: Different treatments with *Cyperus rotundus*

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Plat-9: Different treatments with *Parthenium histerophorus*

*A Study on Biological Control of leaf spot of Lycopersicon esculentum* ..............
A Study on Biological Control of leaf spot of *Lycopersicon esculentum*..................

Plat-10a: Tomato plant infected by *Alternaria alternata* and defoliation

Plat-10b: *Alternaria alternata* leaf spot and necrosis