2. REVIEW OF LITERATURE

The success or failure of infection is determined by dynamic competition and the final outcome is determined by the sum of favourable and unfavourable conditions for both the pathogen and host cells. In fact, if one considers the multitude of microorganisms to which plants are being continuously exposed in nature, the significance of specificity becomes more apparent (Chakraborty, 1988). Disease resistance in plants depends on multiple defense mechanisms which include preformed defense barriers such as the cuticle, the cell wall or constitutive antimicrobial compounds. Purpose of this review is to present briefly the observation of previous workers in concord with the present line of investigation. Two major aspects such as (a) Role of phenolic compounds in plant disease resistance and (b) Potential use of phylloplane microorganisms for biocontrol of plant diseases, have been discussed in the following pages :

Role of Phenolic Compounds in Plant Disease Resistance

Phenols are significant components of the host response which involve the isolation, identification from substantially large amounts of tissues following inoculation. Antibiotic phenols have been found in plants that occur constitutively and function as preformed inhibitors associated with non-host resistance (Millar and Higgins, 1970; Schonbeck and Schlosser, 1976; Mansfield, 1983; Stoessl, 1983). Others are formed in response to ingress of pathogens and their appearance is considered as part of an active defense response. Since the phenolic intermediates have a role in the active expression of resistance, an underlying problem in ascertaining that such secondary metabolites are of primary importance has been the localization and timing of the host response. The problem of response localization and localization of phenolics relative to the sequential disease development stages that lead ultimatly to resistance expression (Nicholson and Hammerschmidt, 1992).

Paschenko (1978) demonstrated the role of phenols in resistance of Nicotiana didebta to Peronospora tabacina. From a study of the effects of pyrogallol, pyrocatechol and hydroquinone and aqueous extracts from leaf tissues of N. didebta and leaf washing on conidial growth, no direct realtionship was found between their quantity and the resistance of mature plants to P. tabacina. Pyrocatechol and hydroquinone showed extremely high fungitoxicity in relation to P. tabacina. Spore growth was more strongly inhibited by extracts from tissue of receptive cultivars. Pyrogallol somewhat stimulated conidial growth. Polyphenoloxidase of resistant cultivars were highly activated during infection.

In potato tubers chlorogenic acid accumulates slower following inoculation with P. infestans than in non-inoculated controls, regardless of cultivar resistance (Gans, 1978). In contrast, in some susceptible cultivars chlorogenic acid accumulates at an accelerated rate after inoculation (Henderson and Friend, 1979). The differentiation of the responses of plants to pathogens based on host and non-host interactions has also been argued by Heath (1980).

Friend (1981) suggested that chlorogenic acid may act as a reservoir for the caffeoyl moiety that, as an activated phenylpropanoid, could be shunted to the synthesis of other phenolics possibly involved in containment of the pathogen. The accumulation of chlorogenic acid may represent a general rise in phenolic biosynthesis. Such synthesis can ultimately result in the accumulation of compounds with sufficient toxicity to be involved in resistance. Mayama et al. (1981) demonstrated that oat produces nitrogen-containing phenolic phytoalexins, the avenalumins, and that these compounds accumulated only in incompatible host-pathogen interactions. Mayama and Tani (1982) took the advantage of the UV-absorbance and autofluorescence spectra of the avenalumins and used microspectrophotometry to reveal the presence of intense fluorescence only in cells immediately associated with the infection site. When carrot root

slice is infected with Botrytis cinerea, the infection leads to the production of inhibitors such as 6-methoxymellein, p-hydroxybenzoic acid and falcarinol (Harding and Heale, 1981).

Rapid accumulation of phenols may result in the effective isolation of the pathogen (or non-pathogen) at the original site of ingress (Legrand, 1983; Ride, 1983). For most plants it is low molecular weight phenols, especially the phenylpropanoid, that are involved in the initial response to stress. In potato, phenols accumulate as an initial response to infection (Hachler and Hohl, 1984; Hammerschmidt, 1984). The accumulation of polymerized phenols also occurs as a rapid response to infection. Hydroxycinnamic acids and their derivatives are thought to contribute to the discoloration and autofluorescence of host tissues at the site of infection (Bolwell et al., 1985; Farmer, 1985). Esterification of phenols to cell-wall materials has been considered as primary theme in the expression of resistance (Fry, 1986; Fry, 1987).

Change in phenolics in maize leaf after inoculation with Helminthosporium carbonum and their antifungal activity was studied by Werder and Kern (1985). Maize inbreds Pr1 (resistant) and Pr (susceptible) to H. carbonum race 1 were inoculated and phenolic material was extracted from maize leaf tissue. The components were then analyzed and resistance was studied with respect to phenol metabolism and accumulation of fungitoxic compounds. Host responses could be differentiated by changes in content of phenolic compounds. The pattern of changes of total phenolic content (hydrolyzed and unhydrolyzed ethylacetate-soluble phenols) of resistant and susceptible inbreds did not differ much between 0 and 96 h after inoculation. However, phenolics content in the resistant inbred increased between 96 and 120 h after inoculation to a level two to three times higher than that of susceptible and non-infected control inbreds. They isolated four antifungal compounds, A, B, C and D from hydrolyzed maize leaf extracts. All four compounds were fungitoxic to

H. carbonum in spore germination and chromatographic bioassays. Compounds A and B were inhibitory to H. carbonum only in high concentrations. The investigators suggested a role of the phenol metabolism in the resistance of maize to H. carbonum based on different content of total phenolics in resistance and susceptible inbreds. The compounds C and D were supposed to play a role in the resistance mechanism as fungitoxic components.

Change in phenolics of two each of resistant and susceptible varieties of wheat leaves in response to Puccinia recondita causing brown rust were evaluated by Saxena et al. (1986). They found that resistant varieties exhibited higher concentration of phenolics than the susceptible one.

Biochemical analysis of pea varieties resistant and susceptible to Erysiphe polygoni causing powdery mildew disease revealed that the quantity of total phenol and orthodihydroxyphenol was high in stem and leaves of resistant varieties as compared to susceptible ones which decreased as the age of plant increased in all the varieties (Parashar and Sindhan, 1987).

The kinds of phenolic compounds that accumulate prior to the active defense response as well as their origin has been addressed by Matern et al. (1988) using parsley cell suspensions as a model system. Inoculation of parsley leaves with P. megasperma f. sp. glycinea (Pmg) or treatment of parsley cell suspensions with a Pmg elicitor results in the accumulation of substantial concentrations of coumarin phytoalexins as well as esterification of phenylpropanoids, in particular ferulic acid, to cell walls (Matern and Kneusel, 1988). Treatment of parsley cells with the Pmg elicitor cause the synthesis of the coumarin phytoalexins isopimpinellin, psoralen, bergapten, xanthotoxin and graveolone.

The temporal and spatial differences in the accumulation of phenylalanine ammonia-lyase (PAL) mRNA occurred as a response to infection which was rapidly elevated in interactions involving an incompatible race of fungus, whereas a significantly different profile of mRNA accumulation occurred in interactions involving a compatible race (Cuypers et al., 1988).

The healthy leaves of Morinda tomentosa contained the two methoxyflavonols 4'-OMe kaempferol and 3',4'-diOMe quercetin, and the four phenolic acids - vanillic, syringic, gentisic and ferulic. The Colletotrichum gloeosporoides infected leaves contained the hydroxyflavonols kaempferol and quercetin along with four phenolic acids found in healthy leaves. The diffusates of both the pathogen and non-pathogen (F. solani) treated leaves contained quercetin and kaempferol (Abraham and Daniel, 1988).

Matern and Kneusel (1988) have proposed that the defensive strategy of plants exists in two stages. The first is assumed to involve the rapid accumulation of phenols at the infection site, which function to slow (or even halt) the growth of the pathogen and to allow for the activation of "secondary" strategies that would more thoroughly restrict the pathogen. Secondary responses would involve the activation of specific defenses such as the de novo synthesis of phytoalexins or other stress-related substances. They argue that the initial defense response must occur so rapidly that it is unlikely to involve de novo transcription and translation of genes, which would be characteristic of the second level of defense. The sequence of events in a defense response can be thought to include - host cell death and necrosis, accumulation of toxic phenols, modification of cell walls by phenolic substituents or physical barriers such as appositions or papillae, and, finally, the synthesis of specific antibiotics such as phytoalexins.

There is often a greater increase in phenolic biosynthesis in

resistant host species than in susceptible host and it is sometimes postulated that the increase in phenolic compounds is part of the resistance mechanism. Some of these compounds are toxic to pathogenic and non-pathogenic fungi and have been considered to play an important role in disease resistance (Vidyasekharan, 1988).

Prasada et al. (1988) reported that after infection total phenol increased in green and ripe tomato fruits in course of rotting due to Sclerotium rolfsii. Changes in phenol contents was also determined by Oke (1988) in young, matured, healthy and Cassicola corynospora and Colletotrichum nicotianae infected leaves of tobacco. After infection the quantity of total phenols and orthodihydroxyphenol increased in both stem and leaves of susceptible and resistant varieties.

Polyphenol content in sweet cherry bark was drastically changed after infection by Cercospora personii (Bayer, 1989). Infected tissue and closely neighbouring areas were characterised by the appearance of phenolic aglycons which inhibited growth of both the pathogen. Mechanically wounded bark tissue showed different phenolic patterns than infected ones. Tore and Tossi (1989) investigated the changes in phenolic and nitrogen metabolism in healthy and infected (with Thielaviopsis basicola) tobacco roots and leaves. The chlorogenic acidcontent increased in infected root and leaves compared with the control beginning on the 8th day after inoculation.

Quantitative changes in phenolic compound on barley varieties inoculated with Puccinia hordei were determined at different time intervals by Etenbarian (1989). There were no significant difference in total phenol content between healthy and infected leaves of barley cultivars two days after inoculation. However, the phenol content of leaves were increased after nine days of inoculation in relation to healthy control. Luthra (1989) determined the levels of total phenol

in sorghum leaves, resistant and susceptible to Ramulispora sorghicola at 15 days interval after 25 days of sowing. Resistant variety exhibited high phenol content in comparison to susceptible ones at all stages of growth.

Phenolic compounds inhibitory to the germination of spores of Colletotrichum graminicola were shown to leach from necrotic lesions on corn leaves caused by the fungus. Primary components of the phenolic mixture were identified as esters and glycosides of p-coumaric and ferulic acids as well as the free compounds themselves. Spores of C. graminicola produced in acervuli on infected leaves were shown to be surrounded by a mucilaginous matrix as is the case when the fungus is cultured in vitro. It is suggested that the mucilage protects spores from the inhibitory effects of the phenols by the presence of proline rich proteins that have been shown to have a high binding affinity for a variety of phenols (Nicholson et al., 1989).

Toxic phenylpropanoids, such as ferulic acid, can form rapidly without the involvement of the traditionally accepted route of phenylpropanoid synthesis and conversion to CoA esters (Hahlbrock and Scheel, 1989). The relatively non-specific disruptive effects on cells that result from wounding lead almost immediately to a variety of physiological changes, including oxidation of secondary metabolites. The accumulation of these esters preceded the onset of visible necrosis of infection sites; the concentration of the compounds fell substantially after the onset of necrosis both of which strengthen the argument for their involvement in the browning response (Bostock and Stermer,1989).

Baker et al. (1989) examined specific race interaction with cloves of resistant and susceptible genotypes. It was found that in resistant reaction accumulation of phenolic compounds were higher than susceptible reaction. They suggested that accumulation of phenolics

may play a role in natural and induced interaction involving Colletotrichum trifolii and Medicago sativa.

It has long been recognized that responses are characterized by the early accumulation of phenolic compounds at the infection site and that limited development of the pathogen occurs as a result of rapid (hypersensitive) cell death (Fernandez and Heath, 1989). Mansfield (1990) has proposed that cell death results from irreversible membrane damage that may occur in response to pathogen recognition or as a result of activated host response.

Kumar et al. (1990) analysed certain biochemical changes in the pearl millet shoots infected with downy mildew pathogen (Sclerospora graminicola).The estimation revealed that the total phenol and free amino acids content were found to be low both in diseased shoot and roots of pearl millet (Pennisetum americanum). In maize there is a marked accumulation of two caffeic acid esters after inoculation with Glomerella graminicola or C. heterostrophus in both compatible and incompatible combinations (Lyons et al., 1990). One compound was identified as caffeoyl glucose, whereas the other was a caffeoyl ester of an unknown organic acid moiety. Although neither compound was fungitoxic, a pattern of rapid accumulation followed by a sharp decrease in the amount of both compounds in the tissue suggested that they may serve as a pool of phenols required for diversion to other products.

Low molecular weight phenols, such as benzoic acids and the phenylpropanoids, are formed in the initial response to infection (Niemann et al., 1991). Early after infection, low molecular weight phenols accumulate in both incompatible (resistant) and compatible (susceptible) interactions. Whether these compounds are significant in the ultimate host rsponse presents a perplexing problem. It has been suggested by Perumalla and Heath (1991) that the accumulation of

phenolics as an initial response to infection may reflect a general increase in host metabolism as well as an accumulation of relatively non-toxic secondary metabolites, which could ultimately serve as precursors for compounds essential to expression of resistance.

By histochemical staining of tissues with toluidine blue O together with clearing and fluorescence analysis, Bruzzese and Hasan (1991) demonstrated that accumulation of phenols at the infection site occurred as early as 3 h after inoculation, indicating an association of phenols with the initial stages of the response. The contents of phenols, O-dihydroxyphenols and peroxidase activity in healthy and Curvularia andrepogonis infected leaves of Java citronella (Cymbopogon winterianus) were determined by Alam et al. (1991). As a result of infection, the content of phenols and peroxidase increased two and four-fold, respectively in necrotic lesions compared to healthy leaves. In surrounding tissue of lesions, their increase was one- and half fold only. The peroxidase activity decrease with the maturity of the necrotic lesions. Necrotic lesions produced in response to infection appear to be the consequence of higher accumulation of phenols and their oxidation by peroxidase. In the interaction of potato tubers with Verticillium dahliae, hypersensitive browning and suberization are characteristic of the initial events in resistance rather than production and accumulation of phytoalexins (Vaughn and Lulai,1991).

Resistant cotton cultivar contained fairly high amount of total as well as orthodihydroxyphenol than susceptible cultivar. After inoculation with Xanthomonas campestris pr. malvacearum, total and orthodihydroxyphenol increased in resistant interaction (Borkar and Verma, 1991).

Changes in carbohydrate, amino acid and phenolic contents in jute plant on inoculation with Macrophomina phaseolina, Colletotrichum

corchori and Botryodiplodia theobromae were studied by Sahabuddin and Anwar (1992). Total sugars, non-reducing sugars, starch and total free amino acids were found to decrease on inoculation with all the three test pathogens of jute, while reducing sugars, total phenols and orthodihydric phenols increased.

The healthy leaves of Trianthema portulacastrum contained 6,7-dimethoxy-3,5,4'-trihydroxyflavone, vanillic acid, p-hydroxybenzoic acid and phytoecdysones. The Fusarium sp. infected leaves, in addition to these compounds, contained quercetin and ferulic acid. By using drop diffusate technique it is found that the pathogen induces the formation of quercetin and ferulic acid (Darshika and Daniel, 1992).

Among fourteen varieties of tea tested separately against Glomerella cingulata, Pestalotiopsis theae and Bipolaris carbonum, TV-18 and TV-26 were highly susceptible and resistant, respectively to both G. cingulata and B. carbonum, while TV-23 and CP-1 were found to be highly susceptible and resistant, respectively, to P. theae. Twelve separate phenolics were detected on thin layer chromatograms after extraction from healthy tea leaves and some were identified as gallic acid, catechol, caffeic acid and p-coumaric acid. Total phenol level decreased by 4.5, 1.2 and 8.5% in the susceptible varieties TV-18, TV-9 and TV-17 respectively after inoculation with B. carbonum, whereas in case of resistant varieties TV-26, TV-25 and TV-16 total phenol level increased by11.1, 5.7 and 12.2% respectively after inoculation. Similar pattern was observed for O-dihydroxy phenol content in healthy and inoculated leaves of resistant and susceptible varieties (Chakraborty et al., 1994b).

The healthy leaves of Tectona grandis contained two flavones : 4'-OMe apigenin and luteolin. The phenolic acids present were syringic, sinapic, vanillic, melilotic and gentisic acids. The other

constituents of the leaves were quinones (lepachol and tectaquinone), proanthocyanidins, iridoids, alkaloids and tannins. The infected leaves did not contain any flavone but a flavonol 3',4'-dimethoxyquercetin instead and phenolic acids such as ferulic, vanillic, melilotic and gentisic acids. They contained the same quinones as of healthy leaves as well as proanthocyanidins, iridoids, alkaloids and tannins. There was no significant chemical difference between the diffusates of control and treated leaves when the healthy leaves were treated with the spore suspension of Curvularia clavata. But when the leaves were treated with a non-pathogen Fusarium solani, the diffusate contained p-hydroxybenzoic acid. Mycelial growth, spore germination and germ tube growth of F. solani and C. clavata is strongly inhibited by p-hydroxybenzoic acid (Daniel, 1995).

Two antifungal compounds isolated from healthy and Bipolaris carbonum-infected tea leaves exhibited clear inhibition zones at R_F 0.8 and 0.65, respectively in a chromatographic bioassay. On the basis of their colour reaction on TLC and UV-spectra these were identified to be catechin and pyrocatechol, respectively. Resistant varieties accumulated 439-510 µg/g fresh weight tissue of catechol in comparison to 187-212 µg/g fresh weight tissue in susceptible varieties after accumulation with B. carbonum. Low concentration of this compound was also detected in healthy leaf tissues (Chakraborty and Saha, 1994a, 1995).

In some host parasite interaction phenolics have been associated with phytoalexin accumulation (Mansfield et al.1974; Langcake and Pryce, 1976; Langcake and MacCarthy, 1979; Holliday et al., 1981; Pierce and Ersenberg, 1987; Baker et al., 1989). Phytoalexin accumulation is believed to be an important early defense response in several plant pathogen interaction A lot of work has been done and several comprehensive reviews have appeared on phytoalexins and their

role in disease resistance (Cruickshank, 1963, 1978, 1980; Kuc, 1966, 1972, 1976; Deverall, 1972, 1976; Ingham, 1972, 1973, 1982; Purkayastha,1973, 1976, 1985, 1986; Van Etten and Pueppke, 1976; Keen and Brueggar, 1977; Harborne and Ingham, 1978; Keen, 1981, 1982, 1990; Van Etten et al., 1982, 1989; Wood, 1982; Bailey and Deverall, 1983; Ward, 1986; Paxton, 1988; Ebel and Grisebach, 1988; Daniel,1995; Purkayastha,1995; Chakraborty et al., 1995).

Phytoalexins constitute a chemically heterogeneous group of substances belonging to various classes of natural products which include isoflavanoids, sesquiterpenoids, polyacetylenes and stilbenoids. Many phytoalexins are absent in healthy, unchallenged plants. It was originally believed that phytoalexins were host specific. With the evidences accumulated so far, concerning the wide spread occurrence, isolation and characterization of phytoalexins during the past 50 years, it is now clear that more than one phytoalexin could occur in a single host species of which one may be dominated (Purkayastha, 1995). Again, similar phytoalexins may also occur in different host species. Plant organs including roots, stem, leaves and fruits have been shown to respond to infection with the formation of phytoalexins. Among plant pathogens, fungi, some bacteria and viruses are capable of inducing phytoalexin production in plants, but involvement of the last two groups of organisms seems to be quite negligible in comparison with the large group of fungi. During incompatible host-parasite interaction, phytoalexin is synthesized rapidly and accumulates at the infection site (Akazawa and Wada, 1961; Cruickshank and Perrin, 1968; Partridge and Keen, 1976; Purkayastha et al., 1983). In contrast, in the compatible host-parasite interaction the plant also synthesises the phytoalexin but relatively slowly and in reduced concentration.

The degree of stimulation of phytoalexin biosynthesis depend on several factors such as quantity of elicitors released by the organism; speed of release; chemical nature of the elicitor; presence

or absence of receptors in the host cell membrane, if present; strong or weak response of receptor; duration of treatment; and environmental conditions. Some selected observations in this line of research have been incorporated in the following paragraphs.

A glucan was isolated from the cell wall extracts of Fusarium oxysporum f. sp. lycopersici (Anderson, 1980) and a polypeptide (monilicolin A) from mycelia of Monilina fructicola (Cruickshank and Perrin, 1968). Both compounds elicited phaseollin production. An elicitor of phaseollin was isolated from the mycelial walls and culture filtrates of Colletotrichum lindemuthianum, which was identified as a polysaccharide. The molecular weight varied between 1 million and 5 million Da, and consisted predominantly of 3- and 4-linked glucosyl residue (Anderson-Prouty and Albersheim, 1975). An amount equivalent to 100 ng of glucose elicited a similar response in the bean tissue.

The isolates of Fusarium solani which differed in their pathogenicity also showed differential pisatin-eliciting potential. It was confirmed when their culture filtrates were tested on pea (Daniels and Hadwiger, 1976). There was a difference in the concentration of elicitor in the culture filtrates of isolates. The elicitor was fairly heat-stable and also stable in freezing, but eliciting activity was reduced significantly by pronase digestion. This strongly suggests that some of the activities were due to proteinaceous components.

An elicitor of glyceollin was isolated from the mycelial wall of Phytophthora megasperma var. sojae by Ebel et al. (1976). This elicitor stimulated the activity of phenylalanine ammonialyase and also induced glyceollin production in soybean cell cultures. They concluded that the action of elicitors is not species or variety specific but is a part of the general defense response of plants. Shiraishi et al. (1978) detected both elicitor and suppressor of

pisatin in the pycnospore germination fluid of Mycosphaerella pinoides.

The regulation system of phaseollin synthesis in cell suspension cultures of dwarf french bean (Phaseolus vulgaris) was studied by Dixon and Christopher (1979). Considerable amount of phaseollin accumulated when french bean was treated with an elicitor from the cell wall of C. lindemuthianum. But the elicitors isolated from the cell walls of P. megasperma var. sojae and Botrytis cinerea were less effective.

Elicitors extracted from the cell walls of Saccharomyces cerevisiae were identified as structural glucans. These are able to stimulate glyceollin accumulation in soybean (Albersheim et al., 1978). Specific elicitors of glyceollin were also detected in the cellular envelops of incompatible races of Pseudomonas glycinea. However, elicitor activity could not be detected in lipopolysaccharide preparations, exopolysaccharide fraction, or the culture fluids of various races of P. glycinea. Elicitors were solubilized with sodium dodecyl sulfate and then preparations from five bacterial races excepting one had similar specificity for elicitation of glyceollin in cotyledons of two soybean cultivars (Bruegger and Keen, 1979). These observations suggests that elicitors are not always race specific.

Glycoproteins were extracted from isolated cell walls of Phytophthora megasperma f. sp. glycinea with 0.1 N NaOH at 0°C and elicited glyceollin in soybean hypocotyls with the same specificity as the fungus races from which they were obtained (Keen and Legrand, 1980). Fraction of the crude extracts on DEAE Bio-Gel and Bio-Gel A-5 m columns showed that specific elicitor activity was associated with the presence of high molecular weight glycoproteins detected by SDS gel electrophoresis.The glycoproteins appeared to contain only glucose

and mannose as neutral sugars. The elicitor activity of the glycoproteins was not diminished by boiling at 100°C or pronase treatment, but was destroyed by periodate, thus indicating that the carbohydrate portions are important for activity. The glycoproteins were the only concanavalin A reactive species detected in the crude cell wall extracts, and fluorescein labelled concanavalin A was hapten-specifically bound to living hyphae of the fungus.

Purkayastha and Ghosh (1983) reported elicitor activity of fresh mycelial wall extract of Myrothecium roridum. Spores were suspended in mycelial wall extract, drops placed on leaf surfaces of soybean, and incubated for 48h. The results of bioassay test revealed that the spores suspended in mycelial wall extract were more inhibitory than the spores suspended in sterile distilled water and incubated on leaf surfaces for a similar period. Mycelial wall extract induced greater production of glyceollin in soybean leaves.

Yamoto et al. (1986) demonstrated that pisatin could be induced in pea leaves by elicitors from Mycosphaerella pinoides, M. melonis and M. lingulicola. Accumulation of pisatin increased after removal of epidermis and application of elicitors from germination fluid of the fungus.

A carbohydrate-rich extracellular component from a race of C. lindemuthianum showed a high level of phytoalexin activity on a resistant cultivar "Dark Red" of kidney bean but not on the susceptible cultivar "Great Northern". Other extracellular components were also recognised as elicitors by both cultivars. It is noteworthy that the two cultivars of Phaseolus vulgaris displayed a differential response to extracellular components. These observations support the hypothesis that both general and specific mechanisms exist in race-cultivar interaction (Tepper and Anderson, 1986).

Metabolites and viable cells of Pseudomonas corrugata from liquid culture medium elicited biosynthesis of the phytoalexin medicarpin in ladino white clover (Trifolium repens) leaflets and callus. The biologically active elicitor components were soluble in 80% ethanol. They were partially purified by removing components greater than 3,500 Da by dialysis and fractionating by preparative reversed-phase HPLC. None of the four fractions separated by HPLC elicited appreciable quantities of medicarpin in callus, but fraction 1 combined with fraction 4 elicited high concentrations of medicarpin. Any combination of fractions 2, 3 and 4 synergistically elicited medicarpin in callus. Elicitor activity was concentration-dependent. The active fractions were acidic in solution, but their elicitor activity was not dependent on low pH. Fraction 1 contained primarily uncharacterized reducing carbohydrate and phosphate. Fractions 2 and 3 were composed primarily of two related, unidentified fluorescent compounds, and fraction 4 contained another unidentified fluorescent compound (Gustine et al., 1990).

Fifteen isolates of Phytophthora parasitica, nine from tobacco (causing black shank disease) and six from other host plants were compared by root inoculation with regard to their pathogenicity to young tobacco plants. A progressive invasion of the aerial parts over 1 week was observed only with the black shank isolates, while the non-tobacco isolates induced leaf necrosis within 2 days. Similar necrosis occurred when the roots of tobacco plants were dipped in diluted culture filtrates from non-tobacco isolates, but not in those from tobacco isolates. The necrosis-inducing filtrates were shown to contain a c.10 k Da protein band which was not present in the other filtrates. This protein (named parasiticein) was purified by ion-exchange chromatography to homogeneity in SDS-PAGE and reverse phase HPLC. Parasiticein was serologically related to cryptogein, a member of the elicitin family of proteinaceous elicitors. Like the other elicitins, parasiticein induced necrosis in tobacco plants and

protected them against black shank. It most closely resembled little leaf necrosis. Ricci et al. (1992) suggested that the absence of parasiticein production by the black shank isolates might be a factor involved in their specific pathogenicity to tobacco.

The phytoplathogenic fungi Phytophthora subspecies elicit hypersensitive-like necrosis on their non-host tobacco (Nicotinia tabacum), with the exception of the tobacco pathogen Phytophthora nicotianae. In culture, these fungi except P. nicotianae secrete proteins, called elicitins, that cause these remote leaf necrosis and are responsible for the incompatible reaction. These proteins protect tobacco against invasion by the agent of the tobacco black shank, P. nicotianae, which is unable to produce such an elicitor. Cryptogein, secreted by Phytophthora cryptogea, has been purified, sequenced and characterized by Terce-Laforgue (1992) as an elicitin, a novel family of 10 k Da holoproteins. The secretion of cryptogein began later than its synthesis and stopped earlier, simultaneously with mycelium growth, when the nitrogen source in the culture medium was nearly exhausted. Electrophoretic patterns of total protein from mycelium extracts and N-terminal sequence analysis showed that cryptogein accumulated in the mycelium in its nature form. Cryptogein was synthesized as a preprotein.

The effects of an elicitor (CG-elicitor) from Colletotrichum graminicola was studied by Ransom et al. (1992). Roots of sorghum (Sorghum bicolor) accumulated 3-deoxyanthocyanidin phytoalexins in response to CG elicitor. Elicitation of the phytoalexin prior to treatment with the elicitor did not prevent infection and development of milo disease symptoms in susceptible seedlings inoculated with conidia of Periconia circinata. However, treatment of roots with the CG elicitor enhanced the synthesis of the 16 k Da proteins in both resistant and susceptible genotypes without expression of disease symptoms.

A glycoprotein elicitor of phytoalexin accumulation in leaves of Phaseolus vulgaris, produced well before lysis in the medium of cultures of Colletotrichum lindemuthianum race delta, has been purified to apparent homogeneity by Coleman et al. (1992). The glycoprotein was a monomer of M.W. 28 k Da with a pI of 4.25. The glycosyl side chains which accounted for 43% of the weight of the holoprotein, were composed principally of galactose, mannose and rhamnose exhibited a minimum degree of polymerization of 8 and were apparently O-linked to abundant serine and/or threonine residues of the peptide backbone. In a P. vulgaris leaf injection bioassay the purified glycoprotein had activity easily detectable at nanomolar concentrations and induced browning of the treated tissue and the accumulation of both phenylalanine ammonia-lyase and the isoflavonoid phytoalexin phaseollinisoflavan. For these three linked defence responses, suboptimal concentrations of the glycoprotein induced respectively 4.2, 7.6 and 9.7 fold more activity in the cultivar resistant to race delta (cv. Kievit) than in a cultivar susceptible to that race (cv. Pinto). Protein integrity was not required for elicitor activity and glycosyl side-chains isolated from the protein were shown to be active elicitor.

Cell walls of germ tubes from wheat stem rust (Puccinia graminis f. sp. tritici) contain a glycoprotein with a molecular mass of about 67 kD referred to as the Pgt elicitor. This glycoprotein induces a hypersensitive-like response in wheat leaves. In elicitor-active intercellular washing fluid (IWF) from compatible wheat-stem rust interactions, several elicitor-active glycoproteins were detected by Beissmann et al. (1992). One of these glycoproteins had an electrophoretic mobility identical to the Pgt elicitor. This IWF-glycoprotein exhibited elicitor activity upon elution from SDS gels. It was recognized by anti-Pgt elicitor antiserum suggesting partial structural identity between the Pgt and IWF elicitors. As with Pgt elicitor, the elicitor-activity of the IWF glycoprotein resides in the

carbohydrate moiety because periodate, but not trypsin or pronase, destroyed activity. These results suggest that the Pgt elicitor is released from hyphal cell walls into the wheat apoplast during stem rust infection.

Effects of the elicitor and the suppressor from a pea pathogen, Mycosphaerella pinodes, on polyphosphoinositide metabolism in pea plasma membranes were examined in vitro by Toyoda et al. (1992). Lipid phosphorylation in the isolated pea plasma membrane was drastically stimulated by the elicitor, but markedly inhibited by the suppressor. A similar inhibitory effect was observed by the treatment with orthovanadate or K-252a that blocked pisatin production induced by the elicitor. Neomycin, an aminoglycoside antibiotic that interacts with the polyphosphoinositide metabolism, also affected the lipid phosphorylation in vitro and blocked the elicitor-induced accumulation of pisatin in vivo. Rapid changes of polyphosphoinositide metabolism in pea plasma membranes is one of indispensable processes during the elicitation of defense responses.

The elicitor-induced incorporation of phenylpropanoid derivatives into the cell wall and the secretion of soluble coumarin derivatives (phytoalexins) by parsley (Petroselinum crispum L.) suspension cultures can be potentiated by pretreatment of the cultures with 2,6-dichloroisonicotinic acid or derivatives of salicylic acid. The cell walls and an extra-cellular soluble polymer were isolated by Kauss et al. (1993) from control cells or cells treated with an elicitor from Phytophthora megasperma f. sp. glycinea. After alkaline hydrolysis, both fractions from elicited cells showed a greatly increased content of 4-coumaric, ferulic, and 4-hydroxybenzoic acid, as well as 4-hydroxybenzaldehyde and vanillin. Two minor peaks were identified as tyrosol and methoxytyrosol. The pretreatment effect is most pronounced at a low elicitor concentration. Its specificity was elaborated for coumarin secretion. When the parsley suspension

cultures were preincubated for 1d. with 2,6-dichloroisonicotinic, 4- or 5-chlorosalicylic, or 3,5-dichlorosalicylic acid, the cells exhibited a greatly increased elicitor response. Pretreatment with isonicotinic, salicylic, acetylsalicylic, or 2,6-dihydroxybenzoic acid was less efficient in enhancing the response, and some other isomers were inactive. This increase in elicitor response was also observed for the above-mentioned monomeric phenolics, which were liberated from cell walls upon alkaline hydrolysis and for "lignin-like" cell wall polymers determined by the thioglycolic acid method. It was shown for 5-chlorosalicylic acid that conditioning most likely improves the signal transduction leading to the activation of genes encoding phenylalanine ammonia lyase and 4-coumarate : coenzyme A ligase. The conditioning thus sensitizes the parsley suspension cells to respond to lower elicitor concentration. If a similar mechanism were to apply to whole plants treated with 2,6-dichloroisonicotinic acid, a known inducer of systemic acquired resistance, one can hypothesize that fungal pathogens might be recognized more readily and effectively.

The elicitor molecules that function *in vivo* for phytoalexin elicitation in soybean (*Glycine max*) infected with *Phytophthora megasperma* f. sp. *glycinea* have been identified as β-1,6- and β-1,3-linked glucans that are released from fungal cell walls by β-1,3-endoglucanase (EC 3.2.1.39) contained in host tissues. Yoshikawa and Sugimoto (1993) identified the putative receptor-like target sites for the glucanase-released elicitor in soybean membranes. The binding was dependent on the pH of the incubation mixture, as well as on the duration and temperature of the incubation. The binding of the glucanase-released elicitor to membranes was abolished by both heat and proteolytic enzymes. Therefore, the binding site is probably composed of proteinaceous molecules.

Resistance or virulence are modelled by multiple biochemical components of two living organisms. *Costus speciosus*, a major

sapogenin bearing medicinal plant was severely affected by Drechslera rostrata causing leaf blight disease. An interesting interaction phenomenon was noticed by Kumar et al. (1995). The HPLC analysis indicated the accumulation of glyceollin II & III as potent phytoalexins by C. speciosus in response of non-pathogenic D. longirostrata. Further the presence of a polysaccharide elicitor, a mycelial wall component seems to be detrimental cause of phytoalexin accumulation. The same elicitor was also present in mycelial wall of pathogenic D. rostrata but in much lower concentration. Additionally it was associated with another polysaccharide component with different identity. The bioassay method of elicitor preparation was expressed in terms of antimicrobial activity mediated through glyceollins. It was determined 88.6% in incompatible which was considerably low (13.7%) in pathogenic reaction. During the pathogenesis of D. rostrata, the susceptibility was not only exercised with low concentration of elicitor but also being mediated with the association of additional carbohydrate component of mycelial wall. Hence expressing the involvement of multiple biochemical components to regulate susceptibility.

Several comprehensive reviews pertaining to elicitors of phytoalexins have also been published (Albersheim and Anderson-Prouty, 1975; Callow, 1977; Keen and Bruegger, 1977; Yoshikawa, 1983; Darvill and Albersheim,1984; Purkayastha, 1986; Yoshikawa et al., 1993; Smith et al., 1995; Yoshikawa, 1995; Paxton, 1995).

Potential Use of Phylloplane Microorganisms for Biocontrol of Plant Diseases

The surfaces of aerial plant parts provide a habitat for epiphytic microorganisms, many of which are capable of influencing the growth of pathogens. These saprophytic organisms play an important part in reducing incidence of foliar diseases on crops in the field (Blakeman and Fokkema,1982). Todate, biological control is more

successful in the rhizosphere than in the phyllosphere. Introduced microbes can be used successfully in various circumstances. The first case is where the habitat is conducive to their growth, a situation comparable to rare establishment of an invading microorganism in a community. The second situation is where growth of the foreign organism is not necessary for biocontrol activity (Andrews, 1990).

Colonization by an antagonist is general requirement for biological control, and thus organisms that grow well on the phylloplane are usually better candidates than those do not. For this reason, efforts typically have been made to promote the activity of naturally occurring or applied populations of resident bacteria (Morris and Rouse, 1985) or yeasts (Fokkema et al., 1979). The role of filamentous fungi as biocontrol agent appears to be limited largely to disease where the pathogen is an opportunistic invader of senescent or dead leaf and floral tissue because the substrates are also colonized by filamentous fungi. For example, some success has been achieved with Epicoccum purpurascens in control of white mold of bean caused by Sclerotinia sclerotiorum (Boland and Inglis, 1989; Zhou and Reeleder, 1989, 1991; Inglis and Boland,1990, 1992; Peng and Sutton, 1991).

The phyllopiane is a dynamic environment with cyclic or non-cyclic environmental variables, including temperature, relative humidity, dew, rain, wind and radiation. Moreover, substantial variation can occur in time and space even on a scale appropriate to microorganisms. Nutrients on the phylloplane originate endogenously or exogenously and include diverse carbohydrates, amino acids, organic acids, sugar alcohols, mineral trace elements, vitamins, and hormones, as well as antimicrobial compounds such as phenols and terpenoids (Blakeman and Atkinson, 1981; Gaber and Hutchinson, 1988; Fiala et al., 1990). Nutrients are important not only because of their direct role as microbial substrates, but also because of their likely

indirect effects (Williams, 1982; James and Gutterson, 1986; Weller and Thomashow, 1990) on synthesis of antibiotics and siderophores on the phylloplane. Much of the nutrient on the phylloplane originate from sources other than the plant; among them are soil particles, dust ions and solutes in rainwater, dead microorganisms and bird and insect excrement.

Exogenous nutrients may stimulate germination, mycelial growth or appressorium formation on the surfaces of leaves, as well as stimulating aggressive basin development in necrotrophic pathogens such as B. cinerea (Clark and Lorbeer, 1977). Nutrients on leaves are primarily derived from leaf tissues as a result of cellular leakage but can be supplemented, usually later in the growing season, by pollen and aphid honeydew deposits. There are numerous reports that pollen grains stimulate infection of, for example, flowers and fruits by B. cinerea and cereal ears and leaves by Fusarium graminearum, C. sativus and Septoria nodorum (Fokkema, 1981).

The chemicals diffusing from inside the leaf on its surface provide nutrition to phylloplane microflora. The activity of both, saprophytes and pathogens in the phylloplane is dependent on the microclimatological conditions as well as the chemical environment on the leaf surface (Blakeman, 1973; Mukherji and Subba Rao, 1982). The leaf surface is a highly dynamic and heterogeneous habitat. Under normal conditions microbial populations in this habitat are held in a dynamic balance by interactions between microorganisms themselves as well as between them and the host plant. The balance between them and the pathogen is maintained perhaps by antagonism or competition or both and some other factors not yet fully known. On the leaf surface, therefore, any change in the populations and activities of these microorganisms should influence disease development.

The effect of extraneous nutrients on pathogens can only be demonstrated on plants with a poorly developed phylloplane microflora, such as freshly-expanded flowers and leaves or leaves of greenhouse-grown plants. On greenhouse-grown rye added cells of Cladosporium spp., A. pullulans, Cryptococcus laurentii and Sporobolomyces roseus could strongly reduce the "pollen effect" of infection by C. sativus (Fokkema, 1973). Where pollen grains are shed within a few days (e.g. rye) the saprophytic population of field-grown plants may not be able to reduce nutrient levels fast enough to prevent infection, e.g., by C. sativus (Fokkema et al., 1975).

Bacteria, yeasts and filamentous fungi may form resident populations on leaves. Of the bacteria most are Gram-negative, often chromogenic, and include the following genera : Erwinia, Pseudomonas, Xanthomonas and Flavobacterium; Gram-positive bacteria such as Lactobacillus, Bacillus and Corynebacterium are isolated less frequently. In addition to saprophytic bacteria, pathogenic bacteria, e.g. Pseudomonas syringae pr. syringae, P. syringae pr. morsprunorum, P. syringae pr. glycinae, Erwinia amylovora and E. carotovora can live in a non-pathogenic epiphytic phase of foliar surfaces (Blakeman and Brodie, 1976).

Reduction of incidence of fire blight in a pear orchard was achieved by repeated spray applications during blossom time with a mixture of cells of three saprophytic pseudomonads and an Erwinia sp. that was a pathogen of sugar beet (Thomson et al., 1976). The latter produced a bacteriocin that killed E. amylovora cells in vitro. The pseudomonads were obtained by spraying aqueous dilutions of orchard floor soil onto pear flowers, isolating bacteria from the flowers on agar media, and selecting those organisms which were present in the highest numbers. Using a mixture of these organisms, almost as good control of fire blight was obtained as when using commercial bactericides.

Mishra and Tewari (1976) studied interactions between a number of phylloplane fungi of wheat and Puccinia graminis tritici. It was observed that several saprophytes, particularly Penicillium notatum, Myrothecium roridum, Cladosporium herbarum and Nigrospora sphaeria caused marked reduction in uredospore germination in vitro, and in number of uredosori on leaves in glasshouse. Spore suspensions and metabolites of these fungi were shown inhibitory to rust fungus.

McKenzie and Hudson (1976) studied interactions between different rust and saprophytic leaf inhibiting fungi. The spores of Alternaria alternata, Botrytis cinerea, Cladosporium cladosporioides, Epicoccum purpurascens and Trichothecium roseum often depressed the germination of uredospores. The effect of B. cinerea was most distinct. The rust species studied were Puccinia graminis tritici (on wheat), Melampsora larici-populina (on polar) and Tranzschelia pruni-spinosae (on plum).

Austin et al. (1977) showed that a number of phylloplane bacteria, particularly Pseudomonas fluorescens, isolated from leaves of Lolium perenne were antagonistic to the pathogen Drechslera dictyoides. The antagonists reduced spore germination and germ tube growth and caused lysis of hyphae, which led to reduction in lesion development.

Doherty and Preece (1978) observed that Bacillus cereus was associated with uredospores of Puccinia allii (on leek) as an antagonist. The bacterium completely inhibited uredospore germination on agar. Spray of bacteria suspension on leaves reduced the disease under the controlled environmental conditions. Reducing the number of bacteria in suspension reduced the extent of disease control and at 10^7 cell/ml., no detectable control was observed.

Omar and Heather (1979) investigated the effect of three

commonly occurring phylloplane fungi, Cladosporium sp., Alternaria sp. and Penicillium sp. on germination of uredospores as well as uredosori development of Melampsora larici-populina on leaf disks. Germination of uredospores and development uredosori on leaf disks were reduced by these three fungi to varying degrees.

Strains of P. syringae and E. herbicola on potato leaves, known to induce ice nucleation and consequently to increase frost damage, could be inhibited both in vitro and in vivo by compounds produced by antagonistic fluorescent pseudomonads and an E. herbicola isolate (Lindow, 1979). Application of these antagonistic bacteria to potato plants in the field was found to be as effective at reducing frost damage as using commercial bactericides or ice nucleation inhibitors.

Variation in density of epiphytic yeasts, filamentous fungi and bacteria on apple leaves collected from eight trees at nine dates for two seasons was determined by Andrews et al. (1980) with respect to three positional factors : height, compass direction from the center of the tree, and lateral proximity to the canopy periphery. Univariate analysis of variance were performed on each of the microbial classes for each date according to a model that excluded tree effect but accounted for the positional factors with interactions. The assumption of no tree effect was explored by residual analysis and examination of the seasonal pattern of microbial densities for each tree. No persuasive evidence was obtained to invalidate this assumption. For filamentous fungi and yeasts, height and lateral position were the most significant factors with $P<0.05$ for yeast at several periods. The two factors appeared to be equal importance. Trends were less clear for bacteria, but all three positional factors and some two-way interactions seemed of some importance. For filamentous fungi and bacteria, frequently no factors were significant at a level of 0.10, but at almost all sampling dates certain positional factors and interactions were significant at a

level of 0.25. Inspection of partial correlation coefficient indicated no apparent linear association between densities of most pairs of microbial classes.

Microorganisms on the surface of Citrus unshium were isolated by Ushiyama (1980). Mycelial growth of Diaporthe citri causing citrus melanose disease was inhibited by 4 of 31 isolates of bacteria and by 36 of 66 isolates of fungi belonging to 12 genera.

Spurr Jr. (1981) considered the role of microbial antagonists in control of foliar plant pathogens. Most examples included phylloplane fungi and bacteria antagonistic to pathogenic Alternaria spp., mainly A. alternata on tobacco leaves. Sprays of Bacillus mycoides, B. thuringiensis and Pseudomonas cepacia (grown in nutrient dextrose broth) were done on leaves of tobacco and pea nut. Field tests were successful in controlling Alternaria leaf spot of tobacco and pea nut cercospora leaf spot.

Kranz (1981) presented a comprehensive account of hyper parasites of biotrophic fungi with special reference to biological control of rusts and powdery mildews. A detailed account of three hyperparasites of rusts, namely Darluca filem, Tuberculina vinosa and Verticillium lecanii was presented including their host range, spatial patterns and incidence in nature, specialisation, modes of infection and entry, post-entry interactions between them and parasites, their effect on parasites, their incubation and latent periods, sporulation and inoculum levels, factors affecting infection, pathogenesis and sporulation, spread and survival, and their potential for biological control.

Parameters affecting production of secondary metabolites will also modify antibiosis in vitro. Since the production of antibiotics is greatly affected by environmental factors, particularly nutrition,

it is not surprising that the assay conditions affect results of antibiosis *in vitro*. The amount of antagonism between certain *Streptomyces* spp. and *R. solani* varied with the assay medium used (Rothrock and Gottleib,1981).

Purkayastha and Bhattacharyya (1982) isolated microorganisms from jute phyllosphere and screened against anthracnose fungus *Colletotrichum corchori*. *Aspergillus nidulans* and *Penicillium oxalicum* showed antagonistic reaction and inhibition was due to a marked change in the pH of these culture filtrates. Among the bacterial isolates, *Bacillus megaterium* completely inhibited *C. corchori*. *Rhizopus stolonifer*, *Aspergillus* spp. and *Alternaria* sp. showed some tolerance to *B. megaterium* B-23. Disease severity was reduced markedly when foliar application of suspension or the culture filtrate of *B. megaterium* was done 24 h prior cell inoculation with *C. corchori*.

Coincidental production of antibiotics *in vitro* and biocontrol ability *in vivo* are not necessarily causally related. Utkhede and Rahe (1983) demonstrated that the isolates of *Bacillus subtilis* inhibited *S. cepivorum in vitro*, suppressed the incidence of onion white rot in the field, and increased onion emergence and yield. They also pointed out that possibly the antibiotic produced by the isolates inhibited the pathogen in the field.

Interestingly, the ascospores of *Chaetomium* do not germinate to any extent on the apple phylloplane, either in nature or under optimal conditions in a growth chamber. The mechanism for control is evidently the water-soluble antibiotic chetomin, which diffuses from the ascospores onto the leaf surface where it inhibits the germination of ascospores or conidia of *Venturia inaequalis* (Cullen and Andrews, 1984). Studies under controlled conditions showed that efficacy is lost if *Chaetomium* is applied more than 3 days after deposition of *Venturia inoculum* (Bourdreau and Andrews, 1987). Variability of

control in nature was attributed to the timing of application of Chaetomium relative to periods of scab infection.

The fungal colonists of the phylloplane and internal tissue of leaves of guava (Psidium guajava) were studied from bud stage to senescent stage, in summer, rainy and winter seasons by Pandey and Dwivedi (1984). The number of composite phylloplane fungi was maximum in rainy and minimum in summer season. Fungi were categorized into three groups : exclusive season fungi, season-sensitive-fungi and season-insensitive fungi. Pestalotia psidii, Fusarium oxysporum f. psidii and Colletotrichum gloeosporioides were isolated from the foliage in different seasons with different grades of dominance. P. psidii was prevalent in all seasons, but the population dynamics was high in winter. C. gloeosporioides and F. oxysporum f. psidii were recorded more frequently in the rainy season. The number of phylloplane mycoflora increased with leaf age.

Three bacterial antagonists Xanthomonas campestris pr. oryzae (Y13), Bacillus subtilis WB and AN 771, inhibited the conidial germination and growth of Helminthosporium oryzae, H. nodulosum, H. sativum, Botryodiplodia theobromae, Pestalotia sp. and Curvularia sp. They also inhibited the growth of Aspergillus niger, A. flavus, Fusarium oxysporum, Fusarium sp. and Colletotrichum sp. The detached groundnut, rice and ragi leaves and cashew twigs, pretreated with the living cells of bacterial antagonists, showed much reduction in disease symptom (Narain and Mohanty, 1983). Sharma (1985) demonstrated the antagonistic ability of some phylloplane fungi against sunnhemp rust (Uromyces decoratus) and triticale leaf rust (Puccinia recondita tritici). Forty four species of fungi and an actinomycete, isolated from phylloplane of sunnhemp (Crotalaria juncea) were tested in vitro against the rust fungus (Uromyces decoratus). Supernatants of spore suspension of all test microorganisms inhibited germination of uredospores to varying extent. Maximum inhibition was caused by

Myrothecium gramineum followed by Chaetomium globosum, actinomycete, Trichoderma harzianum, Fusarium oxysporum, Neocosmospora vasinfecta, Colletotrichum curvatum and Trichothecium roseum.

The pollen mycoflora analysed by Rao and Manoharachary (1985) of fifty plants yielded twenty four fungal species representing the following genera : Alternaria, Aspergillus, Aureobasidium, Cladosporium, Curvularia, Drechslera, Fusarium, Paecilomyces, Penicillium, Pestalotiopsis, Phoma, Rhizopus and Trichoderma. Autoclaved culture filtrates of B. subtilis also suppressed infection of soybean stems by Phomopsis sp. in the field, but not in the greenhouse or growth chamber (Cubeta et al., 1985). The above treatment also controlled significantly the bean rust in the field (Baker et al., 1985). An antibiotic like compound from B. subtilis retarded growth of R. solani on rice-leaf segments and suppressed development of sheath blight (Tschen and Kuo, 1985). Antibiotic substances produced by a strain of Bacillus subtilis previously found to suppress the development of brown rot of stone fruit (Pusey and Wilson, 1984) were soluble in ethanol, methanol, isopropanol and water above pH 7.5; but not soluble in ethyl acetate, acetone, ether or methylene chloride and were found to be biologically active against a wide range of plant pathogenic fungi. When tested for its activity against Monilina fructicola on peach fruit complete suppression of brown rot was evident (McKeen et al., 1986).

Trichoderma spp. produce both non-volatile and volatile antibiotics. Two main antibiotic substances have been identified : "trichodermin", a sesquiterpene antibiotic active against fungi, and various peptide antibiotics, active against fungi and bacteria. Acetaldehyde was believed to be one of the volatile antibiotics produced. The soil fungus Trichoderma has frequently been used for biocontrol in aerial environment (Tronsmo, 1986; Dubos, 1987).

Brown et al. (1987) pointed out that amino acids are required as precursors for the production of epicorazines by Epicoccum purpurascens. A non-siderophore-producing mutant of Pseudomonas putida had no effect on germination of chlamydospores of Fusarium oxysporum f. sp. cucumerinum, while the parental strain significantly suppressed chlamydospore germination (Simeoni et al., 1987).

Among the microorganisms isolated from treated and untreated jute leaves, bacterial population was always highest followed by fungi and actinomycetes. Of these Bacillus megaterium was found to be most antagonistic to Colletotrichum corchori, the causal agent of anthracnose disease (Bhattacharyya and Purkayastha, 1988).

Four different methods were used by Omar et al. (1989) for estimating population of microorganism in the phylloplane of faba bean. The number and type of microflora detected differed depending on the method used. The highest number of leaf microorganisms was obtained using the leaf extract technique. The microorganism population changed during the course of the experiment, the number increased from the second sampling onwards, as the crop progressed from the vegetative stage to the reproductive stage. In vitro studies revealed the antagonistic effect of the isolated phylloplane microorganisms on Botrytis fabae.

Rytter et al. (1989) isolated twelve strains of Bacillus from the leaves of geranium cultivars and tested for their effect on spore germination of Puccinia pelargonii-zonalis, the causal agent of geranium rust. Of these, strain 3 of B. subtilis, isolated from a rust-infected geranium leaf, inhibited spore germination as well as reduced the incidence of rust pustules on inoculated leaves in the greenhouse. The inhibitory substance was present in the culture filtrate of the above strain of B. subtilis which was most inhibitory in decreasing the amount of pustules per leaf area. Washed bacterial

cell treatment also decreased the disease incidence. Cells cultured and applied to leaves in nutrient broth were more effective in reducing rust development compared with a culture filtrate. When bacteria were applied for different periods before inoculation with rust spores, the antagonistic effect persisted for at least 4 days after application.

Erwinia herbicola B247 suppressed Puccinia recondita f. sp. tritici on wheat leaves. Nearly complete protection was attained by application of culture filtrate from the wild type bacterium. Seed treatment with this strain also resulted in about 90% disease suppression in the green house of wheat seedlings caused by Fusarium culmorum which was detected by enzyme-linked immunosorbent assay (Kempf and Wolf, 1989).

Roitman et al. (1990) isolated a chlorinated phenylpyrrole derivatives from a strain of Pseudomonas cepacia collected from apple leaves to detect agents for biological control of fruit spoilage fungi. In vitro testing showed that all four of the phenylpyrroles had antifungal activity toward several fruit pathogens. The new phenylpyrrole showed fungal inhibitory effects on Golden Delicious apple inoculated with conidia of pathogenic organisms. Thomashow et al. (1990) demonstrated the antibiotic (Phenazine-carboxylic acid) produced by Pseudomonas fluorescens 2-79 and P. aureofaciens 30-84 suppressed the take-all disease of wheat caused by Gaeumannomyces graminis var. tritici. Pseudomonas fluorescens strain Hv37a genes for biosynthesis of the relevant antibiotic, oomycin A, have been cloned and the regulation of antibiotic biosynthesis has been altered by Gutterson et al. (1990).

Isolates of Erwinia herbicola, obtained from flowers and leaves of hawthorn (Crataegus monogyna), were screened as potential

control agents of fire blight disease (caused by Erwinia amylovora) by Wilson et al. (1990).Selected isolates were subsequently tested for disease control by infection of hawthorn blossom in the laboratory, and by shoot infection of hawthorn plants grown under controlled (glass house) and fluctuating (polythene tunnel) environmental conditions. Although the immature pear fruit assay provided a general screen for the selection of antagonists for the control of both blossom and shoot blight, it had two major limiations when quantitatively applied. Firstly there were inconsistencies in the relative effects of different isolates on the pear-slice surface, with some isolates being more suppressive than the standard antagonist Eh252 in the first screening and less in the second. Secondly, the assay was not able to predict accurately the level of control in the intact plant - as no correlation occurred between the level of control in the pear fruit assay and the percentage control of either blossom blight or shoot blight. Two isolates of E. herbicola, WL9 and WL40, reduced both blossom - and shoot-blight. WL9 provided over 80% control of blossom blight, equivalent to that provided by chemical agents, and also gave total control of shoot blight when applied at WL9 : pathogen ratio of 10:1.

Handelsman et al. (1990) explored the potential of biological control of alfalfa (Medicago sativa L.) seedling damping-off caused by Phytophthora megasperma f. sp. medicaginis by screening root-associated bacteria for disease suppression activity in a laboratory bioassay. A total of 700 bacterial strains were isolated from the roots of field-grown alfalfa plants by using Trypticase soy agar. A simple, rapid assay was developed to screen the bacteria for the ability to reduce the mortality of Iroquois alfalfa seedlings that were inoculated with P. megasperma f. sp. medicaginis zoospores. Two-day-old seedlings were planted in culture tubes containing moist vermiculite, and each tube was inoculated with a different bacterial culture. Sufficient P. megasperma f. sp. medicaginis zoospores were

added to each tube to result in 100% mortality of control seedlings. Of the 700 bacterial isolates tested, only 1, which was identified as Bacillus cereus and designated UW85, reduced seedling mortality to 0% in the initial screen and in two secondary screens. Both fully sporulated cultures containing predominantly released spores and sterile filtrates of these cultures of UW85 were effective in protecting seedlings from damping-off; filtrates of cultures containing predominantly vegetative cells of endospores inside the parent cell had low biocontrol activity. In a small-scale trial in a field infested with P. megasperma f. sp. medicaginis, coating seeds with UW85 significantly increased the emergence of alfalfa. The result suggest that UW85 may have potential as a biocontrol agent for alfalfa damping-off.

The culture supernatant of Bacillus sp. showed growth inhibition against fungi. Isolation of this bacteria remarkably inhibited the growth of Fusarium sp. on potato-glucose-agar medium. The supernatants of 30-fold dilution also retained the antifungal activity againstPyricularia oryzae and Helminthosporium oryzae respectively (Tsuji et al., 1990b). When culture supernatent was heated at 121°C for 15 min the antifungal activity was retained. The crude powder having an antifungal activity was obtained by both acidification and ammonium sulfate fraction of the culture supernatant. The purified powder also showed a strong antifungal activity to Helminthosporium oryzae and Pyricularia oryzae (Tsuji et al., 1990a).

An isolate of Bacillus subtilis inhibited in vitro growth of Eutypa lata, the causal organism of dieback in grapevines. The bacterium caused 91.4% inhibition of mycelial growth of E. lata and 100% inhibition of ascospore germination. Ascospore germination correlated negatively with time of exposure to B. subtilis.

Malformation of hyphae of E. lata occurred in the presence of B. subtilis. An antibiotic substance in ethanol extract from B. subtilis totally inhibited germination of ascospores of E. lata. Thin layer chromatography of crude antibiotic extract showed five bands, two of which inhibited mycelial growth of E. lata. Spraying a suspension of the bacterium on pruning wounds before inoculation with ascospores of E. lata significantly reduced infection as compared with the unsprayed, inoculated controls (Ferreira et al., 1991). Hassanein and Elgoorani (1991) also detected five isolates of Bacillus subtilis to be antagonistic to 6 isolates of Agrobacterium tumefaciens in vitro. Inoculation of E. subtilis in wounded castor bean plants 30 min. before or simultaneous inoculation with A. tumefaciens resulted in excellent control of crown gall symptoms on the host within 50 days of inoculation. Application of B. subtilis 30 min. after inoculation with A. tumefaciens did not result in appreciable disease reduction. Treatment of the tested plants by B. subtilis did not induce any phytotoxic injury or growth retarding side effect.

Post-harvest peaches as well as post-harvest apples and grapes when coated with Bacillus subtilis B_3, inhibited growth of the pathogens causing brown rot, grey mold and bitter rot respectively (Pusey, 1991). The microbial load on leaves of seven varieties of rice at tillering and boot leaf stages was studied by Viswanathan and Narayanasamy (1991). The population of bacteria was higher than that of fungi and actinomycetes. Of the twelve fungal and four bacterial isolates, Bipolaris zeicola isolated from IR20 rice variety inhibited under in vitro conditions mycelial growth of Sarocladium oryzae Sawada causing sheath root of rice.

Antagonistic effect of 187 isolates of Coryneform bacteria from red smear cheese were screened against Listeria species. Culture filtrates from Brevibacterium linens, Arthrobacter nicotianae and A. nucleogenes showed clear zones of inhibition. A. nicotianae and A.

nucleogenes were more effective against Disteria innocua and L. ivanovii than against L. monocytogenes. No species specificity was observed for B. linens, but there was a difference regarding the inhibitory activity of individual culture filtrates. The culture filtrates of the antagonists lost their inhibitory activity upon heating (Valdes et al., 1991).

The antifungal compound pyrrolnitrin, isolated from Pseudomonas cepacia, an antagonist known to control grey mold (incited by Botrytis cinerea) and blue mold (incited by Penicillium expansum) of apples and pears, was assayed by Janisiewicz et al. (1991) for its efficacy in controlling these diseases on wounded fruit at two temperatures. The compound was applied to wounded fruit after harvest at concentrations ranging from 6 to 200 µg/ml in dip solutions containing conidia of P. expansum and B. cinerea (1×10^4 conidia/ml). Pyrrolnitrin provided effective control of both diseases on apples and pears. The type of wound had a profound effect on control; infections at cut wounds were the easiest and those at "bruise" wounds were the most difficult to control. Higher concentrations of pyrrolnitrin were required for control at 24°C than at 2°C. Pyrrolnitrin at 200 µg/ml, when applied up to 34 h after inoculation, eradicated infections by both pathogens on Golden delicious apples with cut wounds. The pH of the pyrrolnitrin solution, throughout a range corresponding to the pH of the fruit juice, did not change its fungicidal activity in in vitro tests.

In an attempt Vanneste et al. (1992) sprayed Erwinia herbicola (Eh252) into apple blossoms before inoculation with E. amylovora which reduced incidence of fire blight disease. The strain (Eh252) produced an antibiotic on minimal medium that inhibited the growth of E. amylovora. This antibiotic was inactivated by histidine but not by Fe(II), was sensitive to proteolytic enzymes, and showed a narrow host range of activity. To determine the role of this antibiotic in the control of fire blight, two prototrophic Tn5-induced mutants, 10:12 and 17:12, that had lost their ability to inhibit E. amylovora on

plates (Ant-mutants) were compared with the wild-type strain for their ability to suppress fire blight in immature pear fruits. The two mutants had single Tn5 insertions in the chromosome, although they grew in immature pear fruits at a rate similar to that of the wild-type strain, neither of these mutants suppressed fire blight as well as Eh252 did. The Tn5-containing fragment isolated from 10:12 was used to mutagenize Eh252 by marker exchange. Derivatives that acquired the Tn5-containing fragment by homologous recombination lost their ability to inhibit E. amylovora on minimal medium. Furthermore, the three Ant-derivatives tested were also affected in their ability to inhibit E. amylovora in immature pear fruits. The results obtained suggests that antibiotic production is a determinant of the biological control of E. amylovora by E. herbicola (Eh252).

Pseudomonas fluorescens (strain CHAO) suppressed a number of plant diseases including Thielaviopsis basicola which induce black root rot of tobacco (Laville et al., 1992). Out of several antibiotic metabolites produced by this strain only two have been identified as pyoluteorin (Plt) and 2,4-diacetylphloroglucinol (Phl) by Maurhofer et al. (1992).

Antagonistic activities of Bacillus subtilis (NB22) were examined in vitro by Phae et al. (1992), which strongly suppressed the growth of 19 plant pathogenic fungi and 8 plant pathogenic bacteria. Its application to the biological control of crown and root rot and bacterial wilt of tomato caused by Fusarium oxysporum f. sp. radicislycopersici (FoR) and Pseudomonas solanacearum (Ps), respectively, was carried out in the field. When rice straw was immersed in cultural suspension of NB22 and then mixed into soils infested with FoR, the occurrence of crown and root rot of tomato was significantly reduced, and when combined with steam sterilization the suppressive effect was conspicuously enhanced and three to four times greater amount of yield was obtained compared with unsterilized control. Occurrence of bacterial wilt of tomato was also suppressed

remarkably when the culture filtrate of NB22 was poured into heavily infested soils.

The influence and mechanisms of action of Bacillus subtilis on Colletotrichum trifolii, a causal agent of anthracnose of alfalfa (Medicago sativa) were studied in vitro and in vivo by Douville and Boland (1992). In growth room conditions, B. subtilis significantly reduced disease incidence and severity on alfalfa seedlings from 56% to 16% and from 2.0 to 1.2 respectively. Treatment of seedlings with washed cell suspensions of B. subtilis had no influence on disease. Applications of crude filtrate on alfalfa leaflets inoculated with C. trifolii were associated with reduced germination of conidia, lysis of conidia and reduced formation of appressoria. Under in vitro conditions, crude filtrate reduced germination of conidia and induced lysis of conidia and the formation of inflated germ tubes on germinating conidia. An antibiotic of the iturin family, iturin D, was tentatively identified as the active compound responsible for the suppressive effect of B. subtilis on C. trifolii.

Several species of Streptomyces were evaluated by Hodges et al. (1992) for their ability to control dollar spot caused by Sclerotinia homoeocarpa and leaf spot caused by Bipolaris sorakiniana (leaf spot) on the phylloplane of Poa pratensis. Species evaluated included S. diastaticus (S32), S. galbus (S35) and S. hygroscopicus (S28, S13). Isolate S28 of S. hygroscopicus showed erratic antagonism of both pathogens, depending upon how the isolate was prepared for use. Streptomyces diastaticus and S. galbus were antagonistic to S. homoeocarpa only in whole culture form.

Da Silva and Pascholati (1992) studied the protection of maize leaves against Colletotrichum graminicola, the causal agent of anthracnose, by previous or simultaneous applications of suspensions or filtrates of Saccharomyces cerevisiae. The parameters evaluated

involved conidial germination and appressoria formation by C. graminicola, lesioned leaf area, number of lesions, and sporulation capacity of the fungus on the leaves. Suspensions from washed or non-washed S. cerevisiae cells and filtrates of these suspensions reduced the development of C. graminicola as well as the expression of anthracnose on leaves when they were previously or concomitantly treated with these preparations. In vitro experiments showed that S. cerevisiae cells exhibit a possible antagonistic activity against C. graminicola due to antibiosis.

Characterization and medium suitability of extracellular amino acid producing strains of Micrococcus luteus and Micrococcus roseus have been described by Sen et al. (1993). At the onset, 400 microorganisms have been isolated from 125 soil samples. They were grown in liquid medium following which cell free extracts have been collected by centrifugation and their extracellular amino acid producing potentially were tested by paper chromatography using ninhydrin. Growth and amino acid yield of the isolates in varying media have also been compared.

Azevedo et al. (1993) isolated a new strain of Bacillus subtilis C126 from sugarcane fermentation which produced an antibiotic that inhibited the growth of Micrococcus flavus. The antibiotic produced by B. subtilis in the culture medium was extracted with n-butanol. Polypeptide nature of this compound was confirmed by thin layer chromatography and microbiological test. Further analysis by column chromatography and HPLC confirmed the product as bacitracin complex.

A strain of Bacillus subtilis which produces an antibiotic metabolite was also found to produce a volatile compound(s) which was antifungal to Rhizoctonia solani and Pythium ultimum. Growth of the

fungi were severely impaired in the presence of the volatiles and physiological abnormalities of the hyphae were observed, including hyphal distortion and vacuolation. A range of media were tested for volatile production and potato dextrose agar (PDA) was found to be the most active. Temperature had a considerable effect on antifungal volatile activity with the greatest inhibition occurring at 30°C. Addition of $FeCl_3$ to Sabouraud's glucose agar (SGA) also enhanced the antifungal effect. The volatiles were found to be water soluble and remained active when trapped in SGA (Fiddman and Rossall, 1993).

Bacillus cereus SM3 was isolated by Maloney et al. (1993) on a mineral salts medium with Tween 80 as the primary carbon source which was able to produce non-insecticidal products. The enzyme responsible for the hydrolytic reaction was named permethrinase. Permethrinase was purified by ion-exchange chromatography and gel filtration chromatography. The molecular mass of native permethrinase was 61 ± 3 kDa, as estimated by Sephadex G-100 gel filtration, optimum pH and temperature being 7.5 and 37°C respectively. No cofactors or coenzymes were required for permethrinase activity.

Two strains of Pseudomonas cepacia (RJ3 and ATCC 52796) have been identified by Jayaswal et al. (1993) as potential antagonist of fungal pathogens. Although both strains displayed high levels of antagonism, ATCC 52796 was slightly more antagonistic than RJ3. The antifungal compound was isolated from RJ3 and after purification by HPLC and characterization by UV, IR, NMR and mass spectroscopy was identified as pyrrolnitrin.

The effect of different bacterial strains from rice fields in the tropics on rice seed germination and on radicle and hypocotyl development of four rice cultivars was determined by Rosales et al. (1993). There was a varietal difference in response to seed bacterization with the different bacterial strains. Germination of cv.

IR58 increased from 78 to 93%, that of cv. IR64, from 89 to 97%. Less effects on germination of cvs. IR42 and IR36 were observed. All strains inhibited the mycelial growth of Rhizoctonia solani in vitro. The three strains, identified as Bacillus subtilis, inhibited the mycelial growth of eight fungal pathogens whereas the other strains were pathogen specific. Seed bacterization with these bacterial strains provided a sheath blight protection of 4.5 to 73% in the glass house trial. Bacterial strains were identified as Bacillus subtilis, Bacillus laterosporus, Bacillus pumilus, Pseudomonas aeruginosa, Erwinia herbicola - like and Serratia marcescens.

Biocontrol of Botrytis cinerea in strawberry leaves were achieved by the application of Gliocladium roseum, Trichoderma viride and Penicillium sp. (Sutton and Peng, 1993). In the greenhouse the antagonists suppressed the number of conidiophore of B. cinerea by 97-100% in attached leaves, while in field plots they suppressed sporulation incidence of the pathogen by 58, 48 and 64% respectively, in semisenescent over wintered leaves, but 81-100, 53-87 and 59-100% respectively, in green leaves. Biosuppression generally increased as temperature increased from 10 to 25°C, but G. roseum was highly suppressive at 10 and 15°C. Germination rate of conidia and growth of germ tubes of each antagonist on the leaves increased with temperature.

Two mycoparasites, Pythium oligandrum and Coniothyrium minitans have been tested by Whipps et al. (1993) for the control of damping off in cress and sugar-beet caused by Pythium ultimum and Aphanomyces cochlioides respectively in glass house pot trials; pre-planting application of a solid substrate preparation of C. minitans gave reproducible control of sclerotinia disease in the glass house. At higher disease levels biocontrol was lost. C. minitans survived in the soil for over one year and continued to degrade and reduce apothecial production.

The antagonism of five bacterial isolates (Acinetobacter sp., Bacillus polymyxa, Bacillus subtilis, Pseudomonas cepacia and Pseudomonas putida) against Sclerotinia sclerotiorum, Sclerotinia minor, Gaeumannomyces graminis and other fungi was reported by Oedjijono et al. (1993). They showed that in each case, the mode of antagonism appeared to result from antibiotic rather than siderophore production. Partial isolation and characterization of the two antibiotics produced by one of the bacteria (P. cepacia) indicated that they were different from any previously reported pseudomonads.

Pseudomonas cepacia decreased the incidence of diseases caused by Rhizoctonia solani, Sclerotium rolfsii and Pythium ultimum by 85, 48 and 71% respectively under green house conditions. An active and stable β -1, 3-glucanase was detected from P. cepacia. The optimal temperature and pH values for its activity were 60°C and 5.0, respectively. The induction of β -1,3-glucanase by different fungal cell walls as sole carbon source in synthetic medium was correlated with the biocontrol of the respective fungi by P. cepacia. The damage caused to R. solani hyphae was observed under light and electron microscopes (Fridlender et al., 1993). P. cepacia and P. fluorescens also decreased root rot of chickpea caused by Phytophthora megasperma f. sp. medicaginis as described by Myatt et al. (1993).

Fluorescent pseudomonads isolated from cucumber and bean roots, 1107 and 648 in number respectively, were tested against Pythium aphanidermatum and Rhizoctonia solani by Wölk and Sarkar (1993). No antagonistic effect against P. aphanidermatum could be detected by 934 strains of Pseudomonas isolated from cucumber and 549 strains isolated from bean against R. solani.

Pseudomonas fluorescens (A506) and Erwinia herbicola (strain C9-1) established epiphytic populations on pear blossoms which were found to be effective antagonists for the biological control of fire

blight (Johnson et al., 1993). Wilson and Lindow (1993) also reported that in the green house, P. fluorescens (strain A506) effectively colonized the pistils of pear blossoms and significantly reduced colonization of pear nectaries by Erwinia amylovora. Although the inhibition of E. amylovora on pear nectaries may involve preemptive utilization of a growth-limiting resource, as well as other factors, such as induced cessation of nectar secretion or accumulation of a host toxin. P. fluorescens A506 probably prevents fire blight infection of pear in the field by preventing epiphytic build-up of pathogen inoculum on pistils and by inhibiting the growth of inoculum deposited on nectaries.

Screening for potential antagonists of Pseudocercosporella herpotrichoides, the causal agent of eye spot disease of cereals were reported by Clarkson and Lucas (1993a,b). Two isolates of Pseudomonas fluorescens, along with a commercial strain of Streptomyces griseoviridis, showed antifungal activity both in vitro and in vivo. Among the fungal isolates 13 fungi and a commercial strain of Streptomyces griseoviridis showed biocontrol activity against the pathogen. Potential antagonists were selected on the basis of inhibition or overgrowth of P. herpotrichoides on several contrasting nutrients media. Co-inculation of straw with the pathogen and tested antagonist, reduced disease severity in pot trials.

Saikia and Chowdhury (1993) evaluated different cell concentrations of phylloplane microflora for the control of bacterial leaf blight of rice caused by Xanthomonas oryzae pr. oryzae. All the microorganisms including the heat killed and avirulent cells of the pathogen showed antagonistic effect towards X. oryzae pr. oryzae. Erwinia herbicola controlled the pathogen most effectively even at lower ratio (1:1) and registered more than 90% reduction in disease development at 50:1 ratio. Bacillus subtilis also performed better than other antagonists in reducing the bacterial leaf blight of rice.

Tip clipping method of inoculation exhibited significantly more protection than swab and infiltration methods.

Potential microbial antagonists of Colletotrichum gloeosporioides were isolated by Koomen and Jeffries (1993) from blossom, leaves and fruit of mango and screened using a series of assay techniques. In total 648 microorganisms, including bacteria, yeasts and filamentous fungi, were isolated and tested for their inhibition of growth of C. gloeosporioides on malt extract agar. In vitro, 121 microorganisms inhibited the fungus and of these isolates, 45 bacteria and yeasts inhibited conidial germination. These were inoculated onto mangoes and artificially infected with C. gloeosporioides in order to assess their potential use to reduce the development of anthracnose. The final screening procedure yielded Bacillus cereus and Pseudomonas fluorescens. Both were tested in post-harvest trials in combination with different application methods including the addition of adhesive material, peptone, fruit wax or sucrose polyester. Application of B. cereus did not reduce disease development, whereas P. fluorescens reduced anthracnose development. No additional benefit was achieved by incorporating the bacteria in adhesive material peptone, fruit wax or sucrose polyester.

Phyllosphere microorganisms of Brassica napus were isolated by Chakraborty et al. (1994a) and their antagonism against Leptosphaeria maculans, causal agent of black leg disease, was tested in vitro. In paired culture Erwinia herbicola was found to be highly antagonistic to L. maculans. Bioassay of the culture filtrate of the bacterium against the test fungus revealed that E. herbicola secrete an antifungal substance into the culture medium. This substance was partially thermolabile and markedly reduced the germination and germ tube length of L. maculans. When aqueous cell suspension and/or cold sterilized cell free culture filtrates of E. herbicola applied to the seedlings prior to inoculation with L. maculans, significant reduction in the severity of black leg disease was noticed.

A strain of Bacillus subtilis has been found to produce potent antifungal volatiles (AFV) which were active against a range of growth media and in loam-based compost. In vitro AFV activity on nutrient agar was enhanced with the addition of D-glucose, complex carbohydrates and peptones. However, the addition of L-glucose led to significantly less AFV activity than comparable levels of D-glucose. Growth studies in liquid culture revealed that B. subtilis failed to grow in response to L-glucose. Further growth studies on solid media showed no clear correlation between enhanced bacterial growth and increases in in vitro AFV activity in response to supply to substrates. Low level AFV activity was also detected from oil seed rape roots inoculated with B. subtilis. Gas chromatography mass spectrometry headspace analysis of B. subtilis cultures grown on various substrates revealed common similarities between substrate promoting AFV activity, although it was not possible to isolate individual antifungal compounds (Fiddman and Rossall, 1994).

Application of cell suspension of Saccharomyces cerevisiae obtained from commercial baker's yeast were found to be highly effective in protecting maize leaves against the necrotrophic pathogen Exserohilum turcicum (Stangarlin and Pascholati, 1994). In vitro experiments showed that S. cerevisiae cells and filtrates of these suspensions were able to inhibit conidium germination. In vivo experiments also showed that yeast cells inhibited conidium germination and penetration of the host by the fungus, although appressorium formation was stimulated. They suggested that S. cerevisiae protected maize leaves against E. turcicum possibly through antibiosis (inhibition of conidium germination) and/or activation of resistance mechanisms in the host.

Phyllosphere microorganisms of tea were isolated, identified and screened for antagonism to Glomerella cingulata in vitro. Among the large number of microorganisms tested, Aspergillus nidulans,

Aspergillus niger, Penicillium oxalicum, Pseudomonas spp., Flavobacterium, Bacillus, Microbacterium, Micrococcus, Alcaligens and Aureobacterium spp. were found to be highly antagonistic. Pseudomonas spp. was selected to be the most potent among these. Bioassay for effect of the culture filtrate of this bacterium on the pathogen revealed that the bacterium secretes an antifungal substance into the culture medium. Among many solvents tested for extracting the active principle from the culture filtrate, diethyl ether was most effective. In vivo tests with this bacterium showed that when applied prior to inoculation with G. cingulata, it reduced infectiousness of the pathogen significantly (Chakraborty et al., 1994c, 1995b).

Potato cultivar Kufri Chandramukhi supported growth of nine phylloplane fungi belonging to seven genera. They were Penicillium spp., Rhizopus spp., Fusarium spp., Aspergillus spp., Trichoderma spp., Alternaria spp. and Helminthosporium spp. Out of these first five were found to be antagonistic to late blight fungus (Phytophthora infestans) in vitro. The population of phylloplane fungi in general increased with increasing doses of nitrogen fertilizer (Urea). Late blight resistant cv. K. Badshah and JH-222 supported far higher counts than susceptible cv. K. Chandramukhi, K. Bahar and K. Jyoti. With increase in host age there was increase in population of phylloplane fungi. On plants sprayed with Dithane M-45, Blitox-50 and Ridomil MZ-72 there were fewer colonies of phylloplane fungi as compared to unsprayed plants. These three fungicides when tested at 100, 200 and 500 ppm caused 6, 20 and 50% inhibition of growth of antagonistic fungi (in vitro) isolated from potato phylloplane (Mishra and Pundhir, 1995).

The efficacy of four microbial inoculants to suppress white rust disease of mustard (Brassica juncea) cv. Burgundy caused by Albugo candida race 2 was determined by Goyal et al. (1995) in both growth chamber and field. The field trial consisted of four bacterial

treatments (Strain Ral-3 of Pseudomonas cepacia and strains U-14, 63-49 and Gl-3 of P. fluorescens) with 4 replicates. Each bacterial inoculum was sprayed on the seedlings and after 24 h plants were sprayed with a germinating zoosporangial suspension (8.5 x 10^4 zoospores/ml) of A. candida. White rust disease severity ratings were recorded 3 weeks after fungal inoculation. Strain Ral-3 significantly (P=0.05) suppressed white rust compared to the controls.In the growth chamber, bacteria were applied similar to the field study and also as a seed treatment to compare the efficacy of bacteria for A. candida suppression. Strain Ral-3 of P. cepacia and U-14 of P. fluorescens significantly suppressed white rust when used as foliar application.

A number of reviews pertaining to biological control of plant diseases on the phylloplane have also been published by different workers (Blakeman and Fokkema, 1982; Bhattacharyya and Purkayastha, 1983; Sharma, 1985; Fravel, 1988; Andrews, 1992; Fokkema, 1993; Powell and Jutsum, 1993).