Elettaria cardamomum Maton

Distribution

Cultivation of cardamom is mostly concentrated in the evergreen forests of Western Ghats in Southern India. Besides India, cardamom is grown as a commercial crop in Guatemala and on a small scale in Tanzania, Sri Lanka, El Salvador, Vietnam, Laos, Cambodia and Papua New Guinea (PNG). Earlier, India accounted for 70 per cent of the world production and now it is 41 per cent only, while Guatemala contributes around 48 per cent world production. The cultivation of cardamom is mostly under natural forest canopy. The important areas of cultivation in India are Uttara Kannada, Shimoga, Hassan, Chickmagalur and Kodagu (Coorg) districts in Karnataka states; northern and southern foot hills of Nilgris, hill region of Madurai, Salem and Tirunelveli, Anaimalai and Coimbatore district of Tamil Nadu; Wyanad and Idukki districts as well as in the Nelliampathy hill of Palghat district of Kerala.

Botany

E. cardamomum belongs to the monocotyledonous family Zingiberaceae (ginger family) of the natural order Scitamineae. The genus Elettaria consists of about six or seven species distributed in India, Sri Lanka, Malaysia and Indonesia.

Rhizome stout or fairly stout, the intervals between leaf-shoots often short. Leaf-shoots tall, with many blade-bearing: petioles short. Inflorescences from rhizome close to the base of a leaf-shoots, long and slender, prostrate, either just at the surface of the ground or just below it (not bearing roots), protected by alternate fairly large leaves, in the axils of which cincinni arise, their attachment being sometimes supra-axillary. Cincinni short, bearing a close succession of tubular bracts, each of
which encloses entirely the next flower and also the next bract; the flowers in two close rows on one side of the composite axis of the shoots, all pointing in the same direction, and curved, opening in succession. Calyx tubular, split about ¼ of its length down one side, shortly 3-toothed; in some species joined at the base to the corolla-tube about as long as calyx; lobes not very brad, sub equal, the upper with a concave apex. Labellum as in *Amomum*, with yellow median band and red stripes, sometimes so curved that it stands as a hood over the top of the flower. Staminodes none, or short and narrow. Filament of anther very short, broad. Anther longer than filament, the connective produced at the apex into a small crest. Stigma small, in close contact with the distal end of the pollen sacs. Fruit globose or ellipsoid, thin-walled, smooth or with longitudinal ridges when ripe.

**Varieties**

Based on the nature of panicles, three varieties of cardamom are recognized (Sastri, 1952). The var. *Malabar* is characterized by prostrate panicle and var. *Mysore* possesses erect panicle. The third var. *Vazhukka* is considered a natural hybrid between the two and its panicle is semi-erect or flexuous.

**Ecology**

The optimum altitudinal range for growing cardamom is from 1500 m above MSL (Anonymous, 1982). The var. *Malabar*, the traditional cardamom of Karnataka, possesses the capacity to be productive at lower elevations of 500 - 700 m above sea level. The other cardamom varieties, *Mysore* and *Vazhukka* are not productive below 700 m elevation (Abraham and Tulsidas, 1958). Cardamom is highly sensitive to elevation and the wrong choice of cultivars, or wrong location, can severely affect the growth and productivity. The optimum growth and development of cardamom is observed in the warm and a humid condition at a temperature varies from 10 – 35°C (Anonymous, 1976). The upper
temperature limit will normally be around 31 – 35° c. Cold conditions would result in almost poor or no setting of capsules. Thus extremes of temperature and wide diurnal variations are not conducive for healthy growth of cardamom plants. It is also grown under rainfall conditions ranging from 1500 to 5750 mm.

**Diseases of *E. cardamomum***

Cardamom is affected by a number of diseases caused by various pathogenic fungi, bacteria, virus and nematodes. Based on the severity, spread and extent of damage, these are grouped as major and minor diseases occurring in the main plantation and in nurseries. Among the fungal diseases capsule rot, rhizome rot and stem rot seriously affect the plant and cause considerable yield lose.

**Capsule rot**

Capsule rot popularly known as *Azhukal* (Malayalam) means rotting, is perhaps the most serious disease of cardamom. Menon et al. (1972) reported it for the first time from plantations of Idukki district in Kerala state, India.

**Symptoms**

Disease symptoms develop on the capsules, young leaves, panicles and tender shoots. The first visible symptom appears as discolour water-soaked lesions on young leaves or capsules. These lesions enlarge and the affected portions decay. Infection takes place on capsules or tender leaves simultaneously or first on capsules followed by foliar infection (Thomas et al., 1991a). When foliage infection occurs, water-soaked lesions coalesce to form large patches. Immature unopened leaves fail to unfurl following infection. As the disease advances, the infected areas turn necrotic, the leaves decay and shrivel and finally they give a shredded appearance. Infected capsules also show water soaked discoloured areas; which turn brownish and later such capsules decay and drop off. Such rotten capsules emit a foul smell. Capsules of all ages are susceptible to infection. However, this disease seriously affects young capsules.
During favourable climatic conditions the disease is aggravated and infection extends to panicles and tender shoots also. In extreme cases, the whole panicle or the whole pseudostem decay completely. In such cases the rotting extends to underground rhizomes also. The root system of such plant gets decayed and the entire plant collapses. Nair (1979) described similar symptoms and observed that the disease severity is uniform in the three major cardamom types, viz., *mysore*, *vazhukka* and *malabar*. Nambiar and Sarma (1976), who studied the disease earlier, have reported a crop loss of 30 per cent. However later it has been shown that as high as 40 per cent crop loss can occur in severely affected plantations (Anonymous, 1989a)

**Causal organism**

Menon *et al.* (1972) first reported *Phytophthora* sp. as the causal organism. Thankamma and Pillai (1973) identified it as *P. nicotianae* Brede de Haan var. *nicotianae* Waterhouse and as *P. palmivora* Butler (Radha and Joseph, 1974). Nambiar and Sarma (1976) reported the association of *Pythium vexans* and a *Fusarium* sp. along with *Phytophthora* sp. However later studies by Nair (1979) showed that *P. nicotianae* var, *nicotianae* is the causative organism that could be successfully isolated from all infected plant parts.

**Disease management**

As the outbreak of disease occurs during the monsoon season, disease management measures have to be initiated well in advance i.e. before the primary infection starts. Various types of fungicides have been extensively used for controlling the disease during earlier years. Spraying and drenching of copper fungicides such as one per cent Bordeaux mixture and 0.2 per cent copper oxychloride (Menon *et al.*, 1973; Nambiar and Sarma, 1974; Nair 1979; Nair *et al.*, 1982) were recommended as the control measures Thomas *et al.* (1989, 1991a) evaluated a number of contact and systemic fungicides under field
conditions and concluded that two to three rounds of sprays including one round of prophylactic spray with one percent Bordeaux mixture or 0.3 percent Aliette (Fosetyl Aluminum) after proper phytosanitation effectively controlled the spread of disease.

**Biological control**

Bioagents play an important role in an eco-friendly system of disease management to fight against plant pathogens in a totally safe manner avoiding the use of expensive and hazardous chemical fungicides. Inhibition of *P. meadii* under laboratory conditions and disease suppression in cardamom nurseries have been studied by Thomas *et al.* (1991b) using *Trichoderma viride, T. harzianum, Latisaria arvalis* and *Bacillus subtilis*. Suseela Bhai *et al.* (1993) achieved field control of capsule rot disease of cardamom using *T. viride* and *T. harzianum* and developed simple carrier cum multiplication medium for *Trichoderma* (Suseela Bhai *et al.*, 1994, 1997). The isolates of *T. viride* and *T. harzianum* harbouring native cardamom soils have been screened and effective strains for high biocontrol potential have been developed (Dhanapal and Thomas, 1996).

**Rhizome rot**

Rhizome rot is a common disease occurring in cardamom plantations during monsoon period. The disease was first reported by Park (1937). Subba Rao (1938) described it as clump rot disease. The disease is widely distributed throughout cardamom plantation.

**Disease symptoms**

The disease makes its appearance during monsoon period. The first visible symptom is the development of pale yellow colour in the foliage and premature death of older leaves. These leaves show wilting symptoms. The collar portion of the aerial shoots becomes brittle and the tiller breaks off at slight disturbance. Symptoms of rotting develop at the collar region, which becomes soft and brown coloured. At this stage the
affected aerial shoots fall off emitting a foul smell. Mayne (1942) reported the incidence of the disease in cardamom hills of Kerala. The tender shoots or the young tillers also turn brown coloured completely. As the disease advances, all the affected aerial shoots fall off from the base. The panicle and young shoots attached to this also are affected by rot. The rotting extends to the rhizomes and roots also. The rhizome infection becomes severe during July-October months. In severely affected areas as much as 20 percent disease incidence was recorded.

**Causal organism**

With regards to causal organism, there are contradictory reports given by several authors. Subba Rao (1938) observed that cardamom rhizome rot is caused by *Rhizoctonia solani* and it was associated with a nematode. Ramakrishnan (1949) reported *Pythium vexans* as the causal organism. Thomas and Vijayan (1994) reported that *Fusarium oxysporum* is also occasionally found to cause rhizome rot and root rot infections.

**Disease management**

The disease is usually observed in areas previously affected by rhizome rot disease. Therefore phytosanitation plays a major role in disease management. Presence of inoculum in the soil and plant debris, overcrowding of plants and thick shade are congenial conditions for disease development. Soil drenching with one percent Bordeaux mixture or 0.25 percent copper oxychloride at one-month interval has been reported to be very effective for controlling the disease (Thomas and Vijayan, 1994).

**Biological control**

The rhizome rot disease was managed through the application of *Trichoderma* sp. *T. viride* and *T. harzianum* were reported to be effective against rhizome rot incidence in plantations. A formulation of this bioagent in a carrier medium consisting of farmyard manure and coffee husk mixture has been developed for field application in the integrated management system for control of rot diseases of cardamom (Thomas *et al.*, 1997).
Stem rot

A relatively new disease affecting the stem (tillers) of cardamom has been found to occur in several plantations in Idukki district of Kerala and in Lower Pulney area in Tamil Nadu (Dhanapal and Joseph Thomas, 2000). This disease appears usually during post–monsoon period.

Disease symptoms

The disease attacks the middle portion of tillers in the form of pale discoloured patches, which lead to a sort of dry rotting. The stem is weakened at this portion and leads to partial breakage. The partially broken tillers bend downwards and hang from the point of infection. Where infection occurs at lower region of tillers, they fall off giving a lodged appearance. In such tillers, leaves and leaf sheaths dry up soon.

Causal organism

The pathogen was identified as *Fusarium oxysporum*. Symptom observed were similar to the stalk rot disease of sorghum caused by *Fusarium moniliforme*.

Disease management

The disease is usually noticed in areas previously affected by stem rot disease. Therefore phytosanitation plays a major role in disease management. Adequate shade should be provided followed by the three round spraying of carbendazim in a month interval to control the disease effectively.

Uses

The major use of cardamom is on a worldwide basis is for domestic culinary purposes in whole or ground form. In Asia, cardamom plays a major role in a variety of spiced rice, vegetable and meat dishes. Indian cardamom is low in fat and high in protein, iron, vitamin B and C (Pruthy, 1993). Cardamom flavoured coffee (Gahwa) and aromatic tea are prepared by adding cardamom. It is believed that this drinks cools down body heat. Cardamom seeds are chewed after meal towards off foul smell and as a mouth freshener. A variety of cardamom flavoured products
have come to market such as biscuits, chocolates, milk, and cheese and so on. It is also used for making garlands in India and Arab countries for special occasions to present distinguished quests.

Cardamom is effectively used as a powerful aromatic stimulant, carminative, stomachic and diuretic. It also checks nausea and vomiting. Powdered seeds of cardamom boiled in water with tea powder impart a very pleasant aroma to tea and the same can be used as a medicine for scanty urine, diarrhea, dysentery, palpitation of heart, exhaustion due to overwork, depression etc., (Singh and Singh et al., 1996). Medicated cardamom oil and powder can retard various types of hypo-pigmentation on the face. It also finds a place in the formulation of lozenges for the management of common cold and associated symptoms (Nair and Unnikrishnan, 1997)

**Piper nigrum L. (Black pepper)**

Black pepper is one of the important agricultural commodities. This crop is major sources of income and employment for rural households. Pepper producing countries are Sri Lanka, Indonesia, Malaysia, Brazil, Vietnam, Thailand, China and Federal states of Micronesia. In India, Kerala, Karnataka and Tamil Nadu are the major pepper producing states in the country.

**Botany**

*P. nigrum* belongs to the family Piperaceae. The genus *Piper* is a large genus with over 1000 species. The stem is dimorphic. The leaves are alternate and simple. The flowers are unisexual, with monocious or diocious forms or hermaphorodite, as occur in many cultivars. The fruit is a sessile, globose drupe, 4 – 6 mm in diameters with a pulpy pericarp, borne in spike 5 – 15 cm lion.

**Varieties**

*P. nigrum* show considerable variations in the size of the internodes, leaves, inflorescences and fruits. Based on these variations,
the important pepper varieties are Karimunda, Kottanadan, Narayankkodi, Aimpriyan, Neelamundi, Kuthiravalli and Balancotta.

**Ecology**

Pepper grows successfully between 200 North and 200 South of equator and from sea level to 1500 m MSL. Total rainfall and its distribution play an important role in pepper cultivation and productivity. An annual rainfall of 2000 – 3000 mm with uniform distribution is ideal. Rainfall of 70 mm received in 20 days during May-June is sufficient for triggering off flushing and flowering process in the plant. The crop tolerates between 10 – 40°C. The ideal temperature is 23 – 32°C with an average of 28°C. It grows well on soils ranging from heavy clay to light sandy clays rich in humus with friable nature, well drained, but still with near neutral pH, high organic matter and high base saturation with Ca and Mg enhanced the productivity.

**Diseases of pepper**

Pepper is affected by a number of diseases caused by various pathogenic fungi, bacteria and nematodes. These are grouped as major and minor diseases occurring in the main plantation and in nurseries based on the severity, spread and extent of damage. Among several diseases foot rot disease of pepper is the major disease causing considerable crop loss. The diseases such as slow decline, “Fungal pollu” and blight are considered as minor diseases and easily controlled through phytoanitation followed by spraying of fungicides.

**Foot rot disease**

In India, the foot rot of pepper previously known as quick wilt disease was first reported as early as 1902 when severe vine death was noticed in wyanad region of erstwhile Madras state (Menon, 1949). This disease occurs in all pepper growing tracts (Sarma et al., 1991). The yield loses of 20 to 30 percent have been estimated due to this disease (Samraj and Jose, 1966; Nambiar and Sarma, 1977).
Causal organisms

Muller (1936) first identified the causal agent of foot rot as *Phytophthora* sp. and named it as *P. palmivora* var. *piperina*. The first authentic report of *Phytophthora* wilt of pepper in Kerala was reported by Samraj and Jose (1966) who adopted Muller's identification of *Phytophthora*. Kasim (1978) identified the causal agent of foot rot of pepper from Lumpung as *P. capsici*. Finally based on the detailed description of the isolates the so-called *P. palmivora* MF4 was renamed as *P. capsici* (Tsao, 1991).

Disease symptoms

Expression of disease symptoms depends upon the site of infection and extent of damage. On leaves one or more dark spots with characteristic fimbriate margins at the advancing edges could be seen during the rainy season. These spots enlarge rapidly within two or three days and the leaves are shed before these spots cover the entire leaf. Spikes are also affected, black spots appear on the spikes at the point of attachment or anywhere on the length of the spike and are shed. The tender shoot arising at the base of the vines and tailing on the ground are affected causing blighting of the tender shoots. Infection may also occur on the main branch or lateral branch resulting in the yellowing and defoliation of the branches beyond the point of attack. Collar infections may occur either through the runner shoots or through the roots results in sudden wilting of the vine.

Disease management

An integrated management strategy has been developed which include phytosanitation, cultural practices, and chemical and biocontrol methods. The moment the disease is noticed on a few vines, they must be removed along with root system and destroyed. This would reduce the inoculum for next season. At the onset of monsoon, the branches of shade/support trees should be loped to allow better penetration of
sunlight. This would reduce moisture build up and alter the microclimate under the canopy and reduce the incidence of foliar infections. Water stagnation causes adverse effect on the vines results rotting of the vines. Providing adequate drainage and preventing water stagnation are important aspects of disease management. In addition to the methods mentioned above, chemical control is also essential to control foot rot infection in pepper. Wherever foliar infections are severe; Bordeaux mixture (1.0%) may be sprayed once during June and next by August – September. To prevent the soil population build up of the pathogen, drenching with copper oxychloride (0.2%) may be done. Systemic fungicides like Potassium phosphonate (0.3%) may be applied both as foliar spray and soil drench @ 5 liters/vine twice during the monsoon period.

**Biological control**

As the *Phytophthora* inoculum is soil borne, the population build up could be reduced by the use of bioagents such as *Trichoderma*, *Gliocladium* and *Pseudomonas*. Efficient strains of these organisms are identified and are multiplied in large quantities and supplied to farmers. Since *Phytophthora* infects all parts of black pepper, fungicidal spray is required to prevent the aerial infection. As the bioagents survive on the organic matter in the soil, application of organic matter would enhance their population and suppress the population of *Phytophthora* in soil.

**Uses**

The two primary products of pepper are black pepper and white pepper. The use of black and white pepper on a worldwide basis is for domestic culinary purposes. In western countries, both forms find extensive use in the flavouring of processed foods. In ayurveda, fruits, roots and leaves are used as medicine. It is beneficial in the treatment of cold, fever and cough. It is also used as an antidote in snakebite and remedy for muscular pain and impotence.
Seasonal study of AM fungi
Climatic factors

Climatic factors like rainfall, relative humidity, light, temperature and wind strongly influence root colonization and spore population of AM fungus (Furlan and Fortin, 1973; Udaiyan et al., 1996).

Rainfall and relative humidity

The ecological studies pinpointed out very precisely the effect of climatic factors on population dynamics, infectivity and effectivity of AM fungi. A positive and negative relationship between AM fungal spore numbers and relative humidity was reported in *Acacia farnesiana* and *A. planiferons* respectively (Udaiyan et al., 1996) and in *Casuarina equisetifolia* and *Dalbergia latifolia* (Rajesh Kannan, 2002). A significant relationship between AM fungal colonization and rainfall in *Citrus* (Michelini et al., 1993) and a reduction in the intensity and percentage of AM fungal colonization in three species of savannah grasses during dry season (Newman et al., 1986) were reported. False break (rain during summer) decreased mycorrhizal colonization and proportion of length colonized by AM fungal structures (Braunberger et al., 1994). A similar drop in the colonization levels in sugarcane was found in Barbados during the dry season (Chinnery et al., 1987).

Light

AM fungi obtain their carbon source from the host plants and thus rely on the photosynthesis and the translocation of photosynthates to the root. Since light controls the rate of photosynthesis it can strongly affect mycorrhizal formation (Furlan and Fortin, 1977) and shading reduces AM fungal colonization and spore production (Gerdemann, 1968). Day length plays an important role in AM fungi development (Redhead, 1975; Daft and El-Giahmi, 1978), but the effect of light depends on the photosensitivity of host species (Redhead, 1975). Photoperiod may also be
important as the light intensity at optimal day length enhances the root colonization (Daft and El-Giahmi, 1978).

**Temperature**

Temperature has been shown to have significant influence on colonization and sporulation of AM fungi. High temperatures generally increase AM fungal root colonization and spore populations (Muthukumar, 1996). Isolates and species of AM fungi differ in their germination response to temperature. This was demonstrated over the temperature range of 15°C to 34°C by Schenck *et al.* (1975) who found that spore of *Gigaspora heterogama* isolate from Florida did not germinate at or below 20°C, but showed maximum germination at 34°C. In contrast an isolate of *Glomus mosseae* from Washington showed maximum germination at 34°C and very low germination at 20°C. Other studies have also shown that isolates may differ in their optimum temperature for spore germination, root colonization and spore production (Graham *et al.*, 1982; Tommerup, 1983). Furthermore a single isolate may have different optimum temperatures for colonization and sporulation (Schenck and Smith, 1982). Saif (1983) found increased root colonization and number of vesicles in *Eupatorium odoratum* colonized with *Glomus macrocarpum* when the temperature was increased from 28°C to 30°C, but arbuscule production tend to decrease above 25°C. Growth response of soybean tends to vary with soil temperature and AM fungal species (Schenck and Smith, 1982). Schenck and Schroder (1974) reported maximum arbuscules formation in soybean roots at 30°C with *Endogone gigantea* (=*Gigaspora gigantea*), while mycelial development on root surface was greatest between 28°C and 34°C.

**Wind**

AM fungal dispersal is mainly activated by wind. Tommerup (1982) reported that the large spore of AM fungi could be suspended in moving
air currents. Wind dispersal mechanism is responsible for introduction of AM fungi to new geographic locations (Brundrett, 1991). Wind dispersal has been observed in arid ecosystems with strong winds (Warner et al., 1987; Allen, 1988).

**Edaphic factors**

Plants in natural ecosystems have varying levels of dependence on mycorrhizal association that are the result of inherent properties of the plants themselves and the availability of nutrients in the soils which they naturally occur (Janos, 1980). The supply of a particular nutrient depends on its availability and mobility in soils as well as the plant internal requirement to that nutrient (Russell, 1977).

**Water**

Khan (1974) reported that plant species were non-mycorrhizal in permanently waterlogged soils but mycorrhizal on drier soils. Wet habitats may be unsuitable for mycorrhizal formation due to poor aeration as oxygen is necessary for fungal growth (Crawford, 1992). Furthermore in such condition, an array of toxic substances like reduced Mn, H$_2$S, and organic acids etc. are produced that inhibit AM formation (Saif, 1983). AM fungi occur in a wide range of soil water regime, arid desert to aquatic environments (Khan 1974; Bagyaraj et al., 1979; Clayton and Bagyaraj, 1984).

A number of physiological explanations have been suggested for the observed interaction between soil moisture and AMF, like varied root colonization levels in response to soil moisture (Bolgiano et al., 1983; Allen and Allen, 1984) and spore density of AM fungi (Muthukumar et al., 1994 and Rajesh kannan, 2002). The movement of water from soil to roots through the AM hyphal pathway has been the subject of considerable experimentation (Nelson, 1987). The production of copious hyphae by some isolates could have adaptive advantage in arid environments or environments subjected to extremes of soil water
availability. Hyphae are thought to have access to water held in soil aggregates. Controversy about AMF uptake of water is based on the confounding effect of the improved P status of mycorrhizal plants. However, Benthlenfalvay et al. (1988) have shown that growth of plants subjected to severe water stress is attributable to AM mediated increase in water uptake which is associated with increased soil hyphal biomass. Plants P status was similar in mycorrhizal and non-mycorrhizal *Pelargonium* (Sweatt and Davies, 1984). Drought stressed *Citrus* (Levy et al., 1983) and *Trifolium* (Safir et al., 1972) responded to water more rapidly than to non-mycorrhizal controls, and recovered more effectively from water deficits.

Allen (1991) reported that AM fungi have ability to increase water uptake under some circumstances, thus improving the competitive ability of mycorrhizal plants and plant water relations. This can often be ascribed to the increased P absorption thereby increasing shoot and root growth but in some cases there are leaf and root hormonal changes, which have effects on stomatal conductivity (Levy and Krikun, 1980; Auge et al., 1992). Faber et al. (1991) demonstrated a role for AMF hyphae in water transport in soil and suggested that they may be of greater importance in drought situations where water is less mobile and confined to smaller pores to which hyphae may have greater access. Mycorrhizal colonization and number of resting spores were more in water-stress conditions than in the non-stress condition in sunflower, but during extreme water stress condition sporulation is stopped (Reddy et al., 1997).

**pH**

Soil pH plays an important role in availability of nutrients and their uptake by plants. Studies have mainly concentrated on the effect of pH on AM fungal spore germination (Mosse, 1973; Mosse and Hepper, 1975; Hepper and smith, 1976; Daniels and Trappe, 1980), spore
production (Kruckelmann, 1975; Read et al., 1976) and effects on plant growth and P uptake (Hayman and Mosse, 1971; Graw, 1979; Lambert et al., 1980). It also influences the distribution (Nemec et al., 1981) and effectiveness (Hayman and Tavers, 1985) of AM fungi. Many AM fungal isolates as a group function over fairly broad ranges of soil pH. However, most AM fungi appear to be adapted to soil pH condition close to those from which they were isolated (Sylvia and Williams, 1992). It has been established that germination of AM fungal spores is pH sensitive and different species have different pH optima (Green et al., 1976; Daniels and Trappe, 1980; Robson and Abbott, 1989). Mosse (1973) reported that *Glomus mosseae* colonized host root only in soils with a pH > 5, but some species are distinctly acid tolerant. Root colonization is often reported to be pH dependent when single endophytes are used but not so for mixture of endophytes under field conditions (Read et al., 1976; Sparling and Tinker, 1978; Black and Tinker, 1979). However Sparling and Tinker (1978) reported that certain reports also indicate the negligible effect of soil pH on AM status and existence of AMF colonization even at a very low pH of 2.7.

**Nitrogen**

Application of nitrogen plays a significant role on mycorrhizal colonization. Hayman (1975) showed that ‘N’ fertilizers had a large negative effect on the mycorrhizal colonization. Menge (1984) noted that daily fertilization of *Citrus* with more than 100 ppm ‘N’ as a mixture of NO$_3^-$ and NH$_4^+$ retarded mycorrhizal development. Davis and Young (1985) reported NO$_3^-$ salts to be more inhibitory to AM development than NH$_4^+$ salts.

**Phosphorus**

Phosphorus is most important nutrient for plant growth that can be supplied by mycorrhizal associations, because of the many abiotic and biotic factors, which can restrict its mobility in soils (Harley and Smith,
1983; Hayman, 1983; Wood *et al.*, 1984; Marschner, 1986). Sreenivasa and Bagyaraj (1988) observed that rock phosphate at 100 ppm 'P' level increased infective propagules of *Glomus fasciculatum* compared to bonemeal or superphosphate fertilizers. AMF plants that respond greatly to soil fertilization, with tricalcium phosphate, ion phosphate and rock phosphate, seem to indicate an improved use of 'P' from these insoluble fertilizers (Powell and Daniel, 1978; Bolan *et al.*, 1987). Reductions in the benefits provided by mycorrhizal associations (mycorrhizal dependency) to plants caused by increasing soil phosphorus level have often been observed (Crush, 1973; Baylis, 1975; Johnson, 1976; Gerschefskekitt *et al.*, 1988).

**Organic carbon**

Application or availability of organic matter to the soil has been reported to encourage mycorrhizal development and efficiency (Johnson and Michelini, 1974; Ryan *et al.*, 1994). AM fungal spore population was closely correlated with soil organic matter content (Sheik *et al.*, 1975). AM fungi may also be able to grow saprophytically in soil organic matter (Warner and Mosse, 1980; Hepper and Warner, 1983; St. John *et al.*, 1983; Warner, 1984). Weeding compared to mulching surface soils markedly decreased spore number (Nappi *et al.*, 1980) since organic matters are usually higher in soils that are mulched than those that are weeded. Preferential association of AM fungal hyphae with organic matter rich microsites has been previously attributed to the nutrient rich status of the sites (St. John *et al.*, 1983; Muthukumar, 1996). AM fungi are involved in the decomposition of organic carbon (Warner, 1984; Allen and Macmohon, 1985) at localized sites in soil through the proliferation and activity of extraradical hyphae (St.John *et al.*, 1983). Because AM fungal hyphae ramify through the microsites and pores of soil, they change the spatial distribution of carbonaceous compounds (Wright and Millner, 1994).
Climatic and edaphic specificity of AM

Climatic factors and soil conditions influence the occurrence of mycorrhizal associations. Soil factors not only affected mycorrhizal formation but can also influence the diversity of AM fungi in field soils (Cuenca and Meneses, 1996; Brundrett et al., 1999; Muthukumar and Udaian, 2002). Most of these data have been collected using simplified experimental systems, which allow the influence of single factor on one mycorrhizal fungus to be examined, but some field data also exist for comparison. Some evidence of the physiological diversity of mycorrhizal fungi has been provided by comparing experimental responses to soil pH, soil nutrient levels, soil moisture, salinity, temperature, and other factors (Slankis, 1974; Daniels-Hetrick, 1984; Trappe and Molina, 1986; Morton, 1988; Abbott and Robson, 1991a).

There is limited evidence that climatic factors can influence the distribution of mycorrhizal fungal taxa. Ebbers et al. (1987) and Anderson et al. (1984) reported changes in predominance of AM fungi across a soil moisture (soil fertility) gradient in a prairie site, which had a much greater influences on plant populations. Changes to soil properties occur during succession or between sites with similar climates can be correlated with predominance of different species or isolates of AM fungi (Bethlenfalvay et al., 1982; Gerschefske Kitt et al., 1987; Puppi and Reid, 1987; Rose, 1988). Henkel et al. (1989) observed that isolates of AM fungi from adjacent ridge top, mid slope and basal sites and suggested that these isolates had adapted to phosphorus levels or other factors in the soil where they occur. Molina et al. (1978), Graham et al. (1982), Adelman and Morton (1986), Porter et al. (1987) and Stahl et al. (1988) also observed that clonal isolates of AM fungi were more effective when used in their native soil type.

Further, evidence of the physiological diversity of AM fungi is provided by comparing responses of different species or isolates to
physical conditions. These comparisons have demonstrated variations between taxa and intraspecific variability within species of AM fungi in their ability to promote plant growth when exposed to the factors. These fungi apparently have a limited tolerance range to environmental conditions (Stahl et al., 1988) and possess specific adaptations to the soil in which they occur (Lambert et al., 1980). These adaptations apparently can influence the outcome of competition between AM fungal species (Gerschefske Kitt et al., 1987). The effect of low soil pH on AM fungal association is discussed in detail by Howler et al. (1987) and Robson and Abbott (1989). Some endophytes can provide substantial benefits to the host plant in soils with low pH and high aluminium levels, where others are less effective. It has often been observed that the fine endophytes are more abundant in acidic soils (Gianinazzi – Pearson et al., 1980; Wang et al., 1985). Porter et al. (1987) found the distribution of AM fungal taxa in Western Australia to be highly correlated with soil pH. Bethlenfalvay et al. (1989) proposed that the term ‘endophyte’ be used to describe intra specific variants of mycorrhizal fungi isolated from different soils that differ in the mycorrhizal literature about the physiological, ecological or mutualistic characteristics of species of AM fungi that actually only describe one particular clonal isolate (Morton, 1990).

From these surveys it can be seen that the soil in one location normally contains more than one AM fungal species and may contain a fairly wide diversity of these fungi considering that only about 150 species are known (Morton 1990). It is not possible to examine the influence of soil conditions or environmental factors on AM fungal diversity, because of differences in sampling methodologies.

Unfortunately AMF research has been concerned with plant responses to mycorrhizas with little consideration of specific endophytes, thus creating the impression that these are functionally equivalent (Morton 1988; Abbott and Robson, 1991b). This impression has been strengthened
by the classification problems of fungi such as *G. fasciculatum*, which originally had a worldwide distribution and was accredited with remarkable genetic and physiological plasticity (Morton, 1988).

**Biological factors**

**Host plant**

Mycorrhizal associations are generally considered to benefit host plants by enhancing mineral nutrient acquisition especially P. The physiology of mycorrhizal associations has been well discussed by Hayman (1983), Harley and Smith (1983) and Gianinazzi-Pearson (1984). AMF associations may also improve uptake (Barea *et al.*, 1989) and absorption of minor nutrients, such as Mg, Cu and Zn, but Mn uptake can be reduced (Harley and Smith, 1983; Hayman, 1983; Killham, 1985; Pacovsky, 1986; Arines and Vilarino, 1989). Other less specific changes to host physiology – alterations in nutrient requirements, membrane composition and metabolite levels, apparently occur even when nutrient input is negligible (Pacovsky, 1986).

There is little evidence of host fungus specificity in most type of mycorrhizal associations (Harley and Smith, 1983; Gianinazzi-Pearson, 1984; Duddridge, 1987). Ineffective AMF associations have been discovered in only a few of the many host plant and mycorrhizal fungal combinations tried in synthesis experiments (Johnson, 1976; Giovannetti and Hepper, 1985). Genotypic variations within a host species can influence the degree of AMF formation (Azcon and Ocampo, 1981; Krishna *et al.*, 1985; Thomas and Ghari, 1987; Lackies *et al.*, 1988; Sieverding and Glavez, 1988). Root growth is slightly affected by AMF but a detrimental reduction in root elongation occurs in some cases (Jones and Hendrix, 1987). Some AM fungi provide more benefit to the host than others as is hormones, such as ethylene and auxins which may be responsible for the reduced apical growth of mycorrhizal short roots (Gay and Dabard, 1987; Berta *et al.*, 1988; Rupp *et al.*, 1989). Mycorrhizal
associations have been implicated in increased host resistance to disease and other stresses.

**Mycorrhizosphere**

Whipps and Lynch (1986) described three zones in the rhizosphere consisting of (i) the ectorhizosphere (soil in close proximity to roots); (ii) the rhizosplane (root surface) and (iii) endorhizosphere (apoplastic space within roots). In the rhizosphere soil properties changed by enhanced microbial activity of dead cells, mucilages and exudates from roots and these influences are most pronounced near young roots (Newman 1985; Curl and Truelove, 1986; Uren and Reisenur, 1988). Root exudates include inorganic ions, sugars, amino acids and organic acids which escape from root cells (Curl and Truelove, 1986).

The mycorrhizosphere may support substantially altered populations of bacteria, actinomycetes and fungi when compared with non-mycorrhizosphere (Lawley *et al.*, 1982; Ames *et al.*, 1984; Meyer and Linderman, 1986; Secilia and Bagyaraj, 1988). Vancitira *et al.* (1989) observed that soil hyphae of AM fungi had bacteria growing on them and these 'hyphosphere' bacteria were a subset of the host rhizosphere population. Soil microorganisms may enhance the effectiveness of AMF associations in offering benefits to host plants (Azcon-Aguilar and Barea, 1985; Meyer and Linderman, 1986), reduce (Daniels-Hetrick *et al.*, 1987; Bass *et al.*, 1989; Koide and Li, 1989) or have no effect (Paulitz and Linderman, 1989) on the effectiveness of AMF associations in increasing host growth.

**Other soil microorganisms**

The soil microflora may have a significant influence on mycorrhization. Bagyaraj and Menge (1978) reported an increase in rhizosphere populations of bacteria and actinomycetes when plants were inoculated with mycorrhizal fungi. Sutton and Sheppard (1976) showed that non-sterile soil filtrate added to the pasteurized soil increased the extrametrical hyphae of AM fungi in an undermined manner.
AM formations were stimulated by volatile compounds produced by soil microflora (St. John et al., 1983) resulting in qualitative changes in the mycorrhizosphere (Meyer and Linderman, 1986).

**Mycorrhizal association in agricultural crops**

AM fungi increase nutrient uptake and enhance the plant growth (Abbott and Robson, 1982; Mosse, 1973). Onion plants colonized by *G. fasciculatum* exhibited 14-fold increase in the uptake of phosphorus than non-mycorrhizal plants (Mohankumar, 1985). Dual inoculation with *G. fasciculatum* and *Rhizobium japonicum* to soybeans significantly increased dry weight and nitrogen content of the shoot (Bagyaraj et al., 1979). AM fungi mobilize phosphate ions from the soil and convert them to polyphosphate granules in the external hyphae. These granules accumulate in the vacuoles and reach the arbuscules, where hydrolysis has been evidenced in the fungal mycelium (Krishna et al., 1983). Presence of polyphosphate in vacuoles and polyphosphate and ATPase in the arbuscule implies that there is an active mechanism for the transportation of phosphate ions to the host plant cells. Breakdown of arbuscule contributes much to the large molecules of the host cells. According to Morandi et al. (1984) phenolic substances, such as phytotoxins are synthesized when the root is infected by a pathogen.

**Mycorrhizal association in plantation crops and spices**

Cuenca et al. (1990) reported that cocoa seedlings responded well to indigenous AM fungi and exhibited significant increase in plant height, dry weight and increased percentage content of P, Cu and Zn compared to control. Hayman and Travers (1985) demonstrated that at soil pH 4.0 the growth of strawberry was greatly stimulated by *G. fasciculatum*. The perennial crops *viz.*, *Citrus sinensis*, *Cocos nucifera*, *Hevea brasiliensis*, *Manihot esculenta*, *Theobroma grandiflorum*, *Carica papaya*, and *Bixa orellana* were inoculated with AM fungi (*Glomus etunicatum*, *G. intraradices*, *G. manihotis* and *Acaulospora* sp.) in the nursery showed
positive growth response and reduced rates of mortality after transplanting to the field (Feldman et al., 1995). Coffee seedlings inoculated with VAM gave the maximum plant height, dry matter and leaf area (Cruz, 1989). In *E. cardamomum*, maximum leaf area, root length and tillers were recorded in the plant inoculated with *G. monosporum* followed by *G. fasciculatum* (Sreeramulu and Bagyaraj, 1999). Increasing dose of fertilizer in the *E. cardamomum* tended to decrease the intensity and incidence of AM fungal population in soil and roots (Rohini Iyer et al., 1988). Incorporation of *G. fasciculatum* in the rooting medium of sand enhanced rooting of *piper nigrum* (Anandaraj and Sarma, 1994). Sivaprasad et al. (1993) reported that depression due to nematode infestation was minimum when the cuttings of *P. nigrum* were inoculated *G. fasciculatum* and *G. etunicatum*. According to Sreenivasa (1997) inoculation of *G. macrocarpum* enhanced the uptake of Zn, Cu, Mn and Fe in chilli crop.

**Role of mycorrhiza on disease management**

Arbuscular mycorrhiza (AM) associations have been shown to reduce damage caused by soil borne pathogens. Meyer and Linderman (1986) found that the number of sporangia and zoospores formed by cultures of *Phytophthora cinnamomi* was reduced by the application of extracts of rhizosphere soil from AMF plants. Secilia and Bagyaraj (1987) isolated more pathogen- antagonistic actinomycetes from rhizosphere of AMF plants than from nonmycorrhizal controls, an effect that also depended on the AM fungus involved. Furthermore, Caron (1989) reported a reduction in *Fusarium* populations in the soil surrounding mycorrhizal tomato roots as compared with the soil of nonmycorrhizal control. Benhamou et al. (1994) reported that in carrot roots, inoculation of AM fungi reduced the growth of *Fusarium oxysporum* in epidermis and cortical tissues, whereas nonmycorrhizal roots the pathogen reached a higher development, infecting even the vascular stele. In onion,
inoculation with mycorrhizal fungi before planting conferred more complete protection against pathogens (Zhenjia and Xiangdong, 1991).

Schoenbeck and Dehne (1977) inoculated cotton seedlings first with Thielaviopsis badicola followed by AM fungi. The damage caused by pathogen was significantly less in mycorrhizal plants. Baltruschat and Dehne (1972) reported that chlamydospore production in T. basicola was negatively correlated with mycorrhizal colonization of tobacco and alfalfa roots. Devis et al. (1978) studied the difference in mycorrhizal and non-mycorrhizal plants after inoculating with Phytophthora sp. Plant height, top weight and root weight of citrus inoculated with G. fasciculatum alone were greater than those of non-infected seedlings inoculated with P. parasitica or inoculated with both G. fasciculatum and P. parasitica. In peanuts the G. fasciculatum was found to provide resistance against Sclerotium rolfsii (Krishna and Bagyaraj, 1983). Higher number of Endogone spores (August–July) was recorded in rhizosphere soil of strawberry roots growing in a plot without root rot than in the rhizosphere of roots in a plot with root rot or in yellow soil of either plot (Nemec, 1974). According to Morandi et al. (1984) phenolic substances, such as phytotoxins are synthesized when the root is infected by a pathogen. They are non-specific toxic substances, which can be considered to play a role in disease resistance. In mycorrhizal groundnut roots, high concentrations of orthohydroxy phenols were present. This type of phenols has been known to play an important role in plant disease resistance (Krishna and Bagyaraj, 1986).

Pseudomonas fluorescens association in agricultural crops

The beneficial rhizobacteria are termed as Plant growth promoting rhizobacteria (PGPRs) because of their ability to improve plant growth through suppression of deleterious root colonizing microorganisms and by production of plant growth regulators (Kloepper et al., 1981 & 1980; Suslow et al., 1982) There are several reports that Pseudomonas
fluorescens have promoted the growth and reproductive parameters of plant ranging from cereals, pulses, ornamentals and vegetable. Considerable increase in root length and seedling growth was observed by Lazarovitz and Nowak (1997) when they used Pseudomonas species in potato plantlets. Similarly, treatment with *P. fluorescens* of sorghum (Raju *et al.*, 1999) and pearl millet (Umesha *et al.*, 1998) seeds enhanced seed germination and seedling vigor. Results of the experiment conducted under field conditions by Barkaet *et al.* (2000) significant plant growth promotion was recorded in *Pseudomonas* treated plants as against control. Tomato plants treated with *Pseudomonas* strains as industrially formulated seed treatment and/or spore preparation mixed with potting medium enhanced plant growth in tomato (Murphy *et al.*, 2000). Widespread commercial use of *Pseudomonas* has been reported on many crops where *Pseudomonas* is known as yield increasing bacteria (Kloepper, 1994). In cotton, the seed treatment with split application of *P. fluorescens* recorded maximum boll weight/plant and was on par with seed treatment with soil application of *P. fluorescens* as single dose (Jaykumar *et al.*, 2003). Similar significant increase in yield of potato (Burr *et al.*, 1978) and wheat (Beeker and Cook, 1988) due to *P. fluorescens* application were also reported. Seed bacterization of chickpea seeds with *P. fluorescens* isolates resulted in increased seed germination from 90 to 100 percent and enhanced plant growth in terms of length and weight of shoot and root (Pande and Choube, 2003)

**Pseudomonas fluorescens association in horticultural crops and spices**

Plant growth promoting rhizobacteria and their effectiveness has been established on foot rot and slow decline of black pepper and rhizome rot of ginger and clump rot of cardamom. Strains of *P. fluorescens* were found to increase the growth and of these crops apart from suppressing the soil borne diseases (Sarma *et al.*, 2003). They also
observed that growth promoting strains of *P. fluorescens* were found to synthesize phytohormone viz IAA and GA as detected in TLC. The other determinants for growth promotion in black pepper were enhanced production of feeder roots in the plant and also the increased absorptive surface area of the roots. Sipirin (2000) found that the Vetiver could grow and survive without nitrogen and phosphorous application especially in the infertile soil with the help of diazotrophs including the genera of *Pseudomonas*.

**Disease suppression mechanism of Pseudomonas**

Fluorescent *Pseudomonas* spp. make up a diverse group of bacteria that can generally be visually distinguished from other *Pseudomonas* by their ability to produce a water-soluble yellow – green pigment. They are found in soils, foliage, fresh water, sediments and seawater. During the last 25 years, research has illustrated the latent potential of exploiting certain bacteria for the biocontrol of root crop diseases.

**Siderophore – Mediated suppression**

Soil *Pseudomonas* generally produces fluorescent yellow-green, water-soluble siderophores with both a hydroxamate and phenolate group; these siderophores have been classified as either pyoverdins or pseudobactins. Analysis of pseudobactin – and pyoverdin – type siderophores from different fluorescent pseudomonad strains showed the principle is the composition, number, and configuration of the amino acid in the peptide back bone (Neilands. 1981) Fluorescent siderophores, which have high affinity for ferric iron, are secreted during growth under low- iron conditions. The resulting ferric – siderophores complex is unavailable to other organisms, but the producing strain can utilize this complex via a very specific receptor in its outer cell membrane (Buyer and Leong.1986). In this way, fluorescent *Pseudomonas* strains may restrict the growth of deleterious bacteria and fungi at the plant root
(Loper and Buyer 1991). These iron starvation conditions may also prevent the germination of fungal spores. The direct correlation has been observed in vitro between siderophores synthesis in fluorescent pseudomonads and their capacity to inhibit germination of chlamydospores of Fusarium oxysporum (Elad and Baker, 1985). Cline et al. (1982) reported that iron from microbial hydroxamate siderophores might become available to plants in nutrient solution and in soil. Siderophore producing Pseudomonas strains were more effective at protecting potato from diseases than some antibiotic – producing strains.

**Antibiotic production**

Antibiotic production by some Pseudomonas spp. is more recognized as an important factor in disease suppression of many crops. Compounds such as pyoluteorin, phenazine, pyrrolnitrin (Howell and Stipanovic, 1979, 1980), tropolane (Lindberg, 1981), pycocyanin (Dahiya et al., 1988) and 2,4, diacetylphloroglucinol (Keel et al., 1990) have also been isolated from soil fluorescent pseudomonads. Some of these compounds e.g., tropolane have a broad spectrum of activity against many bacteria and fungi. Howell and Stipanovic (1979) found that pyoluteorin from a fluorescent pseudomonads was an effective treatment for the protection of cotton seedling from Pythium induced damping off whereas pyrolnitrin from another pseudomonads was more effective for the protection of cotton seedlings from infection of Rhizoctonia solani. A correlation was also made between the inhibition of pathogens by production of antibiotics in vitro and the in vivo protection of plants from disease by selected strains.

**HCN production**

The production of hydrogen cyanamide by certain fluorescent pseudomonads may influence plant root pathogens. HCN production by fluorescent pseudomonads stimulated root heir formation and also induced plant resistance to certain pathogens.
Application of \textit{Pseudomonas fluorescens} on disease management

The ability of different rhizobacteria to colonize roots offering control of soil borne pathogens has to be reviewed (Weller, 1988). According to Rankin and Pualitz (1994) \textit{Pseudomonas fluorescens} (P150) significantly reduced the \textit{Pythium} root rot of cucumber. Application of \textit{P. fluorescens} as seedling dip and \textit{Bacillus subtilis} as soil application as best delivery systems be incorporated in integrated disease management (IDM) programme for the management of \textit{Fusarium} wilt of banana (Shamaro jahagindar and Siddaramaiah, 2003). It is also proved that \textit{P. fluorescens} isolated from \textit{Lotus corniculatus} rhizosphere produced HCN, siderophores and antibiotics and protected the plant from damping off caused by \textit{Pythium} spp. In \textit{Lens esculantus} (lentil), soil application of \textit{P. fluorescens} reduced the wilting caused by \textit{Fusarium oxysporum}. Application of \textit{P. fluroescens} is well known for its usefulness in the biocontrol of “take – all” disease of wheat caused by \textit{Gaeumannomyces graminis} var. \textit{tritici} (Cook and Ravira, 1976; Gerlagh; 1968; Shipton, 1975). A strain of \textit{P. flourescens} CHAO, isolated from a Swiss soil naturally suppressive to tobacco black root rot, caused by \textit{Thielaviopsis basicola} (Stutz \textit{et al.}, 1986), suppressed the disease in iron-sufficient soils. However, siderophores produced by \textit{Pseudomonas} species have been implicated in the biocontrol of damping off of cotton (Loper, 1988; Laha \textit{et al.}, 1992), root rot of wheat (Becker and Cook, 1988) caused by \textit{Pythium ultimum} and potato seed piece decay by \textit{Ervinia carotovora} (Xu and Gross, 1986) in suppression of several vascular wilts caused by \textit{Fusarium oxysporum} (Kloeppep \textit{et al.}, 1980a; Scher and Baker 1982; Sneh \textit{et al.}, 1984) and in growth responses of potato (Kloeppep \textit{et al.}, 1980b; Baker \textit{et al.}, 1987). Unnamalai and Gnanamanickam (1984) reported \textit{P. flourescens} was antagonistic to \textit{Xanthomonas citri} and \textit{Sarocladium oryzae}. It also suppressed sheath rot disease of rice caused by \textit{Sarocladium oryzae} (Sakthivel and Gnanamanickam, 1986b).
According to Podile and Dube (1988b) *P. flourescens* PN-3 reduced the incidence of stem rot of peanut, caused by *Sclerotium rolfsii* and *Rhizoctonia solani*. Samiyappan reported that *P. flourescens* isolate Pf is applied as seed/soil/foliar application in rice for the control of major diseases such as blast caused by *Pyricularia oryzae*, brown spot caused by *Helminthosporium oryzae*, sheath blight caused by *R.solani* and sheath rot caused by *Sarocladium oryzae*.

**Azospirillum**

The most widely studied group of free-living plant associated bacteria is the N-fixing genus *Azospirillum* (Vande Broek et al., 2000). Five species of *Azospirillum* have been described to date *A. brasilense*, *A. lipoferum*, *A. amazonense*, *A. halopraeferens* and *A. irakense* (Okon and Labandera-Gonzalez, 1994). All *Azospirillum* species can increase plant growth; it is likely that the mechanism of plant growth promotion by *Azospirillum* are controlled by some highly conserved part of genome. The most reproducible effect on plants inoculated with *Azospirillum* is greatly altered root architecture with increased overall root growth, greater production of root heirs and enhanced root surface area (Bashan and Holquin, 1977: Fallik et al., 1994). The contribution of *Azospirillum* to the nitrogen economy of various ecosystems has been recognized (Doberenier and Polli, 1998). However N fixation is not the sole cause of growth responses in *Azospirillum* inoculated plants (Kapulnik et al., 1985a). It has been suggested that these bacteria produce growth-promoting substances (Okon and Kapulnik, 1986), which results in the growth responses. These plant growth substances induce root elongation and the proliferation of lateral roots and root hairs concomitant with enhanced water and mineral uptake, which is in turn results in enhanced plant growth (Kapulnik et al., 1985b). Increased NR activity of the leaf indicated the enhanced nitrogen assimilation of the plants due to *Azospirillum* inoculation. However, this existed strain variation in the
growth response, which may correct with their potential in fixing atmosphere N and production of growth promoting substance. *A. brasilense* is capable of producing gibberellin and cytokinin-like substances (Tien *et al.*, 1979) and also auxins such as Indole Acetic Acid (IAA) when tryptophan was added in culture (Reynders and Vlassak, 1979; Tien *et al.*, 1979).

Several reports indicate isolation of *Azospirillum* from the rhizosphere (Caballero-Mallado and Valdes, 1983). *Azospirillum* has also been isolated from fresh root of various non-gramineous plants such as *Ipomoea batatas, Manihot esculenta* and *Pteridium aquilinnum* (Dobereiner, 1978) *Azospirillum* spp. have been isolated from many tropical trees (Subba Rao, 1984) and root epidermis of *Digitaria decumbens* (Dobereiner and Day, 1974). Three salt tolerant *Azospirillum brasilense* strains were isolated from the roots of finger millet grown in saline calcareous soil (Rai, 1991). The application of *Azospirillum* in agriculture is well established.

**Effect of *Azospirillum* on agricultural crops**

*Azospirillum* is an important biofertilizer in many crops as its contribution to nitrogen economy is in significant level. Mertens and Hess (1984) reported increased yield and higher nitrogen content in grains of *A. lipoferum* inoculated wheat (*Triticum aestivum* L.) both under temperate greenhouse and field conditions, which has been attributed to $N_2$ fixation. Inoculation of *Azospirillum* to seed and seedlings along with soil application was increased growth and yield of rice (Rao *et al.*, 1983; Watanable and Lin, 1984). Several authors confirm the beneficial response of wheat, barley, sorghum and oats, grasses and pearl millet, peanut and onion inoculated with *A. brasilense* alone or in combination with AMF (Bouton *et al.*, 1979; Tien *et al.*, 1979; Kapulnik *et al.*, 1981).

Subba Rao *et al.* (1985) observed a synergistic effect of arbuscular mycorrhizae and *A. brasilense* which resulted in higher growth in barley
and increased uptake of phosphorus in pearl millet compared to inoculation with mycorrhizal or bacterial component. The *Azotobacter* and *Azospirillum* inoculation increased the rooting in culture (Dayakar Yadav and Nagendra kumar, 1991) number of rootlets, length of root system and weight of root mass, shoot length and fresh weight of shoots in leaf yield and growth mulberry (Santhanakrishnan and Oblisami, 1983; Das et al., 1990; Balasubramanian et al., 1992). Seedling height and dry weight were higher in *Azadirachta indica* (Muthukumar et al., 2001) inoculated with *A. brasilense*.

**Effect of Azospirillum on horticulture crop and spices**

The yield increase due to inoculation of *Azospirillum* ranged from 5 to 30 percent in crop species (Bashan and Levanony, 1990; Okon et al., 1994). Inoculation of *Azospirillum* in pepper enhanced plant height, leaf area, shoot and root dry matter production at 90 days after planting (DAP) compared to uninoculated control (Kandiannan et al., 2000; Govindan and Chandy 1985; Bopaiah and Khadar, 1989). According to Merina Premkumar and Balasubramanian (1993), the combined inoculation of *Azospirillum* and VAM (*G. marigarita*) significantly increased the shoot length, root length and total dry weight of coffee seedlings (*Coffea arabica*). It was also supported by Glory swarpa (1996), that combined inoculation of *Azospirillum* with other biofertilizers increased the stem girth and tap root length of coffee seedlings. In organic tea cultivation, the combined inoculation of *A. brasilense*, VAM and Phasphobacteria enhanced the growth and nutrient uptake (Rajagopal and Ramarethinam, 1997). Subba Rao (1983) reported the occurrence of *Azospirillum* in coconut roots. In neem, inoculation of *Azospirillum* reduced fertilizer requirement in plant production (Pacovsky et al., 1985; Okon and Labandera-Gonzalez, 1994). Application of broth culture of *Azospirillum* promoted the growth of cocoa seedlings (*Theabroma cacao*) (Govindan and Vikraman Nair, 1983).
Trichoderma

Isolates of the fungal genus *Trichoderma* are among the most used biocontrol agents. *Trichoderma* spp. is frequently isolated from soil, and most biocontrol trials have done against soil-borne plant pathogens (Papavizas, 1985). However, pathogens on aerial plant surfaces have also been successfully controlled by *Trichoderma* spp. Wood and Tveit (1985) considered using antagonists against pathogens on aerial plant parts. The authors reasoned that antagonists needed to have a high reproductive capacity, to be able to survive under unfavourable environmental conditions, and to be very aggressive or antagonistic.

*Trichoderma* spp. in many ways fulfils these demands as a biocontrol agent. They are fast-growing organisms (radial growth of 15-mm day\(^{-1}\) on solid media is not unusual) and they have simple nutrient requirements. A mineral solution with one of several carbohydrates is sufficient for fast growth. Most isolates tested exhibit one or more of the following mechanisms of antagonism: production of non-volatile or volatile antibiotics, competition for nutrients and hyphal interactions (Dennis and Webster, 1971; Tronsmo and Dennis, 1978). Spores of *Trichoderma* spp. are used for most biocontrol purposes. Most isolates produce masses of spores, either conidia on solid surface or chlamydomspores in liquid cultures. Because biocontrol trials against a pathogen in a field situation must be performed together with chemical control of other diseases, tolerance to agrochemicals is of importance.

The nitrogenous fertilizers such as urea, ammonium sulphate and ammonium chloride favoured the growth and survival of *T. harzianum* in soil (Jayaraj and Ramabadran, 1998). Various types of agricultural wastes and crop residues have been shown as effective carrier media for the mass multiplication and field application of antagonists (Mukopadhyay, 1987, Kousalya and Jeyarajan, 1990). Easily available and least expensive products such as tea waste and coffee husk can be
best utilized in combination with farmyard manure as the carrier media for mass culture of *Trichoderma* spp. (Suseela Bhai *et al.*, 1994).

**Application of Trichoderma for disease management in agricultural crops**

The soil borne pathogens are reduced through the application of effective strains of *Trichoderma* spp. Singh *et al.* (1991) reported antagonistic property of *T. viride* and *T. harzianum* against teliospores of karnal bunt (*Neovossia indica*) of wheat. The antibiotic such as suzukacillin (Ooka *et al.*, 1966), alamethicine (Meyer and Reusser, 1967) and U – 21963, i.e., dermadine (Pyke and Dietz 1966: Meyer, 1966) were extracted from culture filtrate of *T. viride*. Trichodermin, a commercial product completely controlled *Helminthosporium* and *Fusarium* rots of wheat (Krisvoshchekova and Mishchenk, 1990). *T. harzianum* decreased the inoculum potential of *Rhizoctonia solani* and increased disease control in radish (Henis *et al.*, 1978). Significant reduction of collar rot of ground nut caused by *Aspergillus niger* (Lashin *et al.*, 1989), reduction of damping off of cucumber caused by *Pythium aphanidermatum* (Sharif *et al.*, 1988) and reduction of stem rot of sugar beet caused by *polymyxa betae* (D’Ampara *et al.*, 1986) have been reported. Successful control of *R. solani* incited seed rot, damping-off and root rot diseases in tomato, okra, radish, bean and carnation under greenhouse and field conditions was achieved by using *T. harzianum* (Elad *et al.*, 1981: Lifshitz *et al.*, 1985: Lewis *et al.*, 1995: Roberti *et al.*, 1993). According to Ushamalini *et al.* (1997) seed treatment with *T. viride* and *T. harzianum* reduced the charcoal rot incidence of Cowpea. This is in line with the findings of Krishnaveni (1991) who reported that seed treatment with *T. viride* was very effective in controlling charcoal rot of soy bean. The higher yield was obtained in soybean in *Rhizoctonia* infected soil sowing the seeds treated with *T. harzianum* (Kommendahl *et al.*, 1981).
**Application of *Trichoderma* for disease management in horticulture crops and spices**

The beneficial effect of *Trichoderma* includes plant growth promotion, biological control of various diseases by antagonizing the seed and soil borne pathogens and activation of the defense responses of the host plant. Vijayan *et al.* (1994) reported that in cardamom, exotic isolate of *T. harzianum* and native isolate of *T. viride* were found to be most effective and reduced the disease incidence up to 52 to 62 percent. According to Monoranjitham (1999) talc based formulation of *T. viride* could be used for effective control of damping-off of chillies and also obtained more vigorous seedling. Ram *et al.* (1999) observed that soil application of *T. harzianum* and rhizome treatment with fungicides was most effective for the management of rhizome rot of zinger. Several antagonists like *T. harzianum* and *T. virens* reduced the severity of foot rot incidence of black pepper (Rajan, 1999; Rajan *et al.*, 2002). According to Sarma *et al.* (2000) biocontrol consortium for pepper, ginger and cardamom was established. The maximum disease suppression (63%) obtained by the treatment combination *T. harzianum* isolate IISR 1369 and *P. fluorescens* strain IISR-6 in pepper and in cardamom; it was 36% over control. The same treatment could impart 66.2% survival of ginger tillers after challenge inoculation with *Pythium aphanidermatum*. Ramanayagam *et al.* (1999) reported that stem bleeding disease of coconut was reduced through the application of *T. harzianum*, *T. viride* and *T. hamatum* by 66.9, 63.4 and 57.7 percent respectively.

**Induced systemic resistance by microbial inoculants**

Several studies have indicated that plants inoculated with selected endophytic bacteria could induce resistance against vascular pathogens. The defense responses by these bacteria including the production of phytoalexins, accumulation of pathogenesis related proteins, deposition of structural barriers in the cell wall of the host
plants and by production of antimicrobial compounds and siderophores (Manjula, 2002). According to Dowling and O'Gara 1994, some of the rhizobacteria produce wide range of secondary metabolites such as siderophores, HCN and antibiotics which are known to induce pathogen growth inhibition. In pigeon pea B. subtilis induced an increase in PAL and peroxidase activities (Podile and Laxmi, 1998). P. aphanidermatum. Chen et al. (2000) reported that Pseudomonas currugata strain 13 and P. aureofaciens strain 28 – 63 were shown to systemically suppress P. aphanidermatum in cucumber roots. In chilli (Capsicum annuum L), P. chlorophis and B. subtilis triggered the defense related enzymes such as peroxidase (PO), polyphenol oxidase (PPO) and phenylanline ammonia lyase (PAL) and also controlled the damping off disease (Kavitha et al., 2003). According to Srivasava and Arora (2003), the isolates P. fluorescens reduce the incidence of charcoal rot of chickpea when applied as soil drench or seed treatment. The enhanced control of this disease may be due to the secretion resistance inducer such as indole-, salicylic – and aminoburic acid derivatives. Charcoal stump rot caused by Ustulina zonata of tea was significantly reduced when the seedling were inoculated with G. fasciculatum and T. harzianum individually or in combination. The reduction of this disease may due to the increased level phosphorous, phenol and reducing sugar by AM fungi (Roman, 2000) and enhanced activities of defense enzymes such as catalase, peroxidase, PAL along with lignifications of deposition in mycorrhizal root.

**Application of microbial inoculants with fungicides**

Most of the microbial inoculants are highly compatible with fungicides. Foliar spraying can be given to pepper cuttings with either Bordeaux mixture or copper oxichloride or bavistin at 15-20 days intervals during June and July to control the diseases of pepper in the nursery (Sarma, 2000). Thangammal et al. (2003) also reported that combined use of AMF + P. fluorescens (IISR) along with phorate and
copper oxychloride spray resulted better establishment and growth of pepper plants. In *Zizibus mauritiana*, the fruit rot was controlled through the combined application of *P. fluorescens* with fungicides such as thiophenate methyl, captan and aleidine at 50 ppm (Nallathambi and Thakare, 2003). According to Sugavanam *et al.* (1994) application of Fytolan (Copper oxychloride) in groundnut produced a remarkable range of positive responses in VAM infection and spore production, rhizobial nodulation, growth and yield. They also proved that the systemic fungicide like Emisan significantly decreased the growth and yield of the host plant. However, there are conflicting reports on the effects of various fungicides on VAM and rhizobial association in plants. Nemec (1980), Menge (1982) and Hale and Sanders (1982) reported reduced VAM infection with fungicide application while a stimulating effect was reported by Bird *et al.*, (1972), Sutton and Sheppard (1976), Menge (1982), Growth and Martinson (1983), Jabaji-Hare and Kendrick (1985), Afek *et al.* (1990) and Hetrick and Wilson (1991).

Systemic fungicides appear to be more damaging than protectants since may only postpone the infection, but not eliminate it (Menge, 1982). Fungicides in general do not have any bacterial activity. Insensitivity of different strains of *Rhizobium* to fungicides has been reported (Radhakrishnan and Chatrath, 1989: Singh and Agarwal, 1990). The pesticide such as phorate and chlorpyriphos could be safely applied with *T. harzianum* for the management of *Phytophthora* foot rot, nematodes and mealybugs on black pepper (Stephen Jebakumar *et al.*, 2000). In mungbean integration of *T. harzianum* as soil application and seed treatment significantly reduced the dry root rot incidence over soil treatment and seed treatment alone (Rajeswari *et al.*, 1999). Similar observations were confirmed in (Parkhia and Vaishnav, 1986). A combination of *T. viride* and was found to be effective against seed borne fungi in cluster bean (Shivanna and Shetty, 1989). Stem rot of
mustared caused by *Sclerotinia sclerotiorum* was reduced by combined application of *T. harzianum*, *T. viride* and *Gliocladium roeum* in combination with Bavistin (Pathak *et al.*, 2001). Mukhopadhyaya *et al.* (1992) reported reduction in chickpea wilt by treatment with *T. virens* and carbofuran. Similarly Pandey and Upadhyaya (1999) reported a maximum disease reduction of 81% by the application of *T. viride* and *T. harzianum*, next to *T. harzianum* and Thiram. Fytolan applied and inoculated with AMF and *Trichoderma* plants had higher plant growth and biomass compared to other fungicides. But the observed growth response could not be due to the direct effect of Fytolan, since Fytolan applied control plant did not exhibit any significant response in plant growth or biomass. In contrast increased biomass in response to Fytolan application due to the suppression of nutrient immobilizing microorganisms has been reported. (Hertrick *et al.*, 1991)

**Combined inoculation of microbial inoculants for diseases control**

Plant growth promoting rhizobacteria are of increasing importance as inoculants for bio-fertilization, biostimulation and biological control of plant pathogen in sustainable agriculture (Weller, 1988). Siddiqui *et al.* (1998) assessed the effect of *Glomus mosseae*, *Paecilomyces lilacinus* and *P. fluorescens* used alone or in combination to control wilt complex of pigeon pea. According to Prasad *et al.* (2003) a mixture of *P. fluorescens* and *T. viride* at 45,60 and 75 days after sowing significantly reduced blight incidence of sunflower and found better than individual treatments of *Trichoderma* and *Pseudomonas*. They also proved that highest yield (840 kg/hac.) was recorded in treatment with *P. fluorescens* and *T. viride* mixture. Anahosur and Patil (2001) found that the seed treatment with *Trichoderma* spp. and planting such seeds in soil amended with neem cake or FYM in a best eco-friendly practice for the management of cowpea dry root disease. Vanitha and Suresh (2001) reported that the *P. fluorescens* seed treatment @ 19g/kg of seed + soil application of neem
cake and FYM recorded less root rot disease incidence as against control in mung bean. Chandrasekaran et al. (2001) also found that the seed treatment with *T. viride* were on par with *P. fluorescens* treated seed in reducing the black gram seed borne disease. Seed treatment with *T. viride* and *P. fluorescens* alone or in combination with soil amendments like neem cake and vermin compost found to be the best eco-friendly strategy for the management of root rot of cow pea. The efficiency of *P. fluorescens* and *Trichoderma* in mixture for plant growth promotion and disease suppression in different spice crops (Jisha et al., 2003).

According to Hazarika and Phookan (2000) inoculation of tea seedlings with *G. fasciculatum, P. fluorescens* and *T. harzianum* individually or in combination in disease sick plot significantly suppressed the disease and increased seedling stand, plant growth, dry matter production and nutrient uptake of tea seedlings. The effect was more pronounced in combined inoculated compared to individual inoculated seedlings. The reduction of diseases in many crops may be due to antagonistic effect of *T. harzianum* (Mukhopadhyay, 1987) and *P. fluorescens* Dilip Kumar and Benzbaruah, 1997) or due to increased level of phosphorous, phenol and reducing sugar by AM fungi (Roman, 2000) and enhanced activity of defense enzyme such as catalase and peroxidase along with lignification and phenol deposition in mycorrhizal root.