

**Influence of mycorrhizae on root rot in apple
(*Malus domestica* Borkh.)**

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(2005-A-765-M)



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TECHNOLOGY OF KASHMIR**

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**Influence of mycorrhizae on root rot in apple
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(2005-A-765-M)



THESIS

Submitted to

**The Faculty of Postgraduate Studies
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partial fulfilment of requirement for the award of the degree of**

**MASTER OF SCIENCE IN AGRICULTURE
(Plant Pathology)**

2008



to loving Grandmother

*Late Mrs. Hafiza Bano who left to heavenly
abode on 14th April 2000*

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
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Certificate – I

This is to certify that the thesis entitled **“Influence of mycorrhizae on root rot in apple (*Malus domestica* Borkh.)”** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science in Agriculture (Plant Pathology)**, to the Faculty of Postgraduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, is a record of bonafide research work carried out by **Ms. Saba Banday (Regd. No. 2005-A-765-M)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that any help or information received during the course of investigation have duly been acknowledged.

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Title of the Thesis : **“Influence of mycorrhizae on root rot in apple (*Malus domestica* Borkh.)”**

ABSTRACT

Studies were conducted on the “Influence of mycorrhizae on root rot of apple (*Malus domestica* Borkh) during 2006-2007 at SKUAST-K, Shalimar Campus, Srinagar, Kashmir. The pathogens associated with root rot disease were isolated and morphologically characterized, and identified as *Dematophora necatrix* Hartig. and *Pythium ultimum* Trow. *Dematophora necatrix* caused white root rot while *P. ultimum* caused collar and root rot. Both the fungi proved pathogenic and produced characteristic root rot symptoms when inoculated separately. The bioassay of biocontrol agents viz. *Laccaria laccata*, *Suillus granulatus* and *Trichoderma harzianum* against both the pathogens revealed that all the three bioagents inhibited the mycelial growth of *D. necatrix* and *P. ultimum* in dual culture. *T. harzianum* was most effective and mycoparasitized the pathogens. Locally isolated ectomycorrhizae *L. laccata* and *S. granulatus* caused hyphal lysis of *D. necatrix* and *P. ultimum* in dual culture with *L. laccata* proving more effective. Two endomycorrhizae viz., *Glomus mosseae* and *G. calendonius* were isolated from apple tree rhizosphere. *G. mosseae* proved more effective in improving plant growth, root colonization and rhizosphere phosphatase activity. During *in vivo* studies biocontrol agents (*G. mosseae*, *L. laccata* and *T. harzianum*) inoculated individually and in combinations significantly improved the growth and biomass of apple seedlings. Among the bioagents inoculated individually *G. mosseae* showed significantly higher growth followed by *T. harzianum* and *L. laccata*. The combined inoculation of biocontrol agents showed non-additive synergistic growth promoting action and proved superior in increasing apple

seedling growth than individual inoculation. Growth and biomass of apple seedlings was significantly reduced by root rot pathogens (*D. necatrix* and *P. ultimum*). *D. necatrix* proved more pathogenic and caused 35.4 per cent more root rot than *P. ultimum*. Biocontrol agents, however, inoculated individually or in combination reduced the pathogenic effect and significantly improved the growth and biomass of apple seedlings as compared to the seedlings inoculated with pathogen alone. The biocontrol agents inoculated individually reduced the root rot by 52.9 to 37.9 per cent as compared to the seedlings inoculated with *D. necatrix* alone, with maximum reduction by *T. harzianum* and minimum by *L. laccata*. Co-inoculation of all the three biocontrol agents resulted in highest mycorrhizal colonization (43.3%). In presence of root rot pathogen root colonization by mycorrhizae was drastically reduced (30.5%). Biocontrol agents in rhizosphere of apple enhanced soil acid phosphatase activity. *G. mosseae* exhibited higher acid phosphatase activity followed by *L. laccata*. So inoculation of apple plants at nursery stage with appropriate and effective microbial inoculant, alone or in combination can prove effective in enhancing plant vigour and reduce root rot severity.

Key words : Apple, Biocontrol, *Dematophora necatrix*, Ectomycorrhizae, Mycorrhizae, *Pythium ultimum*, Root rot, *Trichoderma harzianum*, VAM

Signature of Student
Dated : _____

Signature of Major Advisor
Dated: _____

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Place : Shalimar, Srinagar

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner during Viva-Voce examination held on 03.10.2008 have been incorporated in the manuscript entitled “**Influence of mycorrhizae on root rot in apple (*Malus domestica* Borkh.)**” submitted by **Ms. Saba Banday (Regd. No. 2005-A-765-M)**.

Dr. G.H. Dar
Chairman
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CHAPTER – 1

INTRODUCTION

Apple (*Malus domestica* Borkh.) is a premier table fruit, rich in nutrients especially fats, carbohydrates, calcium, phosphorus and iron (Hudson *et al.*, 1988). It is cultivated in temperate regions of the world and, to some extent, in cool highlands of some sub-tropical regions such as East Africa, Northern parts of India etc. India in terms of apple fruit production ranks fourth in the world. In India, apple cultivation alone accounts for 55 per cent of the total area and 75 per cent of the total production under temperate fruits in the country (Chadha, 1992). Commercial cultivation of apple fruit in India is confined to the States of Jammu and Kashmir and Himachal Pradesh, and some selected areas of Uttranchal, Arunachal Pradesh, Manipur and Sikkim. In Jammu and Kashmir apple is grown on an area of 1.07 lakh ha with annual production of 12.08 lakh metric tonnes, which constitutes about 84 per cent of total fruit production in the State (Anonymous, 2007).

Amongst the various constraints faced in apple production, diseases have assumed greater significance. Apple is prone to a number of fungal, bacterial and viral diseases which significantly affect its quality and production. However, fungal diseases inflict huge losses to the crop. The

major fungal diseases include scab, powdery mildew, *Alternaria* leaf spot, root rot, collar rot etc. Amongst these diseases, root rot in recent years has posed a serious threat to apple plantation in Jammu and Kashmir State.

The soil borne diseases are more destructive in apple than foliar diseases as the pathogens are capable of killing the plants right from its nursery stage and cause complete death of host. Root rots are incited by many fungi which include *Pythium*, *Phytophthora*, *Dematophora*, *Fusarium*, *Armillaria* and *Rhizoctonia* (Gupta and Sharma, 2000). The annual losses due to root rot in apple in India have been estimated to be approximately 1.3 million rupees (Agarwala and Sharma, 1966).

Root rot pathogens, especially white rot pathogen, attack the host roots and cover root surface with white mycelial growth more prominently during rainy season. The disease eventually leads to the death of whole plant particularly in poorly-drained soils. White root rot as fruit crop disease has been first reported from Germany in 1883 and later in 1891 from France on grapes (Viala, 1891). However, on apple it was first observed from Norwich, England in 1913 (Salmon and Wormald, 1913) and later reported from many countries like Israel, USA, Brazil etc. (Thind, 2001). In India, the fungus was initially encountered in Uttranchal hills by Singh in 1939 (Bose and Sindhan, 1976) and later the disease was noticed

in Himachal Pradesh in 1960 (Agarwala, 1961). The occurrence of perfect stage (*Rosellinia necatrix* Prill) of the pathogen has not so far been reported from India.

Besides white root rot fungus, some other soil-borne pathogens like those belonging to family Pythiaceae cause root, collar or crown rot. Starting its infection from collar the pathogenic attack spread towards roots and eventually causes the death of seedlings (Gupta and Sharma, 2000).

To mitigate the losses caused by these diseases the soil application of chemical fungicides such as carbendazim, aureofungin and copper sulphate have been recommended by various workers (Agarwala and Sharma, 1975; Gupta, 1977). The management of root rot through chemical use has, however, many deleterious effects on beneficial micro-organisms including mycorrhizal fungi. Further, the growing awareness about pesticidal pollution has compelled the growers to reduce chemical use in disease management. Therefore, an integrated approach is considered most suitable approach to effectively manage the root diseases. Biological control as a component of integrated management offers great potential to restrict root rot pathogenic attack. Mycorrhizae and fungal antagonists are feasible biocontrol agents often used against some root rot diseases. Ecto- and endo-phytic mycorrhizae are involved in bipartite symbiotic associations with

their host plants (O’Keefe and Sylvia, 1992). Since mycorrhizae are major components of rhizosphere, therefore it is logical that they could influence the incidence and severity of root diseases. Several field and laboratory experiments have demonstrated that mycorrhizal colonization greatly improves the growth and nutrition of host plants (Sharma *et al.*, 2002) and also effectively suppress the soil borne plant pathogens (Schenck, 1981; Dehne, 1982). Mycorrhizal fungi are reported to reduce soil borne diseases through cross protection mechanism, better competitive ability, release of phytohormones and pathogen-inhibiting chemicals (Zargar *et al.*, 2000).

Various species of *Trichoderma* are known to serve as effective biological control agents against various soil borne plant diseases. *Trichoderma* sp. antagonize fungal pathogenic organisms by producing volatile and non-volatile antibiotics or/and by competing for nutrients and space as well as through mycoparasitism (Martinez *et al.*, 2003). Attempts have been made to explore bicontrol potential of this organism against many root rot diseases in apple (Ashwani *et al.*, 2004), however, no research has been conducted on interaction between root rot pathogen, mycorrhizae and *Trichoderma* in apple in Jammu and Kashmir. Keeping in view the above facts, the present study was aimed at with the objectives as under:

- Isolation, characterization and identification of mycorrhizae from apple tree(s) rhizosphere, their screening and maintenance;
- Isolation and characterization of root rot pathogen from apple tree(s);
and
- Interaction studies between mycorrhizae and root rot pathogen.

CHAPTER – 2

REVIEW OF LITERATURE

The soil borne diseases pose serious threat to apple cultivation as they are more destructive than foliar ones and affect the plant right from its seedling stage. Amongst these diseases, root rots have assumed greater significance due to their more devastating nature. The present review comprehends the information gained on white root rot and *Pythium* root rot diseases and their biomanagement, with special emphasis on mycorrhiza and *Trichoderma*.

2.1 Status of root rot diseases

Root rot in apple is incited by many pathogenic fungi viz., *Pythium*, *Phytophthora*, *Dematophora*, *Fusarium*, *Armillaria*, *Rhizoctonia* etc. (Gupta and Sharma, 1988). Root rot infection is usually deep-seated, therefore visible symptoms generally appear on the foliage when plants are already exposed to severe damage. Sanhueze *et al.* (1988) reported crop loss in apple due to root rot worth 6 million US dollars in Brazil, with *Phytophthora*, *Pythium* and *Fusarium* as the most common root rot causing pathogens. Agarwala and Sharma (1966) reported that upto 15 per cent adult apples trees and 33 per cent nursery seedlings in Himachal Pradesh (India) suffered from root rot thus leading to the monetary loss of 1.3 million rupees. In India, annual loss of apple trees due to root rot disease in

Himachal Pradesh alone has been estimated to be 16-25 per cent (Tapwal *et al.*, 2005). The root rot disease in apple is of greater concern in India.

2.2 White root rot

White root rot is caused by *Dematophora necatrix* Hartig and is one of the dreaded root rot diseases of apple. In the beginning of twentieth century Nattrass (1927) observed the death of some apple trees in a garden at Winscombe, Sommerset (UK), and reported *Rosellinia necatrix* (perfect stage of *D. necatrix*) to be the pathogen responsible for the disease. The first report on the occurrence of white root rot from India was published in the year 1939 by Singh from Uttranchal (Bose and and Sindhan, 1976). Later on, Agarwala (1961) reported the disease from Himachal Pradesh. The pathogen has a very wide host range of about 170 plant species or varieties spread in 63 genera belonging to 30 families of plant kingdom (Khan, 1959). The fungus attacks fruit trees in particular and is of economic importance both in nurseries and orchards.

2.2.1 Disease symptoms of white root rot

Dematophora necatrix Hartig produces root rot and wilt symptoms on apple seedlings. There is a direct correlation between the intensity of underground infection and production of foliar symptoms. The above-ground symptoms of the disease included progressive decline in plant

vigour accompanied by drooping, bronzing, diminution and wilting of leaves followed by defoliation and death of the trees (Thakur *et al.*, 2005). The symptoms are more pronounced and damage severe in nursery. Underground infection starts from fine roots and with the progression of disease the fibrous root system entirely disappears. The older main roots get covered with a greenish grey mycelial mat, which develop a flocculent web of whitish strands or ribbons during monsoon season (Natrass, 1927; Freeman *et al.*, 1990). Natrass (1927) also observed that white flocculent web disappeared after the death of host, leaving behind round black sclerotia. Agarwala and Sharma (1966) reported that the fungus does not infect xylem tissues of apple roots, however bark is completely damaged. Gupta (1977) reported that the pathogen can be isolated from woody parts of diseased apple roots. He further reported that infection occurs when the fungus comes in direct contact with fine root system. From the fine roots it reaches the main root and ultimately devours the root system, as a result of which plants start drying and fruit size remains small. De-Sousa *et al.* (1995) and Lee *et al.* (1995) also observed the development of white mycelial fan which first killed the smaller roots and then invaded the larger ones. They considered it as the characteristic symptom of the disease.

2.2.2 Characteristics of *Dematophora necatrix*

Dematophora necatrix Hartig belong to the Phylum Ascomycota, sub-division Ascomycotina, class Pyrenomycetes, order Sphaeriales and family Xylariaceae (Singh, 2000). The mycelium of fungus is hyaline, branched, septate and knotted. The hyphae are slightly swollen at septa thus giving a characteristic constricted appearance. The mycelial webs are seen on affected roots as mass of white strands but these disappear when roots die. Perithecia may appear occasionally in clusters. Perithecia are globose, black, short pedicillate at base, ostiolate and 1-2 mm in diameter. These are embedded in a ropy subiculum of brown septate hyphae which form a crust on host surface at the base of the perithecium. Asci are cylindrical, long stalked, with a single membrane, 8-spored and measure 250-380 x 8-12 μm in size. Conidiophores are formed independently or in association with perithecia on brown ropy synnemata which project straight outwards as rigid columns. The apex of these branches contains the conidiogenous cell. Conidia are solitary, simple, ellipsoid or obvoid, hyaline to pale brown, 1-celled, smooth and measure 3.0-4.5 x 2.0-2.5 μm in size (Singh, 2000).

2.3 *Pythium* root rot and damping off

Pythium is the most common causal pathogen of seed rot, seedling damping off and root rot of plants including apple. It also incites soft rot of fleshy fruits when fruits are in contact with soil. *Pythium* damping off is a

common problem in crop fields and greenhouses where the organism kills newly emerged seedlings (Jarvis, 1992). Many *Pythium* species, along with their close relative, *Phytophthora* species, are pathogens of economic importance in agriculture. *Pythium* sp. like *Pythium aphanidermatum*, *P. debaryanum*, *P. echinulatum*, *P. glomeratum*, *P. graminicola*, *P. inflatum*, *P. cylindrosporum*, *P. angustatum*, *P. butleri* and *P. ultimum* are reported to cause various root and collar rot (Owen, 2002) They infect papaya, ginger, field crops, cucurbits, mustard, apple etc. Members of genus *Pythium* are common inhabitants of agricultural soils and form diverse associations with a wide range of plant species. *Pythium* spp. tend to be very generalistic and unspecific in their host range (Owen, 2002). The genus *Pythium* encompasses no less than 120 different species, with many crop plants being susceptible to multiple species (Dick, 1990). The *Pythium* species associated with apple roots included *P. intermedium*, *P. ultimum*, *P. irregulare*, *P. heterothallicum*, *P. sylvaticum* and *P. vexans* (Mazzola, 2002), of these many have been reported to reduce the growth of apple (*Malus domestica* Borkh) seedlings, and are cited in numerous geographic regions as elements of pathogen complexes that incite apple replant disease (Dulerck *et al.*, 2002).

In hydroponic systems inside greenhouses where extensive monoculture

plants are maintained in plant nutrient solutions (containing nitrogen, potassium, phosphate and micronutrients) that is continuously recirculated to the crop, *Pythium* spp. cause extensive and devastating root rot (Jarvis, 1992; Owen, 2002). The root rot pathogen affects entire operations (tens of thousands of plants in many instances) within two or four days (Owen, 2002).

2.3.1 Disease symptoms of *Pythium* root rot

Initial symptoms of root rot caused by *Pythium* sp. are the appearance of chlorosis on apple plant. The plants gradually show wilt symptoms and later collapse. The wilted plants when uprooted show severe rotting in root and collar regions. The tissues of cortical regions are severely affected by disintegration.

Small water-soaked lesions first appear on the stem at ground level and attain considerable dimensions which gradually encircle the whole stem in severe cases. The stem is greasy to touch and exudes out latex. The bark at such places is dark brown and easily peeled off thereby exposing the inner tissues of the stem often in semi rotten state. The main root begins to rot and does not progress downward up to the tip. The lateral roots get also attacked. Usually lateral roots do not develop and taproots often become twisted (Owen, 2002; Mishra *et al.*, 2005; Mehrotra and Aneja, 2005).

2.3.2 Characteristics of *Pythium*

The genus *Pythium* was created by Pringsheim (1858) with the description of *Pythium monospermum* Pringhsh. as a type species. *Pythium* belongs to Phylum Oomycota, Sub-Division Mastigomycotina, order Peronosporales, class Oomycetes, family Pythiaceae (Middleton, 1943). The most recent taxonomic description of the genus lists 87 recognised species (Plaats and Vander, 1981).

The mycelium of *Pythium* contains coarse solids granular cytoplasmic contents. Hyphae are coenocytic, hyaline, usually 5-7 μm in diameter (occasionally upto 10 μm) and aseptate except when cultures are old or in the process of spore differentiation. The hyphae are both inter- and intra-cellular and lack haustoria. Sporangia are asexual spores which may germinate indirectly by the formation of motile zoospores or directly by the formation of a germ tube. The sporangia are terminal or intercalary in position. In *P. ultimum*, however, germination of sporangia occurs briefly by germ tube and these sporangia are sometimes referred to as conidia. Zoospores are not produced in *P. ultimum*. Sexual reproduction takes place by well-developed oogonia and monoclinal antheridia which are easily produced in culture (homothallic). After fertilization, oosphere secretes a double wall and develops into a smooth thick-walled spore. The oospore

germinates after a rest period of several weeks.

2.4 Management of root rot disease

In order to check the ravages caused by root rot diseases, various efforts have been made to manage them through cultural, chemical and biological methods. Reports revealed that field sanitation by digging out diseased roots greatly decreased the inoculum source in an orchard (Duan *et al.*, 1990). Similarly, soil amendment in conjunction with solar heating was more effective in reducing the population of *Fusarium oxysporum* f. sp. *conglutinans* than solar heating alone (Remirez and Munnecke, 1988). Apple root rot was also effectively controlled by soil solarization (Xu, 2001). Digging of isolation trenches around the infected trees and removal of affected roots reduced the disease spread (Sharma *et al.*, 2005). Further, it was noticed that improvement in soil drainage and addition of lime helped in reducing the root rot disease of apple. Use of rootstocks which show some resistance has been recommended for grafting (Sharma *et al.*, 2005). Ashwani *et al.* (2002) observed maximum control of root rot in soils amended with sawdust and pine needles which induced increase in population of selected antagonists. Crab apple seedlings grown in pots were partially protected from root rot disease when neem cake (10% w/w) was incorporated in soil one week before inoculation. The disease reduction was

52 per cent and symptom appearance was delayed (Mamta *et al.*, 2002). Sharma and Gupta (1995) observed deodar needles and daphne leaves to be most effective in delayed symptoms expression in seedlings upto 30 days in comparison to 15 days in untreated seedlings.

Metalaxyl, fosetyl-Al significantly reduced the percentage infection of artificially inoculated apple trees on MM-106 rootstock (Utkhede, 1987). Metalaxyl alone or in combination with thiobendazole controlled root rot on McIntosh apple seedlings caused by *Pythium ultimum* (Utkhede and Smith, 1991). Gupta and Gupta (1992) in a field trial advocated that aureofungin (0.02%) + copper sulphate (0.02%) drench is as good as carbendazim, not only in controlling the root rot but also enhancing the yield of rot affected apple trees. The yield of root rot affected trees was improved by aureofungin (0.02%) + copper sulphate (0.02%) drench while carbendazim (0.1%) drench alone or in combination with furadan (30 g/plant) proved best in enhancing the mean annual intension growth (Gupta *et al.*, 1995).

Many fungicides, primarily used to reduce the infection caused by soil borne pathogens, have resulted in increase (Jahangir *et al.*, 1996), no effect (Nemec, 1980) or delay/reduction in beneficial resident organisms including VAM (Clark, 1978) but rarely eliminated them (Menge, 1982). The fungicides applied for the management of soil borne diseases are

vulnerable to physical, chemical and biological interactions in the rhizosphere that makes them ineffective. Most of the fungicides form suspension in soil water and get absorbed to the soil particles when applied as drench thus are unable to reach the infection sites (Sharma, 2000). In addition to being a costly method, frequent and excessive use of chemicals disturbs the soil ecology (Gupta and Sharma, 2004). In present day agriculture, the exclusive use of chemicals for plant disease management is discouraged and thrust is laid on integrated approach. The use of biological control agents is safe method of disease control.

2.5 Bio-management of root rot

Biological control is the reduction of the amount in inoculum or disease producing activities of a pathogen or parasite in its active or dormant stage by one or more organisms accomplished naturally or through manipulation of host, environment or antagonist or by mass introduction of one or more antagonists (Baker and Cook, 1974). Cook (1987) defined biological control as the use of natural or modified organisms, genes or gene products to reduce the effect of pests and diseases. At present biological control is an important component of plant disease management practice. Concerns about the adverse consequences of chemicals on environment have forced researchers to seek alternatives to chemical

control of plant pathogens (Ooijskaas *et al.*, 1998). Several workers have reviewed the various aspects of biocontrol of plant pathogens (Papavizas, 1985; Cook, 1993; Mukhadhyay, 1994; Jayraj and Ramabadran, 1996; Dube, 2001; Sendhilvel *et al.*, 2005; Shalini and Dohroo, 2005; Harman, 2006).

2.6 Biocontrol agents

2.6.1 Mycorrhizae

Frank (1985) was first to coin the term 'Mycorrhiza' (literally means the fungal roots) for designating a symbiotic relationship between fungi and plant roots. Since then many research papers and review articles have been published at national and international levels which have enriched our knowledge in understanding the types and qualities of mycorrhizae and host parasite relationship (Perrin, 1990; Sharma and Mukerji, 1992; Tinker *et al.*, 1992). Two main types of mycorrhizae frequently occurring in nature are (i) endomycorrhiza, in which the fungi are primarily within the roots, and (ii) ectomycorrhiza, wherein fungal partner form sheath/mantle around the host root but do not invade deep.

2.6.1.1 Endomycorrhizae

The endomycorrhizae, commonly known as vesicular-arbuscular mycorrhizae (VAM), are important because of their nutrient solubilizing/

mobilizing and plant growth promoting properties and their large scale association with all kinds of agricultural and horticultural crops including apple. These fungi were named as vesicular-arbuscular mycorrhizae (VAM) by Dangeared in 1900 (Somani, 2005). The fungi forming VAM belong to the class Zygomycetes, order Endogonales and family Endogonaceae. Pyronel (1924) was the first to recognise VAM fungi as species of *Endogone*. Mosse (1956) was the first to experimentally demonstrate that the species of *Endogone* could produce vesicular-arbuscular mycorrhiza. Using sporocraps of unnamed *Endogone* sp. as inoculum, she was able to produce a typical VA mycorrhiza. The important genera of endomycorrhizal fungi reported so far are *Acaulospora*, *Entrophosphora*, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora* (Mortan, 1988). All these fungi are obligate parasites of plant roots and develop symbiotic relationship with their hosts. However, only one VAM fungus *Glomus intraradices* has so far been successfully cultured artificially (Abdoulaye, 2003).

The genera of family Endogonaceae are distinguished at first level on their manner of spore formation i.e. spore morphology and spore bearing structures. Further generic separation is based on sporocarp morphology. *Entrophosphora*, *Acaulospora*, *Gigaspora*, *Glaziella*, *Glomus* and

Sclerocystis do not form zygospores. Different species can be separated on the basis of sporocarp size, spore dimension, presence/absence of hyphal mantles, spore ornamentation (warts, wrinkles, pits, reticulum, spines etc.), spore walls, spore content, hyphal attachment, manner of spore germination, histochemical reaction etc. (Schenck, 1982).

The most important role of VAM fungi is to absorb nutrients from the soil and transfer them to their hosts (Geldermann, 1968). These fungi benefit the host plant primarily by increasing the uptake of P, Zn, Cu, Fe and K (Mosse, 1973). Several studies indicate that VAM fungi such as *Glomus*, *Gigaspora* etc. impart resistance against soil borne plant pathogens, especially root rot and wilt pathogens (Schenck, 1981; Dehne, 1982).

2.6.1.2 Ectomycorrhiza

Ectomycorrhizae have been observed on about 10 per cent of the world flora (Bakshi, 1995). In temperate forests these are found on about 90 per cent of the trees mostly belonging to the families Pinaceae, Fagaceae, Betulaceae, Salicaceae, Juglandaceae, Myrtaceae and Ericaceae (Marx, 1991). In natural ecosystem hundreds of ectomycorrhizal fungi have been reported on a single tree specie and approximately 70 per cent higher plants are obligately dependent on fungal associates, 12 per cent facultatively

dependent and 18 per cent remain typically uninfected (Trappe, 1987). Some ectomycorrhizal fungi such as *Pisolithus tinctorius* and *Cenococcum geophilum* are reported to colonize many hosts belonging to different genera (Marx, 1972).

The main genera of fungi that form ectomycorrhizae in temperate regions belong to Basidiomycetes although some ascomycetous fungi also form hypogeous sporophores (Marx, 1972). Basidiomycetous ectomycorrhizal fungi produce mushroom like epigeous sporophores, puff-balls and truffle-like hypogeous sporophores. Among the basidiomycetous fungi, *Boletus*, *Cortinarius*, *Suillus*, *Russula*, *Gomphidius*, *Hebeloma*, *Tricholoma*, *Laccaria*, *Lactarius*, *Rhizopogon*, *Scleroderma* and *Pisolithus* are most promising so far as the formation of ectomycorrhizae is concerned. Certain orders in the Ascomycetes such as Eurotiales, Tuberales and Pezizales have the species that are capable to form ectomycorrhizae (Marx, 1991).

The ectomycorrhizal fungi are distinguished by their morphology i.e. whether they are simple, bifurcate, coralloid, monopodial, recemose, pyramidal or pinnate; the nature of individual fork whether white, creamish-pale, pale-orange, brown, bright to yellow, chestnut brown or jet-black; surface of mycorrhizae whether smooth with loose weft, surface having white crust or surface densely tomentose (Mehrotra and Aneja, 2005).

Ectomycorrhizal fungi form a compact mantle of hyphae covering the slow growing unsubsized parts of roots and penetrate intercellularly to varying depths into root cortex to form a complex system known as Hartig net (Bakshi, 1995). The mantle varies in thickness from loose wefts of hyphae to dense pseudoparenchyma with firm surface. Ectomycorrhizal fungi produce auxins that help mycorrhizal roots to differentiate morphologically from uninfected roots of similar age such roots are thicker, more brittle and usually differently coloured (Paul and Clark, 1989).

Ectomycorrhizae has been reported to increase the plant nutrient uptake especially N and P contents and phosphatase activity (Abuzinadah and Read, 1986; Koide, 1993; Rajkumar *et al.*, 1990). Sudhakara and Natarajan (1997) reported that ectomycorrhizal association in plants is not simply a symbiosis for nutrition of the host but also for defence against pathogens. Tapwal *et al.* (2005) demonstrated that ectomycorrhizae aid in the uptake of tree nutrition besides protect root tissues from pathogenic invasion.

Marx and Davey (1969) were the first to report the antagonistic properties of mycorrhizal fungi against pathogenic fungi and soil bacteria. They observed that ectotrophic mycorrhizal fungi (*Laccaria laccata*, *Lactarius deteciosis*; *Kucopaxillus cerealis* var. *piceina*, *Pisolithus*

tinctorius and *Suillus luteus*) were antagonistic to *Phytophthora cinnamomi* and other root pathogenic fungi and bacteria. In dual cultures on agar media, diffusible metabolites of a mycorrhizal strain of *Laccaria laccata* inhibited the growth of *F. oxysporum* and caused distortion of its hyphae (Sylvia and Sinclair, 1983). Spore germination of *Fusarium solani* was significantly reduced when treated with the culture filtrate of *Paxillus involutus* and *Laccaria laccata* (Branzanti and Bambonelli, 1994). Quarraqi *et al.* (2005) reported that the culture filtrate of *Suillus cdlinius* and *Hebeloma mesophaeum* exhibited antagonistic effect on the mycelial growth and spore germination of *F. oxysporum*. Similarly, Yamaji *et al.* (2005) found antifungal activity of the culture filtrate of *Paxillus* sp. against *Pythium vexans* and attributed the antifungal activity of *Paxillus* sp. to the production of oxalic acid.

2.6.2 Mycorrhizal use in apple

Geldermann (1968) reported the occurrence of VAM with all types of crop plants belonging to plant kingdom including apple. In India, Thakur *et al.* (2005) reported the association of VAM such as *Glomus mosseae*, *G. calendonius* etc. with apple from Himachal Pradesh.

Experiments conducted so far have proved that VAM inoculations increase the plant growth (Kumar *et al.*, 2002). Several workers have

reported increase in the growth of apple plants by the inoculation of VAM fungi especially *Glomus* spp. (Benson and Convey, 1976; Koch *et al.*, 1982; Geddeda *et al.*, 1984; Sharma, 1994). The application of VAM fungus *Glomus* sp., alone or in combination with green amendments have been reported to significantly improve the plant growth in comparison to green amendments alone (Bhardwaj and Gupta, 2000). Various apple orchards and nurseries surveyed in Himachal Pradesh have revealed the presence of VAM in their rhizosphere with higher VAM colonization and spore population in nurseries (Bharat and Bhardwaj, 2001). Increase in plant growth such as height, stem diameter, leaf area and dry matter etc. in apple through VAM inoculation has been reported by Devi and Sitaramaiah (1991). Root inoculation of apple with *Glomus* sp. enhanced around 15-16 per cent shoot and root dry weight in apple (Granger *et al.*, 1983; Hoepfner *et al.*, 1983). In pot culture studies, Sharma and Bhutani (1993) reported significantly superior (about 25%) growth of apple seedlings when inoculated with *Glomus* sp. Sharma (1994) noticed higher chlorophyll content in the leaves of mycorrhizal seedlings than those of non-mycorrhizal ones.

VAM fungi are known to enhance the uptake of phosphorus in plants. Evidence reveal that P uptake in apple leaves increased from 0.04

per cent in uninoculated plants to 0.19 per cent in VAM inoculated plants (Geddeda *et al.*, 1984; Grekow and Marschner, 1989; Sharma, 1994). A few research workers have reported increase in K uptake in mycorrhizal apple seedlings by *Glomus* specie (Kaushik *et al.*, 2003). Bharat and Bhardwaj (2001) found *Glomus fasciculatum* to be superior over other species with respect to plant growth while Reich (1988) reported the similar observations with *G. intraradices* and *G. epigaem.* The rate of initial root colonization was more with *G. mosseae* than *G. macrocarpum* (Miller *et al.*, 1989).

The increased concentration of phosphorus in host plants like sweet orange and apple by mycorrhizae has been reported to alter the activities of microorganism (Graham and Egel, 1988; Bagyaraj, 1992). Thakur *et al.*, (2005) reported that efficient mycorrhizal colonization of apple roots increased the uptake of nutrients as well as reduced the disease severity.

2.7 Interaction of mycorrhizae and soil borne plant pathogens

Several reports indicate that host plant previously inoculated with VAM fungi exhibited increased resistance to several root diseases (Schenck, 1981; Dehne, 1982; Graham and Eagle, 1988). Similar interactions between VAM and root rot or wilt pathogens have been expressed by various workers (Rosendahl, 1985; Jalali and Chand, 1988).

Davis *et al.* (1978) and Davis and Menge (1981) studied difference

in mycorrhizal and non-mycorrhizal citrus plants after inoculation with *Phytophthora* sp. and reported that plant height, top weight and root weight of seedlings were greatly influenced by *Glomus fasciculatum* x *Phytophthora parasitica* interaction. The mycorrhizal citrus plants exhibited better growth than non-mycorrhizal ones. The number of healthy roots was less in the seedlings inoculated with *P. parasitica* alone. Tolerance to *Phytophthora* root rot (*P. parasitica*) in citrus seedlings was due to the ability of mycorrhizal roots to absorb more phosphorus from the soil. Similar growth promoting effect due to *Glomus fasciculatum* inoculation in *Thielaviopsis basicola* infected plants has been reported by Davis and Menge (1980). However, he observed little influence of *G. fasciculatum* on root rot caused by *Thielaviopsis basicola*.

Several mechanisms by which ectomycorrhizae protect roots from pathogens are: (i) cross protection through fungal sheath formation that acts as a physical barrier, changes the nature and fluxes out root exudates, and subsequently causes reduction in the substrate necessary for the development of pathogen (Zak, 1964), (ii) fungus-induced chemical changes in host and (iii) in some cases production of antibiotics inhibitory to pathogens (Sylvia and Sinclair, 1983; Duchesne *et al.*, 1988).

Veghelyl (1994) studied the interaction between ectomycorrhizae

and root rot pathogens in replanted orchards and vineyards, and reported that ectomycorrhizal fungus *Entoloma clypeatum*, reduced the effects of root rot fungi on the *Prunus* species.

Graham and Egel (1988) inoculated orange seedlings with vesicular-arbuscular mycorrhizal fungus *Glomus intraradices* and then infested them with *Phytophthora parasitica*. They observed fewer rotted root tips on VAM root systems inoculated with *F. parasitica* and there was significant decrease in total tips with root rot in comparison to non-mycorrhizal root systems.

The root rot disease severity in mycorrhiza inoculated 6 month old apple seedlings ranged between 27.5 and 56.5 per cent as compared to 100 per cent in non-mycorrhizal pathogen-inoculated control (Bharat and Bhardwaj, 2001). Minimum disease severity was recorded on the seedlings inoculated with *Glomus* spp. All the mycorrhizal-inoculated apple seedlings revealed efficient VAM colonization in roots within 60 days of inoculation

Three mycorrhizal species viz., *Glomus mosseae*, *Glomus fasciculatum* and *Gigaspora margarita*, inoculated in apple seedlings, resulted in 576, 726 and 370 per cent increase in shoot length; 872, 1184 and 433 per cent increase in shoot fresh weight and 826, 1122 and 410 per cent increase in dry weight, respectively, over control. *Glomus fasciculatum*

was most effective and *G. mosseae* intermediate in promoting plant growth (Geddeda *et al.*, 1984).

VAM fungi can prevent root infections, reduce root knot nematode, suppress apple replant disease caused by phytotoxic Myxomycetes and protect host plant against root infecting pathogenic bacteria (Pal *et al.*, 2006).

2.8 Fungal antagonists

Several fungi such as *Chaetomium globosum*, *Coneothyrium minitans*, *Trichoderma* spp., *Peniophora gigantea*, *Pythium olegandrum*, *Aspergillus niger*, *Penicillium* spp., *Gliocladium virens* etc. have been reported to be good biocontrol agents of various plant pathogenic disease. Among these, *Trichoderma* species have received greater attention with regard to their role in controlling root rots (Elad, 2000).

Various strains of *Trichoderma* species have been found to be strong opportunistic invaders, fast growers and prolific producers of spores and antibiotics (Whipps and Lunsden, 2001). These properties make them ecologically most suitable biocontrol agents. The *Trichoderma* strains showing antagonistic properties have been found in agricultural, native prairie, forest, salt marsh and desert soils of all climatic zones (Monte, 2001). Selected species/strains of *Trichoderma* when incorporated into the

soil and/or applied to seeds have been found more effective against root rot pathogens (James, 1985; Le-Bihan *et al.*, 1997). Three isolates of *Trichoderma viride* (TV₁, TV₂, TV₃), *T. harzianum* and *Gliocladium virens* when screened *in vitro* against white root rot of apple were found effective in restricting the root rot disease. TV₁ proved more effective followed by TV₃, *T. harzianum*, *G. virens* and TV₂ (Ashwani *et al.*, 2004).

Soil inoculation of *Trichoderma harzianum* controlled *Rosellinia necatrix* in apple orchards and greenhouse grown almonds (Freeman *et al.*, 1986). In a greenhouse bioassay, the effects of *Trichoderma* inoculum load and temperature on seedling mortality caused by *P. cactorum* were determined and it was found that 4 per cent *T. harzianum* applied at 24°C were most effective (Roiger and Jeffers, 1991). The antagonist activities of *Trichoderma viride*, *T. harzianum*, *T. hamatum* and *Gliocladium virens* were tested against *Dematophora necatrix* by Mamta *et al.* (2001). They found *T. viride* and *T. harzianum* most effective in inhibiting the mycelial growth and the volatile toxic compounds produced by *T. viride* recorded the highest inhibition in mycelial growth (36.88%) of *D. necatrix*.

Rana and Gupta (1992) reported inhibition of *R. necatrix* by 16 isolates of *Trichoderma* spp. to varying extents. In India, Rana and Gupta (1992) found three species of *Trichoderma* (*T. harzianum*, *T. hamatum* and

T. viride) and *Gliocladium virens* restricted the growth of *R. necatrix* in vitro. Sharma (1993) reported that *T. harzianum*, *T. koningii*, *T. viride* isolate-I and isolate-II overgrew the culture of *D. necatrix*, however, *G. virens* only met and did not overgrow revealing mycoparasitic properties of *Trichoderma* species. He further reported that *Trichoderma* spp and *G. virens* reduced the incidence of *D. necatrix* to varying extent in pot culture. Culture filtrate of various *Trichoderma* spp., *G. virens* and *E. aerogenes* also proved effective, to some extent, in restricting the growth of *D. necatrix*.

CHAPTER – 3

MATERIALS AND METHODS

The present investigations on “Influence of mycorrhizae on root rot in apple (*Malus domestica* Borkh.)” was conducted during 2006-07 in laboratory and experimental field of the Division of Plant Pathology, SKUAST-K, Shalimar, Srinagar. The details of the methods adopted are described as under:

3.1 Collection of root rot affected apple seedlings

The root rot affected apple seedlings were collected from apple nurseries in Government orchard, Department of Horticulture, Zakura and Fruit Research Station, SKUAST-K, Balpora. Two year old apple seedlings showing typical root rot symptoms like chlorosis, wilting and plants not firmly-anchored in soil were collected along with soil, bagged separately in polythene bags and immediately brought to the laboratory. The samples were then carefully uprooted and observations on deviated morphology of plant parts recorded.

3.2 Isolation and purification of root rot pathogen(s)

Diseased samples collected were used for the isolation of associated pathogens. The infected root portions of selected plants were washed in running tap water to remove adhering soil debris. Small bits, approximately

5 mm in size, of healthy bark were taken from the junction of diseased and healthy root parts with the help of a sterilized blade. The bits were surface sterilized in 0.1 per cent mercuric chloride solution for 30 seconds and repeatedly washed with sterilized water to remove the traces of mercuric chloride. Subsequently, these bits were placed on sterilized filter paper to remove excessive moisture and then transferred aseptically to the Petriplates containing potato dextrose agar (PDA) medium. Inoculated Petriplates were incubated at $25\pm 1^{\circ}\text{C}$. Also, some Petriplates were kept in dark at the same temperature and observed regularly for mycelial growth. Fungal growth on the bits, if any, was picked up and purified by hyphal tip method. Various cultural and morphological characteristics of isolated fungi were recorded by making visual observation for colony size, shape and colour and examined microscopically for hyphal septation and pigmentation, shape, size, colour, septation of spores, sclerotia formation, presence of synnemata and pear-shaped swellings at septa. Morphological characteristics of isolated fungi were compared with standard descriptions given by Thomas *et al.* (1957) and Plaats and Vander (1981). The pure cultures were periodically subcultured on freshly prepared PDA slants at every 30 days interval and stored at 4°C .

3.3 Pathogenicity of isolated fungi

A soil:sand (2:1 w/w) mixture was prepared and sterilized in an autoclave for 1 hour at 1.05 kg/cm^3 for 3 consecutive days. One and a half kg sterilized soil-sand mixture was put into ten 2 kg capacity earthen pots. Ten one year old apparently healthy apple seedlings were transplanted in above earthen pots and irrigated as and when required. Once established, the soil around the crown of plant was gently removed and fungal inoculum, multiplied on sand-maize medium (Riker and Riker, 1938), was added @ 50 g/kg to each pot-mixture in the root zone of plant, except control. The pots were incubated in a glasshouse at $26 \pm 3^\circ\text{C}$. The plants were critically examined for the development of root rot symptoms for a period of 9 weeks and typical root rot symptoms on test plants, if any, recorded. The causal pathogens were reisolated and compared with mother isolates. The pathogenicity of pathogens was confirmed by following the Koch's postulates.

3.4 Isolation, screening and characterization of VAM fungi

The soil samples (0-30 cm), taken from the rhizosphere of apparently healthy apple trees in SKUAST-K apple orchard, were collected in polythene bags in March, 2006. The soil samples (about 250 g each) were immediately brought to the laboratory, air-dried and processed for VAM isolation using wet sieving and decantation method (Gerdemann and

Nicolson, 1963). Each soil sample was thoroughly mixed in 1 litre distilled water and kept as such for a few seconds to allow heavier particles to settle down. The supernatant was passed through 750 μm sieve to remove large pieces of organic matter. Then it was passed through 500 μm sieve to remove fine clay particles and through 250 μm sieve to retain the desired spores. The filtrate was again passed through 100 μm sieve to retain smaller VAM spores. The residues of different fractions were shifted to Petriplates by gentle washing and examined under stereomicroscope (25 and 50x) with strong direct light. The spores from different soil samples were picked up by capillary tube technique and transferred to distilled water contained in 200 ml flasks. About 300 identical spores were picked from each sample and maintained at 4°C in a refrigerator for further investigations.

3.5 Identification of VAM isolates

The isolated VAM spores were placed in sterile water on a cavity slide for detailed examination with respect to their shape, colour, size, septation, wall thickness, hyphal attachments, spore dimension, spore ornamentation, spore wall etc. Such examinations were made under a light microscope at 10x to 40x. Crushed specimen were observed for internal cell wall details under oil immersion at 100x. The morphological details were

compared with authentic descriptions of Schenck and Perez (1987).

3.6 Multiplication and infectivity of VAM culture

The VAM multiplication was carried out in polythene bags on maize plants. One kg soil + sand mixture (1:2 w/w) was sterilized in an autoclave at 1.05 kg/cm³ pressure for 1 hour and put into 1.5 kg capacity polythene bags. Surface sterilized healthy maize seeds, pregerminated in Petriplates under aseptic conditions, were sown in these pots. These bags were aseptically inoculated, except control, with identical VAM spores. For inoculation, 50 spores were piped out and placed in soil at 5 cm depth (Jackson, 1973). The bags were kept in a greenhouse at 25±3°C and irrigated with sterile water as and when required. The plants were uprooted after 45 days. The roots were collected, washed with sterile water to remove adhering soil debris and observed for mycorrhizal infection. The infectivity was proved by noticing the presence of Harting net, vesicles, arbuscules or hyphae of endophyte on roots.

3.7 Screening of VAM isolates for acid phosphatase activity and root colonization

Screening of different VAM isolates for their efficiency in plant growth promotion and root colonization was carried out on maize plants. The sterilized soil + sand mixture (1:2 w/w) was inoculated with different

VAM isolates as described in section 3.6. An uninoculated control was also maintained. Each treatment was replicated thrice. The polythene bags were kept in a greenhouse at $25\pm 4^{\circ}\text{C}$ and irrigated with 50 ml sterile water as and when required. The plants were uprooted 45 days after inoculation and roots washed to remove adhering soil debris. The efficacy of different VAM isolates was ascertained on the basis of plant biomass (dry weight), per cent mycorrhizal root colonization and rhizosphere acid phosphatase activity as per the methods described in section 3.13.4 and 3.13.5. The best isolate was chosen for interaction study.

3.8 Isolation and identification of ectomycorrhizal fungi

The sporocarps of ectomycorrhizal fungi were collected from the canopy of apparently healthy apple trees growing in main orchard, SKUAST-K, Shalimar. The sporocarps were brought to the laboratory immediately and gently washed to remove the adhering debris. These were then surface sterilized in 0.1 per cent mercuric chloride for two minutes and washed 3-4 times in sterilized distilled water. A shallow cut 1-2 mm deep was given across the middle of the cap to expose the interior tissue. With the help of a sterilized fine tipped scalpel sporocarp tissue bits of 2.5 mm size were cut and loosened. The tissue bits were aseptically transferred to the Petriplates containing modified Melin Norkan's medium (Marx, 1969)

and incubated at $25\pm 2^{\circ}\text{C}$. The Petriplates were observed daily for mycelial growth. The mycelium from the new colony edges were aseptically transferred onto Melin Norkan's medium (MMN) nutrient agar slants. The slants were incubated at $25\pm 2^{\circ}\text{C}$ for 30 days and then preserved at 4°C . The cultures were revived at three months interval. The cultural and morphological characteristics of isolated ectomycorrhizal fungi were studied for their identification and compared with the descriptions given by Gadbout and Fortin (1985) and Lakhanpal (1988).

3.9 Mass multiplication of ectomycorrhizal fungi

Vegetative inocula of ectomycorrhizal fungi were prepared in peat-moss-vermiculite based medium according to the method of Marx and Bryan (1975). Ectomycorrhizal fungi were grown aseptically in one and a half litre Erlenmeyer flasks containing 750 ml of peat-moss-vermiculite moistened with 375 ml of MMN liquid medium. The flasks were incubated at $25\pm 2^{\circ}\text{C}$ for 10 weeks. The inoculum was then removed from flasks just before inoculation and leached with distilled water to remove unused nutrients.

3.10 Mass production of fungal antagonist

The fungus antagonist *Trichoderma harzianum* was procured from the Division of Plant Pathology, SKUAST-K, Shalimar and multiplied on

sand-maize medium. For this, sand- maize (2:1) @ 250 g was filled in 500 ml conical flasks and sterilized at 1.05 kg/cm³ for one hour for three successive days. The medium in flasks was inoculated with 4 ml spore suspension of *Trichoderma harzianum* Rifai and incubated at 25±2°C for 30 days. After incubation the samples were drawn and, using dilution plate technique, the population of antagonists enumerated on PDA medium in terms of colony forming units (Pramer and Schmedt, 1956).

3.11 Mass multiplication of root rot pathogens

The mass multiplication of fungal pathogens was done on maize: sand (1:2) medium supplemented with sucrose. One hundred ml medium was put in each 250 ml conical flask after autoclaving the medium at 1.05 kg/cm³ pressure for 1 hour for 3 consecutive days. Three fungal discs of 2 week old culture of root rot pathogen(s), grown on PDA medium, were aseptically inoculated in each flask. The flasks were incubated at 25±1°C for 14 days. The medium was hand shaken after every two days to avoid cluster formation and/or to allow thorough mixing of clusters in the medium.

3.12 *In vitro* antagonism between ectomycorrhiza, *T. harzianum* and

root rot pathogens

The *in vitro* antagonism between ectomycorrhizal fungi, *T. harzianum* and root rot pathogens was assessed by dual culture method on PDA medium (Utkhede and Rahe, 1983). A 5 mm disc of one month old mycelial mat of test mycorrhizal fungus was placed 2 cm away from the edge of 90 mm dia. Petriplate containing 20 ml PDA medium. The Petriplates were incubated at $25\pm 2^{\circ}\text{C}$ for 7 and 15 days in case of fast growing and slow growing mycorrhizae, respectively. A 5 mm disc of 7 day old test pathogen was then placed 2 cm away from the edge of Petriplates but opposite to the antagonist. Similarly 5 mm discs of seven day old cultures of test pathogen and *T. harzianum* were taken with the help of a cork borer and placed on fresh PDA 2 cm away from the edge of a 90 mm dia. Petriplate opposite to each other. Pathogenic fungi and ectomycorrhizae/*T. harzianum* individually grown on Petriplates served as control. The Petriplates were incubated at $25\pm 2^{\circ}\text{C}$ for 6 days. Each treatment was replicated four times. The experiment was laid out in a completely randomized design. The radial growth of pathogens in dual culture as well as in control plates was recorded, and mycoparasitism/overgrowth and per cent mycelial inhibition estimated according to the method of Vincent (1947). The morphology of hyphae in interaction zone was observed under a light microscope to

confirm mycoparasitism/ overgrowth, if any.

$$\text{Mycelial growth inhibition (\%)} = \frac{\text{Mycelial growth in control} - \text{mycelial growth in treatment}}{\text{Mycelial growth in control}} \times 100$$

3.13 *In vitro* interaction studies

3.13.1 Preparation of soil

Soil taken from apple orchard of SKAUST-K, Shalimar was crushed and sieved to remove roots, if any. The soil was then mixed with sand in the ratio of 2:1 (w/w). The mixture was autoclaved at 1.05 kg/cm³ for one hour for 3 successive days. Various physico-chemical characteristics of soil were estimated by standard methods (Table 1). One and a half kg sterilized potting-mixture was put into each earthen pot of 2 kg capacity for *in vitro* interaction studies.

3.13.2 Inoculation and seedling transplantation

Two year old healthy apple seedling procured from Government Nursery, Department of Horticulture, Rajbagh were transplanted on 24th February, 2007 in earthen pots containing 1½ kg sterilized potting-mixture. The seedlings were irrigated as and when needed. Once established, the seedling were inoculated with a pathogen, most efficient mycorrhizae and *Trichoderma harzianum* individually and in various combinations. The pots without any treatment served as control. Vesicular arbuscular mycorrhizal inoculation was done in rhizosphere of transplanted apple

Table 1: Physico-chemical properties of soil (soil:sand, 2:1, w/w)

S. No.	Characteristics	Value	Method of analysis adopted
1.	Composition (%)		
	i) Sand	44.8	International pipette method (Piper, 1966)
	ii) Silt	36.7	
	iii) Clay	18.6	
2.	Texture	Loam	Feel method (Gliessman, 2007)
3.	Organic carbon (%)	0.7	Walkley and Black method (Piper, 1966)
4.	pH	6.8	Glass elctrode method (Jackson, 1973)
5.	EC (ds/m)	0.2	Solubridge conductivity meter (Piper, 1966)
6.	Available nitrogen (kg/ha)	280.4	Alkaline permanganate method (Subbiah and Asija, 1956)
7.	Available phosphorus (kg/ha)	16.02	Olsen method (Olsen <i>et al.</i> , 1954)
8.	Available potassium (kg/ha)	242.0	Flame photometer method (Jackson, 1973)
9.	CEC {mol (P ⁺)/ kg}	19.82	Jackson (1973)

seedlings by placing 5 g of inoculum containing spores, hyphae and VAM root fragments. The ectomycorrhizal fungus was inoculated to rhizosphere soil @ 15 g/pot. Similarly, *T. harzianum* was inoculated to potting mixture @ 10 g/kg in the root zone of transplanted seedlings. The pathogenic inoculum, multiplied on sand-maize medium, was inoculated to potting mixture @ 10 g/kg in the root zone of seedlings. The treatment details are given as under:

T ₁	Pathogen
T ₂	Ectomycorrhiza (ECM)
T ₃	Vesicular arbuscular mycorrhiza (VAM)
T ₄	<i>Trichoderma harzianum</i>
T ₅	Pathogen + ECM
T ₆	Pathogen + VAM
T ₇	Pathogen + <i>T. harzianum</i>
T ₈	ECM + VAM
T ₉	ECM + <i>T. harzianum</i>
T ₁₀	VAM + <i>T. harzianum</i>
T ₁₁	Pathogen + ECM + VAM
T ₁₂	Pathogen + ECM + <i>T. harzianum</i>
T ₁₃	Pathogen + VAM + <i>T. harzianum</i>
T ₁₄	ECM + VAM + <i>T. harzianum</i>
T ₁₅	Pathogen + ECM + VAM + <i>T. harzianum</i>
T ₁₆	Control

The inoculation of VAM, ECM and *T. harzianum*, as per the treatment details, was done on 1st March, 2007. The pathogenic inoculation was carried out a week later. The inoculated and uninoculated seedlings were arranged in a completely randomized design with each treatment replicated 3 times. The seedlings were grown under greenhouse conditions at 25±3°C and irrigated as and when required. No fertilizer or protective chemical was applied throughout the study. The seedlings were out-planted 60 and 120 days after transplantation in case of *D. necatrix*. In case of *P. ultimum* only eight treatments (T₁, T₂, T₃, T₄, T₅, T₆, T₇ and control) were taken and seedlings out-planted 120 days after transplantation. The rest experimental conditions were same as above. Root rot index, mycorrhizal root colonization, rhizosphere acid phosphatase activity, plant height, girth, shoot biomass (dry weight), root biomass and root length were estimated soon after the seedlings were out-planted.

3.13.3 Root rot index

Root rot index was calculated on the basis of percentage of root area affected by the rot as per the root rot index rating/scale (Plate 1) described by Bharat and Bhardwaj (2001) as under:

Grade	Percent root area infected
0	Apparently free from infection
1	1-10
2	10-20
3	21-50
4	51-75
5	76-100

3.13.4 Mycorrhizal root colonization

For estimating mycorrhizal root colonization, the root samples were collected and washed carefully to remove the adhering debris. The tertiary roots were cut into small pieces of approximately 1 cm length and subjected to differential staining as described by Phillips and Hayman (1970).

The small pieces of roots were placed in 10 per cent KOH solution in 500 ml beaker and heated for 1 hour at 90°C. The root segments were then rinsed in sterile water, acidified with dilute HCl (2%) and boiled for 5 minutes in cotton blue stain. The roots were destained by keeping them in lactophenol overnight and viewed under a stereoscopic microscope. The estimation of mycorrhizal infection in roots was made by visual observation (Giovannetti and Mosse, 1980). A randomly selected aliquot of stained root

segments, suspended in water, was spread in a Petridish viewed under a dissecting microscope at a magnification of 40 and 100x. For ECM colonization, root segments containing hartig net and number of mycorrhizal short roots were considered infected. In case of VAM colonization, root segments containing vesicles and arbuscules of endophyte and number of mycorrhizal short roots were considered infected as suggested by Beckjord *et al.* (1984).

$$\text{Per cent mycorrhizal infection} = \frac{\text{Number of infected root segments}}{\text{Total number of segments examined}} \times 100$$

3.13.5 Acid phosphatase activity

The acid phosphatase activity of rhizosphere soil was measured by the method of Tabatabai and Bremner (1969). One gram soil sample, drawn from the plant rhizosphere in each treatment, was placed in 50 ml volumetric flask. Then 0.2 ml toluene, 4 ml modified universal buffer (2.8 g citric acid and 1.26 g boric acid dissolved in 100 ml 1 N NaOH and volume made upto 1 litre with distilled water) and 1 ml of 0.025 M p-nitrophenyl phosphate were added. The flasks were incubated at 37±1°C for 1 hour. Enzymatic reaction was stopped by adding 4 ml of 0.5 M NaOH and 1 ml 0.5 M CaCl₂. The contents were filtered through Whatman filter paper No. 42, and absorbance of yellow colour (p -nitro phenol released g⁻¹ soil)

developed was measured spectrophotometrically at 460 nm wavelength. The amount of p-nitrophenol released was calculated with reference to a standard curve prepared by using different concentrations of p-nitrophenol. The enzyme activity was expressed as μM p-nitrophenol (PNP) released/g soil/hour.

3.13.6 Plant growth characteristics

Plant growth characteristics such as plant height, girth, root length and shoot and root biomass were recorded after the seedling were out-planted. The length of main shoot was measured from 1.0 cm above ground level to the top of terminal shoot bud with the help of a measuring tape. Stem diameter was measured with a Vernier calliper 10 cm above soil level. Root length was measured after gentle uprooting of seedlings, spreading them on white paper, marking the end points and measuring distance between the end points with a scale.

For shoot and root biomass estimation, the samples were first washed in distilled water and dried in an oven at $70\pm 1^\circ\text{C}$ for 72 hours. The root and shoot samples were then weighed separately using an electronic balance (Chapman, 1964).

3.14 Statistical analysis

The data collected was statistically analysed by using analysis of

variance technique. Wherever the test was significant at 5 per cent probability level, critical difference was used to compare the treatment means. The software used for analysis was “Minitab”.

CHAPTER – 4

EXPERIMENTAL FINDINGS

The results of the experiments on “Influence of mycorrhizae on root rot in apple (*Malus domestica* Borkh)” are presented as under:

4.1 Collection, isolation, characterization and identification of pathogens

4.1.1 Isolation of pathogens from diseased samples

The root rot affected apple seedlings, collected from apple nurseries in Government orchard, Department of Horticulture, Zakura and Fruit Research Station, SKUAST-K, Balpora, had typical rot symptoms like chlorosis, brown-lesioned/watery-decayed roots, white mycelial growth on roots, not firmly-anchored in soil etc. (Plate 2). Such seedlings were collected along with the soil. The isolation of pathogens, made from such apple seedlings, yielded two root rot fungal pathogens *Dematophora necatrix* Hartig and *Pythium ultimum* Trow. which were identified on the basis of their morphological and cultural characteristics. Besides, many other fungi like *Fusarium*, *Trichoderma* etc. were also isolated but these proved of lesser importance. The isolated fungi were maintained on potato dextrose agar and subculturing was done at monthly intervals.

4.1.2 Characterization of *Dematophora necatrix*

The purified culture of fungus on PDA initially produced cottony white

colonies which attained a growth of 36 mm after 3 days of incubation at $25\pm 2^{\circ}\text{C}$ (Plate 3). The colonies turned slightly blackish after 15 days of incubation. Microscopic observation revealed that mycelium was brown, branched and septate with characteristic pyriform swellings at septa. Hyphae were 5-8 μm in width (Table 2). Conidiophores were hyaline, flexuous, intertwined and dichotomously branched on synnemata. The size of conidiophore ranged from 40-300 μm in size. Conidia were olive brown, solitary, ellipsoid, one-celled, smooth and measured $3.0-4.5 \times 2.0-2.5 \mu\text{m}$ in size. Synnemata brown in colour were rosy and projected as rigid columns. **4.1.3 Characterization of *Pythium ultimum***

The purified culture of *P. ultimum*, grown on PDA, covered 36 mm surface within 24 hours of incubation at $25\pm 2^{\circ}\text{C}$. The fungus produced white fluffy colony (Plate 4). The mycelium was hyaline and on an average measured 10 μm in width. Hyphae were cylindrical, fine, well branched, inter- or intra-cellular, measured 10 μm in dia. and aseptate but turned septate in old cultures. Terminal hyphal swellings were hyaline, globose to sub-globose having a size of about 15-30 μm . (Table 3). Abundant oospores found were spherical, smooth, borne terminally and measured 17.0-25.0 μm in dia. Monoclinous anthredia arose from the original stalk immediately below the oogonium. No zoospores were found.

4.1.4 Identification of pathogens

Dematophora necatrix Hartig was identified on the basis of pear shaped septation in mycelium which distinguished it from other root rot fungi. *Pythium*

ultimum Trow. was identified on the basis of smooth, spherical oospores, non-production of zoospores and monoclinous anthredia.

4.2 Pathogenicity of isolated fungi

Pathogenicity test of isolated fungi (*D. necatrix* and *P. ultimum*) was carried out separately on apple seedlings. The initial disease symptoms developed after 15-16 days of inoculation in case of *D. necatrix* inoculated seedlings, and 20 days after inoculation in case of *P. ultimum*.

The pathogens were reisolated from the inoculated seedlings but not from the uninoculated control seedlings. The *D. necatrix* and *P. ultimum* isolates obtained from inoculated seedlings were compared with their original cultures. The reisolated pathogens completely resembled the original inoculated fungi in their morphological, cultural and pathogenic characteristics so satisfied Koch's postulates.

4.3 Symptomatology

Symptomatology of disease on apple seedlings attacked by the pathogens viz., *D. necatrix* and *P. ultimum* was studied separately. *D. necatrix* infected seedling produced typical root rot symptoms after 15 days of inoculation and such seedlings exhibited stunted growth. The above-ground symptoms of disease included progressive decline in plant vigour accompanied by browning, drooping and wilting of leaves, diminution in leaf size followed by defoliation and death of plant. The examination of under-ground plant parts revealed that all the fibrous

roots were completely devoured while lateral roots turned dark brown in colour. In advanced stage the whole root system was killed leaving behind only tap root. The rotted portions of root showed white mycelial growth.

Seedlings attacked by *P. ultimum* initially showed small water-soaked lesions on collar (stem at ground level) which enlarged with age and gradually encircled the whole collar in severe cases. Such affected portions were greasy to touch and exuded out latex. The bark at such places was dark brown and easy to be peeled off thereby exposing the inner tissues of the stem. The bark usually got sloughed off within a short period after pathogenic attack. The inner bark was often in semi-rotten state. The lateral roots were also affected. Usually lateral roots in such affected plants did not develop and taproots often became twisted. The plants showed wilting symptoms, retarded plant growth, chlorosis and partial or total death of root system. *P. ultimum* attacked growing tips of primary roots. The colour of roots changed from brown to black. The main root began to rot but did not progress downward up to the tip.

4.4 Isolation and identification of vesicular arbuscular mycorrhizal (VAM) fungi

Vesicular arbuscular fungal spores were isolated from the soils collected from rhizosphere of apple trees in Shalimar campus by wet sieving and decantation procedure. The VAM fungi were identified on the basis of sporocarp, spore size, colour, aggregation, spore wall, surface ornamentation and presence and absence of vesicles in soil. Two *Glomus* species were isolated from these

roots (Plate 5). The differentiating characters noticed are given in Table 4. The *Glomus mosseae* did not possess any sporocarp but the spores were found aggregated into loose clusters. The spores were double-walled, measured 110-205 μm in size, pale yellow in colour and had a basal protrusion which was typically funnel shaped. The subtending hyphae at the widest part measured 18-50 μm in dia. The soil borne vesicles were not observed. The cell wall was thick, hyaline and measured 30 μm in width. In case of *Glomus calendonius* sporocarp was present. Spores were dark brown and measured 100-250 μm in size. Surface ornamentation was absent. Subtending hyphae was simple, occluded by spore wall, thick, pigmented and measured 10-20 μm in diameter. Cell wall was of unit type.

4.5 Screening of efficient VAM isolates

The isolated VAM spores, multiplied on maize (Plate 6), were screened for their efficiency in improving plant growth, extent of root colonization and rhizosphere acid phosphatase activity. The results presented in Table 5 revealed that *Glomus mosseae* was more compatible endophyte in terms of above parameters. Both VAM isolates increased plant height, with 9.6 per cent increase over control in *G. mosseae* and 7.8 per cent increase in *G. calendonius*. Further, *G. mosseae* proved superior over *G. calendonius* with regard to increase in plant biomass. *G. mosseae* increased plant biomass (dry weight) by 24.8 per cent and *G. calendonius* increased it by 18.1 per cent over control. Greater colonization was observed in *G. mosseae* (49.6%) and slightly less 45.7 per cent in *G. calendonius*.

Moreover, *G. mosseae* showed 2.9-fold increase in soil phosphatase activity than control whereas *G. calendonius* showed 2.6-fold increase.

Table 5: *In vivo* screening of VAM isolates on maize

VAM isolate	Plant height (cm)	Plant biomass (g/plant)	VAM colonization (%)	Acid phosphatase activity (μ M p-nitrophenol released /g soil/hr)
<i>Glomus calendonius</i>	48.4	3.78	45.7 (42.53)	48.5
<i>Glomus mosseae</i>	49.2	3.99	49.6 (44.77)	52.4
Control	44.9	3.20	0.00 (0.00)	13.6
C.D _(0.05)	0.6	0.17	(0.625)	1.25

Figures in parentheses are angular transformed values

4.6 Isolation and characterization of ectomycorrhizal fungi

4.6.1 Isolation

The ectomycorrhizal fungi were isolated from sporocarps collected from orchards and grown on modified Melan Norkan's (MMN) agar medium. Two ectomycorrhizae isolated and characterized on the basis of their culturo-morphology were identified as *Laccaria laccata* (Broome and Berkeley) and *Suillus granulatus* (fr.) Kuntze.

4.6.2 Morpho-cultural characterization of *Laccaria laccata*

The caps (pileus) of *L. laccata* were 2-5 cm in dia., initially orange brown but changed to buff colour later, obtuse to convex in shape, often had a central depression and minutely scaly with smooth margin (Table 6; Plate 7a). Gills were pink to pinkish, distant or close and attached to the stem. Stipes were pinkish, equal to sub-clavate and measured about 1-2 x 3-7 cm in size. Spores were hyaline, 7-12 μm in size, globose to sub-globose and echinulate (Plate 7b). Basidia were hyaline, clavate with 4 strigmata and measured 25-54 x 6-14 μm in size. The culture of *L. laccata* was white to slightly pinkish (Plate 7c). Mycelial mat was felty thick, interwoven and tightly appressed to agar surface. Colonies attained a diameter of 60-70 mm on MMN agar medium in 2 weeks period.

4.6.3 Morpho-cultural characterization of *Suillus granulatus*

The caps (pileus) of *Suillus granulatus* were yellowish brown to light brown in colour, convex in shape and 4-12 cm in size (Plate 8a). The cap surface was shinny and smooth composed of loosely arranged gelatinised hyphae (Table 6B). Pores were 1.0-1.5 mm wide tubes about 1.0 cm deep and radially arranged. Pore surfaces were whitish at first and soon turned bright yellowish. Stipes were 4.0-8.0 cm long and 1.0-2.0 cm thick initially white but later became yellowish. The stipe surface had brownish to reddish brown glandular dots. Stipes were mostly equal, occasionally tapered to the base, solid and without any veil. The spores were hyaline to dull brown, smooth, elliptical to oblong and measured 7.0-9.0 x 2.5-3.5 μm in size (Plate 8b). Basidia were hyaline, clavate to cylindrical

with 4 strigmata about 18-24 x 5-6 µm in size. The cultures were initially white later turned grey yellow to fawn coloured. Colonies were furry and hyphae grooved and aseptate (Plate 8c). Colonies attained a diameter of 20-30 mm in 25 days of incubation on MMN medium.

4.7 *In vitro* interaction between mycorrhizae/fungal antagonist and root rot pathogens

S. granulatus (fr.) Kuntze and *L. laccata* (Broome and Berkeley), raised on MMN agar, and *Trichoderma harzianum* (Rifai), raised on PDA (Plate 9), were evaluated against *Dematophora necatrix* (Hartig) and *Pythium ultimum* (Trow) separately by dual culture technique on potato dextrose agar (PDA).

In vitro evaluation of antagonists against *Dematophora necatrix* and *Pythium ultimum* in dual culture revealed that all the tested biocontrol agents significantly inhibited the mycelial growth of test pathogens in comparison to control. The mycelial growth inhibition in *D. necatrix* and *P. ultimum* ranged from 36.20 to 65.87 and 39.8 to 65.1 per cent, respectively, on the 6th day of incubation.

Significantly higher mycelial growth inhibition of *D. necatrix* was recorded due to *T. harzianum* (65.8%). This was followed by *Laccaria laccata* and *Suillus granulatus* (Table 7). *T. harzianum* covered the entire surface of the medium and overgrew the growth of *D. nectarix* in 6 days (Plate 10). Thus these were highly antagonistic in nature. The above bioagent also parasitized the test pathogen in dual culture. Microscopic examinations of hyphae revealed rupture

and twisting of hyphae at initial stage with gradual desiccation of protoplasm, shrinkage of cytoplasm, lysis of parasitized hyphae and aggregation of protoplasm with such hyphae. The hyphae of test antagonists either directly penetrated the host hyphae or formed granular mycelial bunches around the entry point. *L. laccata* showed moderate antagonism and was equally effective in restricting the growth of test pathogen. *S. granulatus* did not exhibit any dominance over *D. nectarix* in dual culture so was categorised as slow antagonist.

In case of *P. ultimum* all the antagonists tested significantly inhibited pathogenic growth in dual culture (Plate 10; Table 8). The maximum mycelial growth inhibition on 6th day of incubation was caused by *T. harzianum* (65.1%), followed by *L. laccata* (45.3%). *Suillus granulatus* exhibited minimum hyphal growth inhibition (39.8%). The degree of antagonism of bioagents against *P. ultimum* showed similar trend as observed in case of *D. necatrix*, with *T. harzianum* proving highly antagonist, *L. laccata* moderate antagonist and *S. granulatus* slow antagonist against *P. ultimum*.

4.8 In vivo interaction between biocontrol agents and root rot pathogens

The *in vivo* interaction studies between most effective bioagents (*Glomus mosseae*, *T. harzianum* and *L. laccata*) and root rot pathogens (*D. necatrix* and *P. ultimum*) were conducted on apple seedlings in pots (Plate 11). The impact of bioinoculants on growth parameters (seedling height and root length), root and shoot biomass (dry weight), root rot index, mycorrhizal root colonization and acid

phosphatase activity in rhizosphere of apple seedlings was recorded 60 and 120 days after inoculation in case of *D. necatrix* and 120 days after inoculation in case of *P. ultimum*.

4.8.1 *D. necatrix* – biocontrol agent interaction in apple seedlings

4.8.1.1 Effect on seedling height

The study on the influence of biocontrol agents on seedling height of apple seedlings in presence and absence of root rot pathogen (*D. necatrix*) revealed that *D. necatrix* infection significantly reduced the seedling height (21.1%) in comparison to uninoculated control after 120 days of pathogenic inoculation (Plate 12). The individual inoculation of biocontrol agents alone showed 0.5 to 24.0 per cent increase in seedling height over uninoculated control with maximum increase observed in *G. mosseae* treatment and minimum in *L. laccata* treatment (Table 9).

The inoculation of two or more biocontrol agents, in general, had non-additive synergistic effect on seedling height. The dual inoculation of biocontrol agents showed 18.1 to 32.1 per cent increase in seedling height with maximum increase over control in *G. mosseae* + *T. harzianum* and minimum in *T. harzianum* + *L. laccata*. The combined inoculation of *G. mosseae* + *L. laccata* + *T. harzianum* depicted highest increase of 32.7 per cent over uninoculated control.

The coinoculation of root rot pathogen and individual biocontrol agent depicted significant increase of 23.4 to 38.3 per cent in seedling height over *D.*

necatrix inoculated seedlings. In presence of *D. necatrix*, the inoculation of *Glomus mosseae* proved more effective in improving the seedling height followed by *T. harzianum*.

The combined inoculation of two or more biocontrol agents in *D. necatrix* infested soil showed significantly better seedling height in comparison to uninoculated or *D. necatrix*-inoculated seedlings. A maximum increase of 62.5 per cent in seedling height over *D. necatrix*-inoculated plants was noticed in treatment where *L. laccata* + *T. harzianum* + *G. mosseae* were coinoculated in *D. necatrix*-infested soil, followed by *G. mosseae* + *T. harzianum* (55.9%), *L. laccata* + *G. mosseae* (43.8%) and *L. laccata* + *T. harzianum* (33.0%) (Plate 12).

The seedling height showed gradual increase with the advancement of growth period. The seedlings irrespective of bioagent or pathogenic inoculation attained a mean height of 64.9 cm in 60 days and 78.1 cm in 120 days growth period.

4.8.1.2 Effect on seedling diameter

The perusal of Table 9 revealed that plant diameter was significantly reduced by 18.7 per cent in *D. necatrix*-inoculated seedlings as compared to uninoculated control. The individual as well as combined inoculation of biocontrol agents significantly reduced the pathogenic effect of *D. necatrix*. Among the individual inoculations, *G. mosseae* in presence of *D. necatrix* improved the stem diameter by 11.5 per cent as compared to the seedlings

inoculated with *D. necatrix* alone, followed by *L. laccata* + *D. necatrix* (8.9%) and *T. harzianum* + *D. necatrix* (6.4%). Combined inoculation of *G. mosseae* + *T. harzianum* + *L. laccata* in presence of *D. necatrix* showed highest increase in plant diameter (35.8%) as compared to *D. necatrix*-inoculated plants followed by coinoculation of *G. mosseae* + *L. laccata* + *D. necatrix* (29.5%) and *T. harzianum* + *G. mosseae* + *D. necatrix* (23.1%).

Individual inoculation of biocontrol agents in absence of *D. necatrix* significantly improved plant diameter as compared to uninoculated control. *G. mosseae* depicted maximum increase in plant diameter (8.3%) followed by *T. harzianum* (3.1%) and *L. laccata* (2.1%).

The combined inoculation of *L. laccata* + *G. mosseae* + *T. harzianum* depicted highest increase of 16.6 per cent over uninoculated control followed by *G. mosseae* + *T. harzianum* (13.1%) and *L. laccata* + *G. mosseae* (12.5%).

Irrespective of treatments, a significant increase in seedling diameter of apple seedlings was observed with the advancement of growth period. The mean seedling diameter, irrespective of treatments increased from 7.5 mm in 60 days to 9.7 mm in 120 days of growth period.

4.8.1.3 Effect on root length

The data on the influence of biocontrol agents and root rot pathogen (*D. necatrix*) on root length of apple seedling is presented in Table 10. The root length was significantly reduced by 39.4 per cent due to *D. necatrix* inoculation as

compared to control (Plate 12). Biocontrol agents inoculated individually in absence of root rot pathogen significantly increased root length as compared to the uninoculated control with maximum increase in *G. mosseae*-inoculated seedlings (15.1%), followed by *T. harzianum* (12.5%) and *L. laccata* (2.0%) [Table 10].

Biocontrol agents reduced the pathogenic effect of *D. necatrix* and significantly improved root length of apple seedlings. Individual inoculation of biocontrol agents in presence of pathogen depicted 41.3 to 50.0 per cent increase in root length as compared to *D. necatrix*-inoculated seedling with maximum increase registered in *D. necatrix* + *T. harzianum* and minimum in *D. necatrix* + *G. mosseae*.

The combined inoculation of biocontrol agents in presence of *D. necatrix* showed significantly higher root growth as compared to *D. necatrix*-inoculated seedlings or uninoculated control. The maximum increase (108.7%) in root length over *D. necatrix*-inoculated plants was observed when all the three bioagents (*L. laccata* + *G. mosseae* + *T. harzianum*) were coinoculated. This was followed by *G. mosseae* + *T. harzianum* + *D. necatrix* (78.2%) and *L. laccata* + *G. mosseae* + *D. necatrix* (69.5%)

The combined inoculations of biocontrol agents in absence of root rot pathogen were found most effective in improving the root length of apple seedlings. The coinoculation of *G. mosseae* + *T. harzianum* + *L. laccata* depicted highest increase of 26.3 per cent in root length as compared to uninoculated control. Coinoculation of *G. mosseae* + *T. harzianum* and *L. laccata* + *T.*

harzianum proved next best treatments and exhibited 25.0 and 19.7 per cent increase in root length over uninoculated control, respectively.

Increase in growth period caused decrease in root length only in *D. necatrix*-inoculated apple seedlings due to root rot. The mean root length in this case was 10.1 and 9.2 cm on 60 and 120 day of growth period, respectively.

4.8.1.4 Effect on shoot biomass

The study on the effect of biocontrol agents and root rot pathogens (*D. necatrix*) on shoot biomass of apple seedlings revealed that *D. necatrix* caused 27.3 per cent reduction in shoot biomass of apple seedlings as compared to uninoculated control (Table 10). The individual inoculation of biocontrol agents in absence of root rot pathogen showed significant increase in shoot biomass when compared to control, with maximum increase of 16.8 per cent by *G. mosseae*, followed by *T. harzianum* (12.4%).

The combined inoculation of biocontrol agents in absence of *D. necatrix* showed 19.9 to 28.6 per cent increase in shoot biomass compared to uninoculated control, with maximum increase in coinoculation of *G. mosseae* + *T. harzianum* + *L. laccata* and minimum in *G. mosseae* + *T. harzianum*.

Individual inoculation of biocontrol agents in presence of pathogen depicted 27.3 to 34.1 per cent increase in shoot biomass compared to *D. necatrix*-inoculated seedling, with maximum increase in *D. necatrix* + *L. laccata* and *D. necatrix* + *T. harzianum* inoculated plants.

The combined inoculation of biocontrol agents in *D. necatrix*-infested soil significantly improved shoot biomass as compared to *D. necatrix*-inoculated seedlings. In presence of *D. necatrix* maximum increase in plant biomass was observed in combined inoculation of *G. mosseae* + *T. harzianum* + *L. laccata* (61.5%) followed by *L. laccata* + *T. harzianum* (48.7%), *G. mosseae* + *T. harzianum* (45.2%) and *G. mosseae* + *L. laccata* (42.7%).

Shoot biomass of apple seedlings increased significantly with the advancement of growth period. Irrespective of treatments, shoot biomass increased from 16.23 g in 60 days to 17.3 g in 120 days growth period.

4.8.1.5 Effect on root biomass

The study on the effect of biocontrol agents and root rot pathogen (*D. necatrix*) on root biomass of apple seedlings revealed that *D. necatrix* caused 40.3 per cent reduction in root biomass as compared to uninoculated control (Table 10). The individual inoculation of biocontrol agents in absence of root rot pathogen showed significant improvement in root biomass with maximum increase by *G. mosseae* (18.4%) followed by *T. harzianum* (15.7%) and *L. laccata* (2.7%) as compared to control.

The combined inoculation of biocontrol agents in absence of *D. necatrix* showed 16.6 to 22.8 per cent increase in root biomass as compared to uninoculated control, with maximum increase in coinoculation of *L. laccata* + *T. harzianum* + *G. mosseae* and minimum in *T. harzianum* + *G. mosseae*.

Individual inoculation of biocontrol agents in presence of pathogen depicted 47.0 to 57.3 per cent increase in root biomass as compared to *D. necatrix*-inoculated seedlings, with maximum increase in *D. necatrix* + *T. harzianum* and minimum in *D. necatrix* + *L. laccata* inoculated plants.

The combined inoculation of biocontrol agents in *D. necatrix*-infested soil significantly improved root biomass as compared to *D. necatrix*-inoculated seedlings. In presence of *D. necatrix*, maximum increase in root biomass was observed in combined inoculation of *L. laccata* + *T. harzianum* + *G. mosseae* (104.4%) followed by *G. mosseae* + *T. harzianum* (83.8%) and *L. laccata* + *T. harzianum* (79.5%).

Root biomass of apple seedlings increased significantly with with the advancement in growth period. Irrespective of the treatments, root biomass increased from 9.65 g in 60 days to 12.1 g in 120 days growth period.

4.8.1.6 Effect on root rot index

The scale followed for root rot index in present study is presented in Plate 1. The biocontrol agent - *D. necatrix* interaction revealed that biocontrol agents invariably influenced the development of root rot in apple seedlings (Table 11). All the bioagents when inoculated individually significantly reduced root rot over control, with maximum reduction in *T. harzianum* inoculated seedlings (52.9%) and minimum in *L. laccata* inoculated seedlings (37.9%).

The combined inoculation of biocontrol agents was most effective in

reducing the root rot of apple seedlings than their individual inoculations. Co-inoculation of *L. laccata* + *T. harzianum* + *G. mosseae* depicted maximum decrease in root rot (75.3%) over control, followed by *T. harzianum* + *G. mosseae* (74.1%) and *L. laccata* + *T. harzianum* (64.2%).

The root rot index of apple seedlings, irrespective of treatments, increased with the advancement of growth period. However, the increase in root rot was comparatively less in the treatments inoculated with biocontrol agents individually or in combination.

4.8.1.7 Effect on mycorrhizal root colonization

Both mycorrhizae (*G. mosseae* and *L. laccata*) successfully developed symbiotic association with the roots of apple seedlings and improved the mycorrhizal short root formation with increase in growth period (Table 11). The highest mean mycorrhizal root colonization by mycorrhizae fungus was observed in apple roots inoculated with *G. mosseae* (36.2%), followed by the seedlings inoculated with *L. laccata* (28.8%) when inoculated alone. *D. necatrix* significantly reduced the number of mycorrhizal short roots. The lowest mycorrhizal root colonization of 20.2 per cent was recorded in seedlings inoculated with *L. laccata* in presence of *D. necatrix*.

The coinoculation of *G. mosseae* + *L. laccata* + *T. harzianum* depicted maximum mycorrhizal colonization (43.3%) over control followed by *G. mosseae* + *L. laccata* (40.7%) and *D. necatrix* + *G. mosseae* + *L. laccata* + *T. harzianum*

(40.0%) over control.

4.8.1.8 Effect on rhizosphere acid phosphatase activity

The root rot pathogen *D. necatrix* reduced the acid phosphatase activity in the rhizosphere of apple seedlings by 54.9 per cent as compared to uninoculated control (Table 11). However, the pathogenic effect of *D. necatrix* was significantly reduced by the inoculation of biocontrol agents.

Biocontrol agents inoculated individually alongwith *D. necatrix* significantly increased rhizosphere acid phosphatase activity by 33.6 to 58.6 per cent as compared to *D. necatrix*-inoculated treatment after 120 days growth, with maximum increase in *D. necatrix* + *G. mosseae* and minimum in *D. necatrix* + *T. harzianum* treatment.

The combined inoculation of *G. mosseae* + *T. harzianum* + *L. laccata* caused significantly higher increase in rhizosphere acid phosphatase activity (146.2%) as compared to *D. necatrix*-inoculated treatment followed by coinoculation of *D. necatrix* + *T. harzianum* + *L. laccata* (133.8%).

The biocontrol agents, inoculated individually or in combination, proved most effective in improving rhizosphere acid phosphatase activity. Among the individual inoculations, the highest increase in rhizosphere acid phosphatase activity (183.0%), as compared to uninoculated control, was observed in *G. mosseae*-inoculated treatment, followed by *L. laccata* (169.7%) and *T. harzianum* (85.9%).

The combined inoculation of *G. mosseae* + *T. harzianum* + *L. laccata* in absence of pathogen caused highest increase in acid phosphatase activity (252.0%) in apple rhizosphere as compared to uninoculated control. This was followed by the coinoculation of *L. laccata* + *T. harzianum* with 233.8 per cent increase in rhizosphere acid phosphatase activity over uninoculated control.

Rhizosphere acid phosphatase activity increased significantly with the advancement of growth period, even in control seedlings. The acid phosphatase activity in rhizosphere of apple seedlings ranged from 8.0 to 32.0 and 14.2 to 54.3 $\mu\text{M PNP/g soil/hr}$ in 60 and 120 days growth period, respectively.

4.8.2 *Pythium ultimum*-biocontrol agent interaction in apple seedlings

4.8.2.1 Effect on seedling height

The study on the influence of biocontrol agents on seedling height of apple seedlings in presence and absence of root rot pathogen (*P. ultimum*) revealed that *P. ultimum* significantly reduced the seedling height by 8.4 per cent in comparison to uninoculated control (Plate 12). The individual inoculation of biocontrol agents showed 3.0 to 13.4 per cent increase in seedling height over uninoculated control, with maximum increase in *G. mosseae* inoculated plants and minimum in *L. laccata* inoculated plants (Table 12).

The coinoculation of root rot pathogen and individual biocontrol agents depicted a significant increase of 10.2 to 14.7 per cent in seedling height over *P. ultimum*-inoculated seedlings with maximum increase observed by the inoculation of *G. mosseae* in presence of *P. ultimum* followed by *T. harzianum* treatment.

4.8.2.2 Effect on seedling diameter

The study on the influence of biocontrol agents on seedling diameter of apple seedlings in presence and absence of *P. ultimum* revealed that *P. ultimum* significantly reduced seedling diameter by 11.4 per cent in comparison to uninoculated control (Table 12). The individual inoculation of bioagents showed 5.7 to 17.1 per cent increase in seedling diameter over uninoculated control, with maximum increase in *G. mosseae*-inoculated plants and minimum in *L. laccata*-inoculated plants.

The coinoculation of root rot pathogen and individual biocontrol agents depicted a significant increase in seedling diameter (14.5 to 22.5%) over *P. ultimum* inoculated seedling, with maximum increase due to the *G. mosseae*+ *P. ultimum* and minimum in *T. harzianum* treatment.

4.8.2.3 Effect on root length

P. ultimum significantly reduced root length of apple seedlings by 15.2 per cent as compared to uninoculated control (Table 12). Biocontrol agents inoculated individually in absence of root rot pathogen significantly increased root length of apple seedlings as compared to uninoculated control, with maximum increase observed in *G. mossea* (15.2%) followed by *T. harzianum* (7.6%) and *L. laccata* (1.0%).

Biocontrol agents reduced the pathogenic effect of *P. ultimum* and

significantly improved the root length of apple seedlings. Individual inoculation of bioagents in presence of pathogen depicted 19.0 to 25.0 per cent increase in root length as compared to *P. ultimum* inoculated seedlings, with maximum increase observed in *P. ultimum* + *G. mosseae* and minimum in *P. ultimum* + *T. harzianum*.

4.8.2.4 Effect on root biomass

The root rot pathogen *P. ultimum* caused 12.5 per cent reduction in root biomass as compared to uninoculated control (Table 12). The individual inoculation of biocontrol agents in absence of *P. ultimum* caused significant improvement in root biomass with maximum gain of 15.3 per cent over control in *G. mosseae* inoculation, followed by *T. harzianum* (11.5%) and *L. laccata* (8.6%).

The individual inoculation of biocontrol agents in presence of pathogen significantly improved root biomass from 23.1 to 17.5 per cent in comparison to *P. ultimum*-inoculated seedlings. The highest increase was observed in *P. ultimum* + *G. mosseae* and lowest in *P. ultimum* + *T. harzianum* inoculated plants.

4.8.2.5 Effect on shoot biomass

The root rot pathogen *P. ultimum* caused 17.3 per cent reduction in root biomass compared to uninoculated control (Table 12). The individual inoculation of biocontrol agents in absence of *P. ultimum* showed a significantly improved shoot biomass with maximum increase in *G. mosseae* inoculated plants (13.0%), followed by *T. harzianum* (9.8%) and *L. laccata* (3.3%) and as compared to

control.

The individual inoculation of biocontrol agents in presence of pathogen significantly improved shoot biomass from 17.1 to 26.3 per cent in comparison to *P. ultimum*-inoculated seedlings. The highest increase was observed in *P. ultimum* + *G. mosseae* and minimum in *P. ultimum* + *L. laccata* inoculated plants.

4.8.2.6 Effect on root rot index

The influence of bioagents on root rot index of apple seedlings caused by *P. ultimum* was assessed 120 days after seedling establishment. The biocontrol agents invariably influenced the development of root rot in apple seedlings (Table 13). All the bioagents when inoculated individually reduced apple root rot significantly in comparison to *P. ultimum* inoculated control, with maximum reduction observed in *P. ultimum* + *G. harzianum* inoculated seedlings (63.3%) and minimum in *P. ultimum* + *L. laccata* inoculated seedlings (52.4%).

4.8.2.7 Effect on mycorrhizal colonization

The study on root colonization of apple seedlings by mycorrhizal fungi (*G. mosseae* and *L. laccata*) revealed that these fungi were able to develop symbiotic association with apple roots, and with the passage of time these fungi induced more mycorrhizal short root formation (Table 13). Highest mycorrhizal root colonization was observed in apple roots inoculated with *G. mosseae* (34.2%) followed by *L. laccata* inoculated seedlings (26.5%). *P. ultimum* significantly reduced the number of mycorrhizal short roots formed. The lowest mycorrhizal

root colonization of 17.9 per cent was recorded in seedlings inoculated with *L. laccata* + *P. ultimum*. However, *G. mosseae* + *P. ultimum* inoculated seedlings depicted 23.3 per cent mycorrhizal colonization.

4.8.2.8 Effect on acid phosphatase activity in apple rhizosphere

The study on the impact of biocontrol agents and root rot pathogen *P. ultimum* on rhizosphere acid phosphatase activity of apple revealed 35.5 per cent decrease in acid phosphatase activity due to the presence of *P. ultimum* (Table 13). Among the biocontrol agents inoculated individually in absence of pathogen, maximum increase in rhizosphere acid phosphatase activity over uninoculated control was observed in *G. mosseae* inoculated seedlings (160.4%), followed by *L. laccata* (149.6%) and *T. harzianum* (61.0%).

The biocontrol agents inoculated individually alongwith *P. ultimum* significantly increased rhizosphere acid phosphatase activity by 46.5 to 60.3 per cent as compared to *P. ultimum* inoculated treatment, with maximum increase in *P. ultimum* + *Glomus mosseae* and minimum in *P. ultimum* + *T. harzianum* treatment.

CHAPTER – 5

DISCUSSION

Root rot, caused by soil borne fungi, is one of the major constraints limiting the successful establishment of apple seedlings right from their nursery stage. The root rot affected seedlings turn brown, wither and eventually dry. The disease in case of mild attacks also reduces the yield of plants significantly.

Two fungi were mainly found responsible for causing the root rot in affected apple seedlings. On the basis of their morphological characteristics, pathogenicity and comparison with the authentic descriptions these fungi, isolated from diseased root samples, were identified as *Dematophora necatrix* Hartig and *Pythium ultimum* Trow. The characteristics of isolated fungi corroborated with the descriptions given by Nattrass (1927), Agarwala and Sharma (1966), Gupta (1977), Plaats and Vander (1981) and Freeman *et al.* (1986). *Dematophora necatrix* Hartig, the causal pathogen of white root rot, and *P. ultimum* (Trow), collar and root rot pathogen, are main soil borne pathogens which inflict heavy loss to the apple plants (Agarwala and Sharma, 1966; Utkhede and Smith, 1991). Apart from apple, these pathogens have been reported to infect many other crops including tropical and subtropical plants such as avocado and mango (Khan, 1959; Behdad, 1975; Szejnberg and Madar, 1980; Zentmyer, 1984). In India, while root rot was first reported from the hills of Uttranchal by Singh in 1939 (Bose and Sindhan, 1976) and later encountered in Himachal Pradesh (Agarwala and

Sharma, 1961). The disease has assumed serious threat to apple plantation in Kashmir only in recent years.

The initial typical symptoms of the disease appeared after 15 days of pathogenic inoculation on one year old apple seedlings in case of *D. necatrix* and within 20 days in case of *P. ultimum*. The symptoms of white root rot included drooping of topmost leaves followed by bronzing, drying and ultimately the death of plant (Thakur *et al.*, 2005). The examination of underground plant parts revealed that all the fibrous roots turned brown in colour and were killed. The rotted portions of root showed white mycelial growth. These findings are in agreement with Nattrass (1927). Reports reveal that white flocculent growth of fungus on dead roots is prominent during rainy season (Freeman *et al.*, 1990).

In case of *Pythium ultimum* initially small water-soaked lesions appeared on collar portion of plants which gradually increased and encircled the whole collar in severe cases. The affected portions were greasy to touch and exuded out latex. The bark was easy to be peeled off thereby exposing the inner tissues which was almost in a semi-rotten state. The main root began to rot but did not progress downward up to the tip. The lateral roots were also affected. Usually lateral roots in such affected plants did not develop and taproots often became twisted. The above ground parts showed chlorosis, withering, stunted growth and wilting symptoms. The findings are in agreement with those of Owen (2002); Mehrotra and Aneja (2005) and Mishra *et al.* (2005).

Different antagonists were isolated from the rhizosphere of apple seedlings in view of the fact that the native antagonists usually have better rhizosphere competency and high antagonistic potential. Two endo-mycorrhizal fungi (VAM), isolated from the rhizosphere of healthy apple trees, were identified as *Glomus mosseae* and *G. calendonius* on the basis of their morphological characters compared with the authentic descriptions given by Hall (1979). VAM fungi were identified on the basis of their sporocarps, spore size, colour, aggregation, spore wall, surface ornamentation and presence and absence of vesicles in soil. *Glomus mosseae* did not possess any sporocarp but its spores were aggregated into loose clusters. The spores were double-walled, measured 110-205 μm in size, pale yellow in colour and had a basal protrusion which was typically funnel shaped. The soil borne vesicles were absent. However, in case of *Glomus calendonius* sporocarps were present and spores were dark brown measuring 100-250 μm in size. Subtending hyphae were simple occluded by spore wall, thickened, pigmented and measured 10-20 μm in diameter. Cell wall was of unit type. Such typical characteristics of *G. mosseae* and *G. calendonius* corroborate with those of Schenck and Perez (1987).

Two ectomycorrhizal fungi isolated from the sporocarps, collected from canopy of healthy apple trees, were identified as *Laccaria laccata* Broome and Barkely and *Suillus granulatus* (fr.) Kuntze on the basis of their morpho-cultural characteristics compared with the authentic descriptions given by Lakhenpal (1988) and Mehrotra (1991). *L. laccata* and *S. granulatus* have previously been

isolated from the basidiomata collected from apple orchard growing near forest plantations (Zak, 1964). The caps of *L. laccata*, 2-5 cm in dia., were initially orange brown. Later, these became buff coloured, obtuse to convex with a central depression and minutely scaly with smooth margin. Gills were pink and attached to stem. Stipes were pink and equal to subclavate. Spores were hyaline, globose to sub-globose and echinulate. Basidia were hyaline and clavate with 4 sterigmata. The culture of *L. laccata*, raised on MMN medium, was white to slightly pinkish and fungal colonies reached 60-70 mm dia. after 2 weeks of incubation at $25\pm 2^{\circ}\text{C}$. The mycelial mat was thick, interwoven and tightly appressed to agar surface. Similar characteristic features of *Laccaria laccata* (Broome and Berkeley) has been given by many workers (Schenck, 1982; Sylvia, 1983 and Gadbout and Fortin, 1985).

The caps of *Suillus granulatus* were 4-12 cm in size, yellowish brown to light brown and convex with shining smooth surfaces. Pores were radially arranged having 1.0-1.5 mm wide tubes with pore surfaces whitish when fungus was young but soon turned bright yellow. The stipes were white which later became yellow. The stipe surface had brownish to reddish brown glandular dots. Stipes were mostly equal, occasionally tapering to the base, solid and without any veil. The spores were hyaline to dull brown, smooth, elliptical to oblong and measured $7.0-9.0 \times 2.5-3.5 \mu\text{m}$ in size. Basidia were hyaline, clavate to cylindrical with 4 sterigmata. Somatic cultures were initially white later turned grey yellow. Colonies were furry and hyphae grooved and aseptate. The colonies of *S.*

granulatus attained 20-30 mm dia. in 25 days of incubation on MMN medium at 25±2°C. These observations are in accordance with those of Lakhanpal (1988) and Mehrotra (1991).

On the basis of mycorrhizal colonization, plant biomass and rhizosphere acid phosphatase activity *G. mosseae* proved more effective than *G. calendonius* which formed basis for its selection *in vivo* studies (Meera *et al.*, 1990)

The soil borne pathogens are difficult to control through chemical means alone as the efficacy of chemicals is affected by several factors in a complex system like soil. Further, chemicals when applied in soil may prove lethal to non-target beneficial soil microflora. Therefore, for successful management of soil borne diseases integration of all available practices is desirable. Locally isolated antagonists generally prove better in combating the pathogenic attacks due to their faster acclimatization. *In vitro* evaluation of isolated antagonists under dual culture conditions revealed that antagonists inflicted growth inhibition of root rot pathogens (*D. necatrix* and *P. ultimum*). *T. harzianum* caused maximum mycelial growth inhibition of 65.8 and 65.1 per cent in *D. necatrix* and *P. ultimum*, respectively, in dual culture, exhibited strong mycoparasitic activity and lysed the host mycelia once in contact with them. The formation of hyphal coils by *T. harzianum* on pathogenic colonies was also noticed. These observations are in agreement with those of Bunker and Mathur (2001) and Pandey *et al.* (2005). The differential response of antagonists in inhibiting the root rot pathogens may

probably be due to variation in type, quantity and stability of metabolites produced including antibiotics (Somani, 2005).

Laccaria laccata in dual culture with *D. necatrix* and *P. ultimum* caused hyphal lysis of both the pathogens. Interaction caused rupture and twisting of pathogenic hyphae at the initial stage and showed gradual desiccation and shrinkage of cytoplasm. The mycoparasitic activity of ectomycorrhizal fungi against root rot pathogen has earlier been suggested by Zhao and Kuo (1988).

In the present study apple seedlings showed variable response to the *in vivo* inoculation of *Glomus mosseae*, *Laccaria laccata* and *T. harzianum*. There was a significant increase in seedling growth by the inoculation of mycorrhizal fungi, with *G. mosseae* proving more effective in improving the plant biomass, height and girth as well as in colonizing high proportion of apple roots as compared to *L. laccata*. *G. mosseae* inoculated seedlings showed 17.4 and 23.4 per cent increase in root length and seedling height, respectively, as compared to *L. laccata* inoculated seedlings. The increase in plant growth and biomass may be probably ascribed to the release of growth promoting substances by mycorrhizal fungi, altered root physiology, and cross-protection against the pathogenic organisms. An increase of 31.4 per cent in seedling growth has previously been reported by Bharat and Bhardwaj (2001) in apple seedlings inoculated with *G. mosseae*. A significant correlation between *G. mosseae* inoculation and growth parameters has been reported by Mosse (1972) and Krishna and Bagyaraj (1986) in groundnut.

Rhizosphere acid phosphatase activity was higher in *G. mosseae* inoculated seedlings. Among the bioagents inoculated individually, the VAM fungus *G. mosseae* exhibited good potential to mobilize phosphorus into plants viz-a-viz enhanced the plant growth. The significant increase in VAM colonization, plant growth and soil acid phosphatase activity by the inoculation of VAM isolate suggested the extrinsic external mycelial proliferation which, in turn, enhanced phosphorus uptake (Graham and Egel, 1988.). It seems that acid phosphatase presumably increased the nutrient mobilization and uptake so supported better growth (Dunabeitia *et al.*, 2004). VAM fungi reportedly increase plant growth by enhancing nutrient uptake through reduction of distance between diffusible nutrient and plant roots as well as by accelerating the rate of nutrient absorption and nutrient concentration at the absorbing surfaces and finally by chemically modifying the availability of nutrient for uptake by host through mycorrhizal hyphae (Champawat, 1989).

Ectomycorrhizal fungus *L. laccata* showed significantly higher plant growth with 27.3, 61.9 and 38.4 per cent increase in shoot height, root length and shoot biomass, respectively, as compared to *Dematophora*-inoculated control. The seedlings with considerable ectomycorrhizal colonization rapidly regenerate new lateral roots, create more new sites for ectomycorrhizae and thereby utilize available nutrients more efficiently than non-mycorrhizal seedlings (Marx and Hatchell, 1986). The favourable influence of ectomycorrhizal fungus on plant growth and health may be attributed to the excretion of growth promoting

substances by mycorrhizae (Strzelczyk *et al.*, 1985; Duchesne and Peterson, 1987) or indirectly by alteration in root physiology, mineral uptake and exudation pattern into the mycorrhizosphere (Leyval and Berthelin, 1990).

T. harzianum showed stimulatory effect on seedling growth and biomass when compared to uninoculated control. *T. harzianum* increased seedling height by 16.6 per cent, root length by 12.5 per cent, seedling diameter by 3.1 per cent, shoot biomass by 12.4 per cent and root biomass by 15.7 per cent. The present findings are supported by Roiger and Jeffers (1991) who observed significantly higher plant biomass in apple seedlings inoculated with *Trichoderma* spp. in comparison to uninoculated control. *Trichoderma* species reportedly produce hormone like metabolites and release nutrients from soil or organic matter thereby facilitate better plant growth (Windham *et al.*, 1986).

During *in vivo* study biocontrol agents (*G. mosseae*, *L. laccata* and *T. harzianum*) when inoculated to apple individually or in combination significantly improved the plant growth and biomass. However, the presence of root rot pathogens (*D. necatrix* or *P. ultimum*) reduced the plant growth and biomass in apple seedlings. The combined inoculation of all the three bioagents (*G. mosseae* + *L. laccata* + *T. harzianum*) caused maximum increase in seedling growth and biomass than dual inoculation. The seedling height, root length, seedling diameter, shoot biomass and root biomass of apple seedlings increased by 32.7, 26.3, 16.6, 28.5 and 29.8 per cent, respectively, as compared to uninoculated control. Steep

increase in plant growth may be ascribed to the synergistic growth promoting action of biocontrol agents as well as more solubilization of mineral nutrients.

Significantly reduced seedling growth and biomass was noticed due to the infection of root rot pathogens. However, biocontrol agents individually or in combination reduced the pathogenic effect of root rot fungi and significantly improved the growth and biomass of apple seedlings as compared to the seedlings inoculated with pathogen alone. Combined inoculation of biocontrol agents in presence of root rot pathogen proved superior over individual inoculations because of their synergistic action on root rot pathogen and growth promotion.

Root rot index of apple incited by *D. necatrix* and *P. ultimum* was significantly decreased by the inoculation of biocontrol agents. The roots of apple seedlings were found infected only when *D. necatrix* or *P. ultimum* was inoculated in plant growth medium which suggests that *D. necatrix* and *P. ultimum* incite root rot in apple seedlings. *D. necatrix* proved more pathogenic and caused 35.4 per cent more root rot than *P. ultimum*. Decreased plant growth and biomass and less ectomycorrhizal colonization was observed in pathogen-infected apple seedlings obviously due to less availability of healthy sites for colonization. In present study, the application of antagonists (*G. mosseae*, *L. laccata* and *T. harzianum*) individually as well as in combination significantly reduced the root rot percentage of apple seedlings. *T. harzianum* showed highest antagonistic activity followed by *G. mosseae* and *L. laccata*. *T. harzianum* and *G. mosseae* are reported to have greater rhizosphere competence and ability to parasitize the

pathogenic fungi (Naik, 2003; Tapwal *et al.*, 2005). The antagonists in rhizosphere are likely to compete with the pathogen for the host surface and nutrients as well as inhibit the pathogenic growth through antibiosis/mycoparasitism (Howell, 2003). This accordingly seems to have reduced the seedling root decay. These speculations are substantiated by our *in vitro* biocontrol studies on *D. necatrix* and *P. ultimum* with respect to *L. laccata* and *T. harzianum*. The principal mechanisms of these bioagents for disease control have been assumed to be those primarily acting upon the pathogens which include mycoparasitism, antibiosis and competition for resources and space (Harman, 2006). Thus reduction of root rot in apple seedlings incited by *D. necatrix* and *P. ultimum* may be due to the biological control action of antagonist on these pathogens. Our findings are in agreement with the findings of Sharma (1994), Bhardwaj and Gupta (2000) and Ashawani *et al.* (2004). Further, reduction in root rot due to *L. laccata* may be attributed to the protection, induced resistance and release of antimicrobial compounds by ectomycorrhiza. Fungal sheath around roots seems to have restricted the advancement of pathogen into mycorrhizal cortex (Zak, 1964). Protection of apple seedlings against *D. necatrix* due to *L. laccata* has been attributed to the possible production of antifungal phenolic compounds by the host in presence of mycorrhizae (Sylvia and Sinclair, 1983; Sampangi and Perrin and Garbaye, 1983).

The combined inoculation of biocontrol agents, in general, showed non-additive synergistic effect in root rot infected seedlings with respect to growth

promotion and nutrient uptake. Roots of apple seedlings were colonized by mycorrhizae *G. mosseae* and *L. laccata* when inoculated as vegetative inoculum. Generally their co-inoculation increased the total number of mycorrhizal short root in comparison to individual inoculations. The highest root colonization was observed in treatment wherein all the three bioagents (*T. harzianum* + *G. mosseae* + *L. laccata*) were inoculated. It seems that *T. harzianum* might have stimulated the mycorrhizal development in apple roots (Granger *et al.*, 1983; Windham *et al.*, 1986; Graham and Egel, 1988).

In the present study mycorrhizal colonization was decreased in presence of *D. necatrix* and *P. ultimum*. Decreased mycorrhizal colonization may be attributed to the competition for space as well as reduction in root mass due to root decay by rot causing organisms, thereby leaving less surviving root area for symbiosis (Veghelyi, 1994; Thakur *et al.*, 2005). Also, it seems that probably the pathogen might have altered the host metabolism and depleted their nutrients. Chakravarty and Unestam (1987) reported that development of mycorrhizal species was strongly suppressed by the root rot pathogens.

During the present investigation it was found that presence of biocontrol agents in rhizosphere of apple seedlings enhanced acid phosphatase activity in soil. The combined inoculation of biocontrol agents resulted in higher acid phosphatase activity than individual inoculations. Acid phosphatase is solely an extra-cellular enzyme and is believed to be involved in the mineralization of soil organic phosphorus. Among the bioagents, *G. mosseae* exhibited higher

phosphatase activity followed by *L. laccata*. The phosphatases produced by mycorrhizae solubilize insoluble forms of phosphorus and other nutrients not readily available to mycorrhizal plant roots (Bartlett and Lewis, 1973). Dunabeitia *et al.* (2004) have also observed greater acid phosphatase activity in the rhizosphere of mycorrhizal plants than non-mycorrhizal ones. In present study, the rhizosphere acid phosphatase activity was significantly higher in *G. mosseae*-inoculated seedlings than uninoculated control. These findings are in accordance with the results of Bhardwaj and Gupta, (2000) who reported that *G. mosseae* possess significant phosphorous solubilizing and mobilizing activity. *T. harzianum* has also the ability to solubilize many plant nutrients including rock phosphate from their solid phase compounds by its enzymatic activity (Altomare *et al.*, 1999). The synergistic effect of biocontrol agents in combined inoculation resulted higher phosphatase activity in the rhizosphere of apple seedlings.

CHAPTER – 6

SUMMARY AND CONCLUSION

The results on the study of “Influence of mycorrhizae on root rot in apple (*Malus domestica* Borkh)” are summarized hereunder.

The causal agents of root rot of apple were isolated from the infected seedlings and grown on potato dextrose agar medium. On the basis of cultural and morphological characters, pathogenicity and comparison with authentic descriptions, two pathogens found responsible for the disease were identified as *Dematophora necatrix* Hartig and *Pythium ultimum* Trow. The pathogenicity of isolated fungi (*D. necatrix* and *P. ultimum*) was proved by Koch’s postulates. The characteristic disease symptoms in case of *D. necatrix* were noticed 15 days after inoculation whereas in case of *P. ultimum* the symptoms appeared 20 days after inoculation.

Two endomycorrhizae and two ectomycorrhizae were isolated from the rhizosphere of apple trees. The vesicular arbuscular mycorrhizae identified on the basis of various physico-morphological characters such as shape, colour, size, septation, wall thickness, hyphal attachment, spore dimension, spore ornamentation, spore wall etc. were *Glomus mosseae* (Gerdemann & Nicolson) and *G. calendonius* (Gerdemann & Nicolson). On the basis of mycorrhizal colonization, plant biomass and acid phosphatase activity, *G. mosseae* proved more effective.

Two ectomycorrhizal fungi *Laccaria laccata* (Broome and Berkley) and *Suillus granulatus* (fr.) Kuntze were isolated from the sporocarps collected from the canopy of apple trees and identified on the basis of their morpho-cultural characteristics.

All the biocontrol, except VAM, tested under *in vitro* conditions showed mycelial growth inhibition of root rot pathogens (*D. necatrix* and *P. ultimum*) in dual culture, with maximum inhibition due to *Trichoderma harzianum* followed by *L. laccata* and *S. granulatus*. All the antagonists exhibited strong mycoparasitic activity and lysed host mycelia in dual culture.

During *in vivo* study biocontrol agents (*T. harzianum* + *L. laccata* + *G. mosseae*) inoculated individually or in combination significantly improved the plant growth (seedling height and root length) and biomass. The root rot pathogens (*D. necatrix* and *P. ultimum*) significantly reduced the growth and biomass of apple seedlings. Among the bioagents inoculated individually, the endomycorrhiza *G. mosseae* showed significantly higher plant growth, followed by *T. harzianum* and *L. laccata* as compared to uninoculated control. The combined inoculation of biocontrol agents, in general, showed synergistic growth promoting activity. The highest increase in growth and biomass was observed due to the combined inoculation of all the three bioagents (*L. laccata* + *G. mosseae* + *T. harzianum*). Growth and biomass of apple seedlings was significantly reduced by root rot pathogens (*D. necatrix* and *P. ultimum*). However, biocontrol agents inoculated individually or in combination reduced the pathogenic effect of both

the pathogens and significantly improved the growth and biomass of apple seedlings as compared to the seedlings inoculated with pathogen alone.

Among the bioagents inoculated individually in presence of pathogen, *G. mosseae* was most effective in improving the growth of apple seedlings, followed by *T. harzianum* and *L. laccata*. However, combined action of all the three bioagents resulted in maximum inhibition of root rot pathogen, thereby improved plant growth and biomass.

Root rot of apple seedlings incited by *D. necatrix* and *P. ultimum* was significantly decreased by the inoculation of biocontrol agents. *D. necatrix* proved more pathogenic and caused 35.4 per cent more root rot than *P. ultimum*. Application of biocontrol agents (*T. harzianum* + *G. mosseae* + *L. laccata*) individually or in combination significantly reduced the root rot of apple seedlings. The biocontrol agents when inoculated individually reduced the root rot of apple seedlings by 37.9 to 52.9 per cent as compared to the seedlings inoculated with *D. necatrix* alone, with maximum reduction by *L. laccata* and minimum by *T. harzianum*. The combined inoculation of biocontrol agents (*L. laccata* + *T. harzianum* + *G. mosseae*) was most effective and reduced the root rot of apple seedlings upto 75 per cent as compared to the pathogen-inoculated control. Co- inoculation of *G. mosseae* + *L. laccata* + *T. harzianum* resulted in highest mycorrhizal root colonization of 43.3 per cent due to their synergistic action.

Presence of biocontrol agents in the rhizosphere of apple seedlings enhanced the rhizosphere acid phosphatase activity. The combined inoculation of biocontrol agents resulted in higher phosphatase activity than individual inoculations. Among the bioagents tested, *G. mosseae* exhibited higher acid phosphatase activity followed by *L. laccata*.

In light of present investigation it may be deduced that root rot of apple seedlings is prevalent in apple orchards of Kashmir valley which needs urgent attention. The white root rot is incited by *D. necatrix* and collar and root rot by *P. ultimum*. Isolated antagonists have high rhizosphere competence and biological suppressive activity against these root rot pathogens of apple seedlings. Inoculation of apple plants at nursery stage with an appropriate and effective microbial inoculant, alone or in combination can be advocated for enhancing the plant vigour and reducing the root rot severity. Combined inoculation of *G. mosseae*, *L. laccata* and *T. harzianum* significantly improved the growth and reduced the pathogenic infection of apple seedlings as compared to individual inoculations. The biomanagement of disease can ensure the successful plantation of apple trees. These diseases need to be managed through integrated strategy involving biological control as well.

Table 9: Impact of pathogen (*Dematophora necatrix*) x bioagent interaction on plant height and seedling diameter of apple

Treatment	Plant height (cm)		Seedling diameter (mm)	
	60 d*	120 d	60 d	120 d
Pathogen (P)	50.4	54.5	7.4	7.8
ECM (E**)	54.0	69.4	7.8	9.8
VAM (V***)	69.8	85.7	8.2	10.4
<i>T. harzianum</i> (T)	64.7	80.6	7.9	9.9
P + E	56.9	67.3	7.6	8.5
P + V	62.6	75.4	7.8	8.7
P + T	58.5	70.0	7.6	8.3
E + V	73.7	89.7	8.4	10.8
E + T	67.6	81.6	8.1	10.4
V + T	75.2	91.3	8.5	10.9
P + E + V	64.9	78.4	7.8	10.1
P + E + T	61.4	72.5	8.2	9.6
P + T + V	71.7	85.0	8.2	9.6
E + T + V	76.2	91.7	8.6	11.2
P + T + E + V	74.3	88.6	8.3	10.6
Control	57.9	69.1	7.8	9.6
CD(p= 0.05)	4.07	5.95	0.24	0.52

* Days after inoculation

**E = *Laccaria laccata*

***V= *Glomus mosseae*

Table 10 : Impact of pathogen (*Dematophora necatrix*) x bioagent interaction on root length and dry biomass of apple

Treatment	Root length (cm)		Root biomass (g)		Shoot biomass (g)	
	60 d*	120 d	60 d	120 d	60 d	120 d
Pathogen (P)	10.1	9.2	6.4	6.8	11.8	11.7
ECM (E**)	11.2	14.9	7.9	11.7	13.2	16.2
VAM (V***)	13.0	17.5	10.2	13.5	17.7	18.8
<i>T. harzianum</i> (T)	14.8	17.1	10.0	13.2	17.1	18.1
P + E	10.2	13.5	8.9	10.0	14.7	15.7
P + V	9.7	13.0	9.4	10.5	15.1	15.1
P + T	10.8	13.8	9.3	10.7	14.9	14.9
E + V	14.3	18.2	11.1	14.0	18.3	19.8
E + T	15.1	18.7	10.0	13.6	17.3	20.6
V + T	15.8	19.0	10.7	13.3	18.4	19.3
P + E + V	11.9	15.6	10.3	11.9	16.9	16.7
P + E + T	11.1	14.5	9.6	12.2	15.1	17.4
P + T + V	12.7	16.4	10.2	12.5	17.7	17.0
E + T + V	16.8	19.2	10.9	14.8	18.6	20.7
P + T + E + V	16.0	18.3	10.5	13.9	18.4	18.9
Control	11.6	15.2	9.0	11.4	14.5	16.1
CD (p=0.05)	1.43	1.55	1.03	1.48	1.06	0.95

* Days after inoculation

**E = *Laccaria laccata*

***V= *Glomus mosseae*

Table 11 : Impact of pathogen (*Dematophora necatrix*) x bioagent on root rot, mycorrhizal colonization and rhizosphere acid phosphatase activity in apple

Treatment	Root rot (%)		Mycorrhizal colonization (%)		Acid phosphatase activities $\mu\text{M PNP g}^{-1} \text{ soil hr}^{-1}$	
	60 d*	120 d	60 d	120 d	60 d	120 d
Pathogen (P)	38.7 (38.45)	71.0 (57.42)	-	-	15.3	22.0
ECM (E**)	-	-	20.2 (26.70)	28.8 (32.075)	24.2	38.3
VAM (V***)	-	-	27.3 (31.49)	36.2 (36.98)	25.3	40.2
<i>T. harzianum</i> (T)	-	-	-	-	16.2	26.4
P + E	28.2 (32.07)	44.04 (41.55)	12.5 (20.68)	20.2 (26.70)	21.2	32.2
P + V	21.6 (27.68)	35.0 (36.27)	19.0 (25.83)	25.7 (30.46)	26.1	34.9
P + T	18.9 (25.76)	33.4 (35.30)	-	-	18.0	29.4
E + V	-	-	30.6 (33.50)	40.7 (39.64)	29.4	42.3
E + T	-	-	21.2 (27.40)	29.9 (33.14)	22.4	47.4
V + T	-	-	28.4 (32.20)	38.2 (33.56)	27.4	38.4
P + E + V	20.2 (26.70)	31.4 (34.10)	27.4 (31.56)	30.5 (38.17)	31.2	44.4
P + E + T	17.4 (24.65)	25.04 (30.26)	20.5 (26.91)	25.4 (30.26)	24.4	51.3
P + T + V	14.0 (21.97)	18.4 (25.40)	26.2 (30.78)	27.4 (31.56)	29.0	42.5
E + T + V	-	-	32.4 (34.69)	43.3 (39.98)	32.0	54.3
P + T + E + V	12.9 (21.04)	17.5 (24.72)	27.9 (31.88)	40.0 (39.23)	31.7	50.3
Control	-	-	-	-	8.0	14.2
CD (p=0.05)	(2.68)	(2.52)	(2.14)	(2.91)	1.49	1.73

* Days after inoculation

**E = *Laccaria laccata*

***V= *Glomus mosseae*

Values in parentheses are arc sine values

Table 12 : Impact of pathogen (*Pythium ultimum*) x bioagent interaction on seedling growth and dry biomass of apple

Treatments	Plant height (cm)	Seedling diameter (mm)	Root length (cm)	Root biomass (g)	Shoot biomass (g)
	120 d*	120 d	120 d	120 d	120 d
Pathogen (P)	42.8	6.2	10.0	9.1	7.6
ECM (E**)	45.3	7.4	11.9	11.6	8.9
VAM (V***)	53.0	8.2	13.6	12.0	10.4
<i>T. harzianum</i> (T)	52.1	7.7	12.7	11.3	10.1
P + E	47.2	7.4	12.3	10.9	8.9
P + V	49.1	7.6	12.5	11.2	9.6
P + T	48.5	7.1	11.9	10.7	9.1
Control	46.7	7.0	11.8	10.4	9.2
CD (p=0.05)	2.60	0.53	1.20	1.06	1.25

* Days after inoculation

**E = *Laccaria laccata*

***V= *Glomus mosseae*

Table 13 : Impact of pathogen (*Pythium ultimum*) x bioagent interaction on root rot, mycorrhizal root colonization and rhizosphere phosphatase activity in apple

Treatments	Root rot (%)	Mycorrhizal colonization (%)	Acid phosphatase activity μM PNP/g soil/hr
Pathogen (P)	52.4 (46.37)	-	20.2
ECM (E**)	-	26.5 (30.97)	37.2
VAM (V***)	-	34.2 (35.76)	38.8
<i>T. harzianum</i> (T)	-	-	24.0
P + E	24.9 (29.95)	17.9 (25.02)	30.5
P + V	20.3 (26.77)	23.3 (28.86)	32.4
P + T	19.21 (25.99)	-	29.6
Control	-	-	14.9
CD (p=0.05)	(1.77)	(1.82)	(2.44)

Values in parenthesis are arc sine values

**E = *Laccaria laccata*

***V= *Glomus mosseae*

Table 6: Morphological and cultural characteristics of ectomycorrhizal fungi collected from the rhizosphere of apple trees
A) *Laccaria laccata* Broome and Berkeley

Character	Colour	Shape	Size
Cap (Pileus)	Orange brown later changing buff	Obtuse to convex; becomes plane to uplifted, often with a central depression; minutely scaly, margin smooth	2-5 cm dia.
Gills	Pink to pinkish brown	Attached to stem, distant or close	-
Stipe	Pink	Equal, subclavate	1-2 x 3-7 cm
Spores	Hyaline	Globose to sub-globose; occasionally broadly ellipsoid, echinulate	7-12 μm (Av. 9.5 μm)
Basidia	Hyaline	Clavate with 4 sterigmata	25-54 x 6-14 μm (Av. 39 x 9 μm)
Colony	White to slightly pinkish	Mycelial mat felty thick, interwoven, tightly appressed to agar surface, occasionally forming cottony to felty aerial layer	60-70 mm after 2 week on MMN agar

Contd...

B) *Suillus granulatus* (fr.) Kuntze

Character	Colour	Shape	Size
Cap	Yellowish brown to light brown	Convex becoming broadly convex; slimy, smooth surface composed of loosely arranged gelatinised hyphae	4-12 cm dia.
Pore surface	Whitish at first soon turn yellowish	Radially arranged	1.0-1.5 mm wide tubes about 1.0 cm deep
Stipe	White become yellowish to bright yellow; surface with brownish to reddish brown glandular dots	Equal, sometimes with tapering base, solid, veil absent	4-8 cm long, 1-2 cm thick
Spores	Dull brown	Smooth elliptical to oblong	7.0-9 x 2.5-3.5 μm (Av. 8.25 x 2.75 μm)
Basidia	Hyaline	4-sterigmata, clavate to cylindrical	18-24 x 5-6 μm (Av. 20 x 5.2 μm)
Colony	White soon turn to grey yellow to fawn coloured	Furry colonies, grooved hyphae, aseptate	Reached 20-30 mm dia. in 25 days on MMN medium at 25 °C.

Table 4. Morphological characteristics of VAM isolates

S. No.	Characteristics	<i>Glomus mosscae</i> (Nicolson and Gerdemann)	<i>Glomus calendonius</i> (Nicolson and Gerdemann)
1.	Sporocarp	-	+
2.	Spore		
i.	Size	110-205 μm (avg. = 190 μm)	100-250 μm (avg. 210 μm)
ii.	Colour	Pale yellow	Dark brown
iii.	Aggregation	Aggregated into loose clusters	-
iv.	Surface ornamentation	-	-
3.	Subtending hyphae	+, funnel shaped at widest part 18-50 μm (basal protrusion) closed by septum	+, simple hyphal attachment occluded by spore wall thicken, pigmented, and 10-20 μm in dia.
4.	Soil borne vesicle	-	-
5.	Cell wall type	Thick walled (30 μm), hyaline	Unit type (single layered) rigid wall

+ means present

- means absent

Table 2 : Morphological characteristics of white root rot fungus *Dematophora necatrix* (Hartig)

Fungal propagule	Colour	Shape	Size	Septation	Growth
Colony	Initially white later turn slightly black	Cottony	-	-	36 mm after 3 days of incubation at 25°C
Mycelium	Brown	Branched septate and knotted slightly swollen at septa i.e. pyriform swellings	5-8 µm (wide) (Av. 6.5 µm wide)	Septate	-
Conidiophore	Hyaline	Flexous intertwined, repeatedly branched threads formed independently on brown ropy synmmeta which project as rigid columns	40-300 µm (Av. 260 µm)	-	-
Conidia	Olive brown	Solitary, ellipsoid,1 celled, smooth	3.0-4.5 x 2.0-2.5 µm (Av. 3.5 x 2.5 µm)	-	-
Synemmata	Brown	Projecting as rigid columns component threads flexous, intertwined and dichotomously branched	-	-	-

Table 3 : Morphological characteristics of root rot fungus *Pythium ultimum* (Trow)

Fungal propagule	Colour	Shape	Size	Septation	Growth
Colony	White	Fluffy mass		-	24 hours radial growth 36 mm at 25°C
Mycelium	Hyaline	Fine, well branched, slendrical	Av. 10 µm (width)	Aseptate	
Hyphal swelling	Hyaline	Globose or sub-globose, terminal	15-30 µm (Av. 20 µm)	-	-
Oospore	-	Smooth spherical, terminal	17-25 µm dia. (Av. 19 µm dia.)	-	-
Anthredia	-	Monoclinous	-	-	-

Table 7: *In vitro* effect of bioagents on radial growth of apple white root rot pathogen *Dematophora necatrix*

Treatments*	Average radial growth (mm)	Inhibition over control (%)
<i>Laccaria laccata</i>	51.38	42.90 (40.9)
<i>Suillus granulatus</i>	57.38	36.20 (36.9)
<i>T. harzianum</i>	30.71	65.87 (54.2)
Control	90.00	-
CD (p=0.05)	-	(2.97)

Values in parentheses are arc sine values.

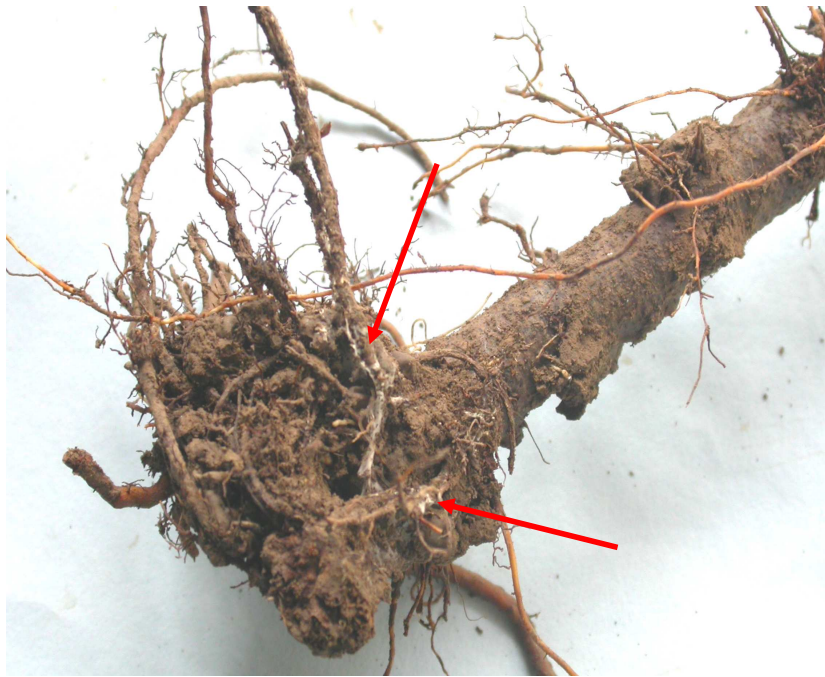
* *L. laccata* and *S. granulatus* inoculated on PDA 7 and 15 days, respectively, prior to pathogenic inoculation where as *T. harzianum* and pathogen inoculated on PDA simultaneously

Table 8 : *In vitro* effect of bioagents on radial growth of apple root rot pathogen *Pythium ultimum*

Treatments*	Average radial growth (mm)	Inhibition over control (%)
<i>Laccaria laccata</i>	49.20	45.3 (42.3)
<i>Suillus granulatus</i>	54.15	39.8 (39.1)
<i>T. harzianum</i>	31.38	65.1 (53.7)
Control	90.08	-
CD (p=0.05)	-	(2.92)

Values in parentheses are arc sine values.

* *L. laccata* and *S. granulatus* inoculated on PDA 7 and 15 days, respectively, prior to pathogenic inoculation where as *T. harzianum* and pathogen inoculated on PDA simultaneously



(a)

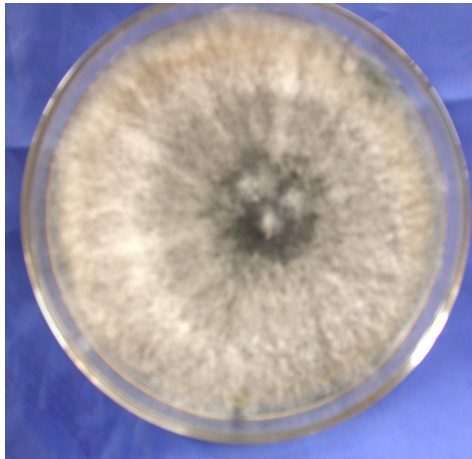


(b)

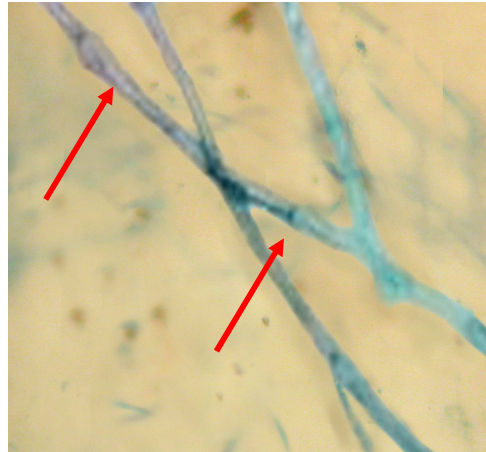


(c)

Plate 2: Rot affected apple seedlings showing (a) typical white mycelial growth and browning; (b) drooping of leaves and wilt symptoms; and (c) decayed roots



(a)

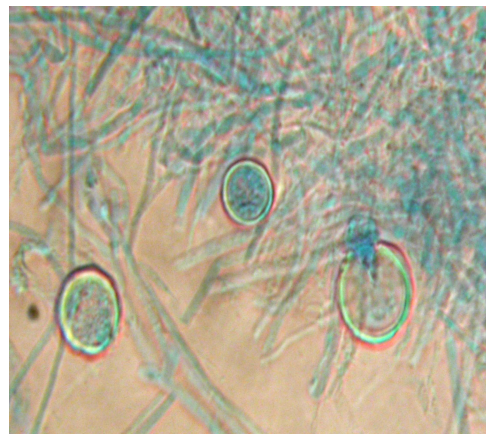


(b)

Plate 3: Pure culture of *Dematophora necatrix* (a) with typical pyriform swellings at septa (b)



(a)

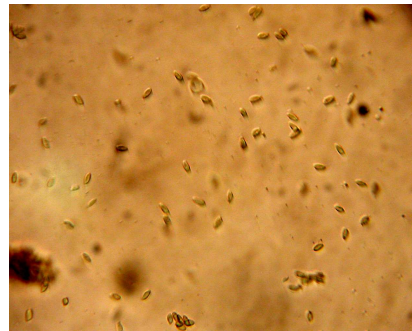


(b)

Plate 4: Pure culture of *Pythium ultimum* (a) with coenocytic mycelium and oospores (b)



(a)



(b)

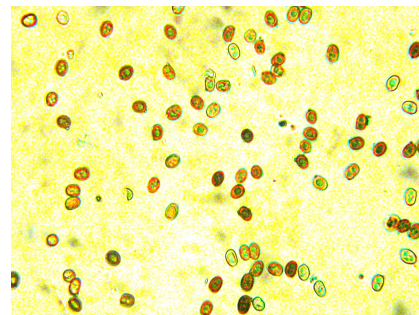


(c)

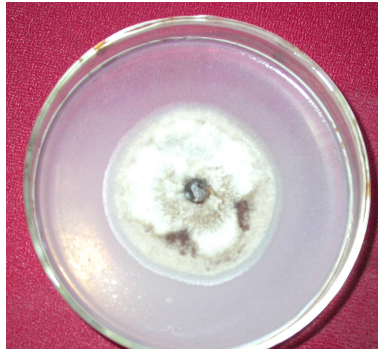
Plate 7: (a) Sporocarp (b) spores and (c) pure culture of *Laccaria laccata*



a)

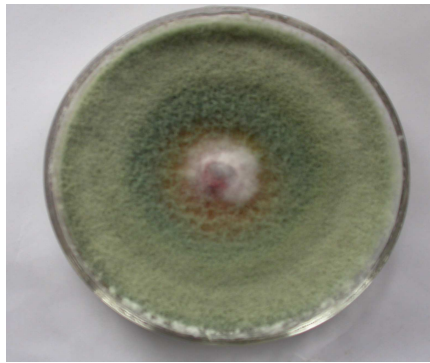


b)



(c)

Plate 8: (a) Sporocarp (b) spores and (c) pure culture of *Suillus granulatus*

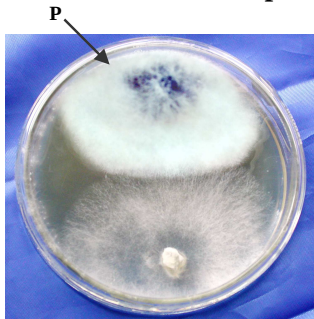


(a)



(b)

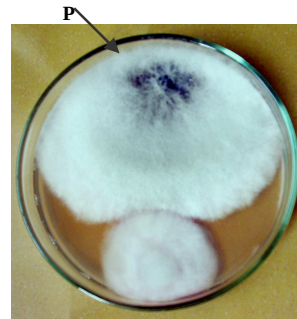
Plate 9: Pure culture of (a) *Trichoderma harzianum* and their (b) conidiophores



(a)

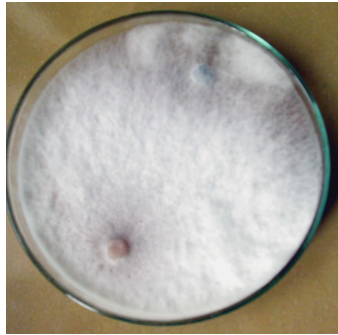


(b)

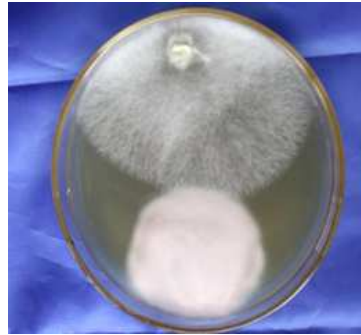


(c)





d)

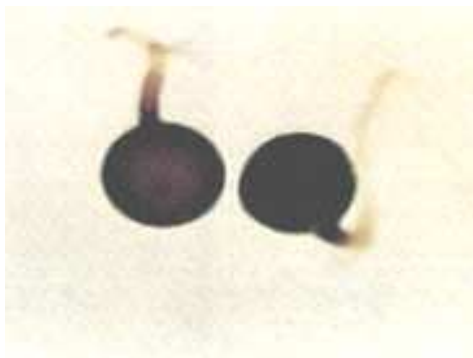


e)

P = Pathogen

Plate 10: *In vitro* interaction of pathogens and bioagents in dual culture

- a) *Dematophora necatrix* + *Laccaria laccata* b) *D. necatrix* + *Trichoderma harzianum*
- c) *D. necatrix* + *Suillus granulatus*
- d) *Pythium ultimum* + *L. laccata* e) *P. ultimum* + *S. granulatus*



(a)



(b)

Plate 5: Spores of endomycorrhizae (a) *Glomus calendonius* (b) *Glomus mosseae*



***PLATE 6: MASS MULTIPLICATION OF
VAM ON MAIZE PLANT***



**Pots inoculated with : V + E + T; V +
T; E +T; P₁ + T + E + V; P₁; C
(from left to right)**

Pots inoculated with : V+E+T;P₁;C



(1) C (2) V + E + T (3) E + V



**Pots inoculated with V; T; P₂ + V;
P₂ + T; P₂; C (from left to right)**

**V = *Glomus mosseae*; E = *Laccaria laccata* ; T = *Trichoderma harzianum* ;
P₁ = *Dematophora necatrix* P₂ = *Pythium ultimum* and C = Control**

**Plate 12 : Comparison of different treatments in case of *Dematophora
necatrix* and *Pythium ultimum***



0 : Healthy; 1 : 1-10% rot; 2 : 11-20% rot; 3 : 21-50% rot; 4 : 51-75%; 5 : > 76%

Plate 1 : Scale (0-5) for assessment of root rot of apple



PYTHIUM ULTIMUM - ANTAGONIST INTERACTION TRIAL



DEMATOPHORA NECATRIX - ANTAGONIST INTERACTION TRIAL

Plate- 11 : A view of experimental trials

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