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Review article

BIOLOGICAL CONTROL OF DISEASES OF TEMPERATE FRUIT CROPS

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ABSTRACT

In modern era of organic farming the use of chemicals is being discouraged especially in fruit and vegetable production as these are consumed as raw. The chemical pesticides are posing threat in the form of environment and health hazards. The development of resistant pathogen populations against the chemicals has also stimulated the interest in formulation of integrated disease control system. Biological control would appear to have significant potential in terms of both environmental and economic issues for incorporation into organic and conventional temperate fruit production systems. Biological control of soil borne pathogens with antagonistic fungi and bacteria has been under intensive investigations for the last many years. This method has gained considerable attention and appears to be promising as a viable supplement or alternative to chemical control. A number of studies have demonstrated benefits resulting from application of plant growth promoting and disease suppressive rhizobacteria to subsequent growth of apple in replant soil. There is no chemical control for crown gall and hence the only management practice available is biological control. Perhaps the best documented success of biological control agent targeted for a soil borne pathogen has been the use of *Agrobacterium radiobacter* strain K-84, which has been used over 20 years to control crown gall on various plants all over the world. The mechanism of biological control of powdery mildew fungi by *Ampelomyces quisqualis* has been established as hyper-parasitism as this fungus posses the ability to colonize the mycelium of powdery mildew and produce reproductive structures. Successful biological control of postharvest diseases of fruits has been obtained through application of yeasts. In apple and other temperate fruits diversity of yeasts have been studies for biological controls of post harvest decays. Control of *Penicillium expansum, Botrytis cineria and Mucor spp.* has been reported using yeasts *Condida guilliermondii.* This is apparent that extensive ecological research will be required in order to achieve optimum utilization of the biological resources resident to orchard ecosystems for managing disease of temperate fruits.

KEY WORDS: Biological control, temperate fruits, Disease management

INTRODUCTION

Agro-climatic conditions in hilly states offer immense natural potential for increasing area and production under temperature fruits, especially apple. The production of these fruits is influenced to a great deal due to various diseases caused by fungi, bacteria and viruses. These diseases result in heavy losses to the farmers. To minimize the losses due to the diseases, farmers employ chemical control measures in fruit production system. Chemical fungicides which are used on fruit crops are posing threat in the form of environment and health hazards. In modern era of organic farming the use of chemicals is being discouraged. Also the development of resistant pathogen populations against the chemicals has stimulated interest in the formulation of integrated disease control system. Biological control would appear to have significant potential in terms of both environmental and economic issues for incorporation into organic and conventional temperate fruit production systems. Perennial cropping systems such as temperate fruits production provide unique opportunities and impediments to the use of biological control measures especially during the process of plant establishment. Several opportunities will exist in the nursery environment for the application of microbial inoculants such as biocontrol agents or mycorrhizal fungi. An additional opportunity for introduction of biocontrol agents exists at the time of orchard establishment. In this chapter, the application of biological measures for the control of some specific diseases of temperate fruits is discussed.

SOIL-BORNE DISEASES

Replant Problem

Cultivation of fruit trees is rapidly changing, with growers planting high density orchards of trees on dwarfing rootstocks instead of low density orchards with vigorous trees. Because of the limited land available to plant new orchards, growers are compelled to lay out new plantation on the old orchard site. Due to this replant problem has become major concern of growers. The problem of establishing fruit trees in old nurseries or orchard sites is generally known as replant problem or replant disease. There is confusion about the terminology of replant problem and replant disease. Replant problem includes biotic and abiotic factors that cause poor growth and delayed fruit production. Replant disease is caused by biotic factors and is one of the components of the replant problem.

Replant problem is a complex malady of temperate fruit crops. It is caused by abiotic and biotic factors. Abiotic

factors including phytotoxins, imbalance nutrition, poor soil structure, poor drainage, cold or draught stress and excess or lack of soil moisture contribute to the occurrence of replant problem. Among biotic factors various fungi, bacteria, nematodes and actinomycetes have been reported to be associated with replant disease. Various species of fungi like *Fusarium equiseti, F. oxysporum, F. solani, Rhizoctonia* spp., *Cylindrocladium spp., Rosellinia necatrix, Penicillium claviforme, P. janthinellum, Phytophthora* spp., *Puthium* spp., *Cylindrocarpon* spp., of bacteria like *Pseudomonas* spp. and *Bacillus* spp., and of nematodes like *Pratylenchus penetrans* and X*iphinema* spp. have been found associated with replant disease by various workers (Utkhede, 1996). The cause of the replant problem varies from region to region. Depending upon the causal factor involved in the replant problem different control measures including cultural practices, chemicals, bioagents and host resistance have been suggested to control this. Chemical control with soil fumigants is the most adopted method of controlling replant disease all over the world. But it is not an attractive approach as the effectiveness of volatile fumigants is influenced by temperature and moisture. Also the application of fumigants is difficult, expensive and hazardous. Moreover, soil fumigation is believed to destroy the natural equilibrium between pathogens and antagonistic microorganisms in soil. In this regards attempts have been made to develop a biological control of replant problem. A number of studies have demonstrated benefits resulting from application of plant growth promoting and disease suppressive rhizobacteria to subsequent growth of apple in replant soil (Caesar and Burr, 1987; Utkhede and Li, 1989b; Janisiewicz and Covey, 1983). A diversity of bacterial species has been identified that suppress individual causal elements and enhance growth of plants in replant soil. Biological control of *Phytophthora cactorum* which contributes to replant disease (Mazzola, 1998) has been reported in response to application of *Enterobacter aerogenes* (Utkhede and Smith, 1991). Application of strain B8 of *E. aerogenes* alone was found increasing growth of apple seedlings in replant soil (Utkhede and Li, 1989a). Similarly, BACT-1, EBW and B10 strains of *Bacillus subtilis* and B8 strain of *E. aerogenes* applied as soil drench increased plant growth over and above that of formalin fumigation (Utkhede and Li, 1989b). The inoculation of roots of young apple plants with *Agrobacterium radiobacter* has eliminated replant problems in green house and nursery experiments (Catska and Hudska, 1990). This biocontrol agent reduced the number of colonies of phytotoxic micromycetes which contribute towards replant disease. *Pseudomonas putida* strain 2 CB isolated from apple roots was found to inhibit growth of each element of the fungal complex reported to incite replant disease and enhanced growth of M-26 root stock in multiple apple replant soil (Mazzola *et al*., 2002). Casear and Burr (1987) identified two fluorescent pseudomonads and an enteric bacterium possessing the ability to promote growth of apple in replant soils. Enhanced growth by these rhizobacteria was associated with a reduction in root infection of *Cylindrocarpon destructans*, a fungal pathogen known to contribute apple replant disease (Jaffe *et al*., 1982; Mazzola, 1998). Application of *Bacillus subtilis* and *Enterobacter aerogenes* has also been found effective in promoting growth of apple plants even under filed condition in British Columbia, Canada (Utkhede and Smith, 1994). These results indicate that these species of rhizobacteria have a potential for biological control of replant disease.

Various studies have indicated that phosphorus is an essential nutrient for early growth of young plants in replant soil. Mycorrhizae symbiosis can improve nutrient uptake, particularly for immobile ions such as phosphate (Mosse, 1973). As a result of increased uptake of mineral nutrients from soil, mycorrihizal plants grow more vigorously and appear healthier than non mycorrhizal plants. However, these beneficial fungi are eliminated when replant disease is controlled by soil fumigation (Nemec, 1980). Inoculation of apple seedlings with arbuscular mycorrhizal fungi (AMF) was increased their growth is replant disease soil (Catska and Taube-Baab, 1994). Inoculation of apple seedlings with AMF *Glomus fasciculatum* and *G. macrocarpus* suppressed the population of phytotoxic micromycetes, responsible for replant disease and increased plant biomass (Catska, 1994). Two AMF, *Glomus intraradices* and *G. mosseae* significantly increased total shoot length and number of shoots per rootstock in replant soil. The seedlings inoculated with *G. mosseae* showed increased growth in replant soil which was neither pasteurized nor fertilized (Utkhede *et al*., 1992). Preplant sterilization of soil and subsequent inoculation with AMF, *Glomus epigaeum* significantly controlled the replant problem of apple and peach. It was observed that the growth promotion by inoculation with AMF was more in autoclaved replant soil (Bingye and Shengrui, 1998).

Root rot

Root rot is a very serious soil-borne disease infecting temperate fruits especially apple. It is caused by *Rosellinia necatrix* Berl. ex Prill. (Anam. *Dematophora necatrix* Hartig). The perfect stage of the fungus is not known to occur in India. The fungus has a wide host range of about 158 plant species belonging to over 45 families (Ito and Nakamura, 1984) comprising of fruit plants, forest trees and vegetable and field crops. In India the disease was first observed by Singh in 1939. Agarwala (1961) observed the disease on apple trees in Himachal Pradesh. The annual losses estimated due to this disease are about Rs. 1.3 million (Agarwala and Sharma, 1966) which are expected to be much more as the disease is reported to occur in all apple growing regions of the country. The pathogen affects the under ground parts of the trees. The lateral roots turn dark brown and are covered with greenish gray or white mycelial mat and with the progress of disease all the roots are attacked and fibrous root system disappears. Whitish mycelial mat like fungal growth is visible during monsoon on the affected parts. The affected plants show bronzing of the leaves and progressive decline and ultimately die with in 2-3 years of infection.

The pathogen survives in the form of mycelium or sclerotia in the infected roots. The infection of new roots takes place by the fungal mycelium present in the sol on debris or by the contact of new plant roots with the old dead roots. The disease is more serious in water logged acidic soils.

Management of root rot is very difficult because of deep seated infection. It is very difficult to make the reach of

remedial measures up to the point of infection. It can be managed by practicing preventive as well as curative measures consisting of cultural, biological and chemical methods and resistant root stocks. Biocontrol of soil borne pathogens with antagonistic fungi and bacteria has been under intensive investigations for the last many years. This method has gained considerable attention and appears to be promising as a viable supplement or alternative to chemical control. Ieki *et al*. (1969) have reported the inhibition of *R. necatrix* by different isolates of *Trichoderma* spp. Species of *Trichoderma* viz., *T. harzianum* and *T. hamatum* isolated from naturally roots rot infected root inhibited the growth of the fungus *R. necatrix* (Freeman *et al*., 1986.) The use of antagonistic *Trichoderma* spp. against the disease has also been explored along with soil solarization (Sztejnberg *et al*., 1987). Reduction of root rot by using the antagonists like *T. harzianum, T. koningii* and *T. viride* has also been noticed under pot culture studies (Sharma, 1993). Fungal antagonists *T. harzianum* and *T. viride* and bacterial antagonists *Pseudomonas fluorescens* and *Bacillus* spp. have proved effective in controlling root rot under pot culture studies (Sharma, 2000). Other bacterial antagonists *Enterobacter aerogenes* have been found to protect the plants from *D. necatrix* (Gupta and Jindal, 1989). Repeated application of these antagonists enhanced their efficacy against the pathogen and the effect persisted for longer period. Besides antagonistic fungi and bacteria the arbuscular mycorrhizal fungi (AMF) have also been used against soil borne diseases. Bharat and Bhardwaj (2001) while studying the interactions between AMF and root rot pathogens *D. necatrix* on apple seedlings under pot culture found that the apple seedlings previously inoculated with local AMF isolate of *Glomus* spp. showed less root rot severity as compared to the seedlings which were not inoculated with *Glomus* spp. The mycorrhizal seedlings also exhibited increased growth.

Crown rot

Crown rot, also known as collar rot is prevalent in all apple growing regions of the world. It is caused by a fungus *Phytophthora cactorum* (Lebert- Cohn) Schroeter. The disease causes extensive losses sometimes resulting in death of trees. The infection starts from the collar region and spread mostly to under ground parts and the above ground stem. Bark at the soil level becomes slimy and rots resulting in cankered area. The attacked trees show chlorotic foliage with red colouration of veins and margins. The causal fungus in known to survive in the orchard soils as chlamydospores in plant debris or soil. The fungus produces oospores which serve as the source of primary inoculum. Moderate temperature and high soil moisture favour the disease.

For the management of this disease attempts have been made by using chemicals, cultural practices and through host resistance. But the use of biological agents for controlling *P. cactroum* has also been tested by various workers. Roiger and Jeffer (1991) evaluated *Trichoderma* spp. against *Phytophthora* crown rot of apple and found that *T. virens, T. koningii* and *T. harzianum* were effective in controlling the disease. Other species of *Trichoderma, T. longibrachiatum, T. viride* also proved effective in controlling *P. cactorum* in apple (Kumar, 2002).

Some bacterial antagonists have also been found effective in checking the disease. Utkhede (1983) showed that bacterium *Enterobacter aerogenes* was antagonistic to *P. cactorum*. When this antagonistic bacterial cells were applied as soil drench, reduced the infection of *P. cactorum* in McIntosh apple seedlings. Other bacterial antagonists such as *Pseudomonas* spp., *Bacillus subtilis* were also found affective against *Phytophthora* crown rot (Janisiewicz and Covey, 1983; Utkhede, 1984). Antifungal activity of *E. aerogenes* and *Bacillus* spp. against *P. cactorum* was also observed by Gupta and Utkhede (1986) and with the treatments of apple plants with these antagonists even managed the crown rot infection under field conditions (Utkhede, 1987). Effect of soil drenching of apple trees with *E. aerogenes* was evaluated for three years by Utkhede and Smith (1991) in British Columbia with god control of the disease. Apple trees treated with strain B8 of *E. aerogenes* for two consecutive years not only reduced *P. cactorum* infection but also increased the plant height (Levesque *et al*., 1993). It has also been demonstrated that antibiotics produced by *E. aerogenes* provide protection to apple trees from infection by other soil borne pathogens (Marchi and Utkhede, 1994). Another species of antagonistic bacterium *Enterobacter*, *E. agglomerans* have also been reported to reduce disease severity and increase the trunk girth and fruit yield in McSpur apple on MM 106 rootstock which is otherwise susceptible rootstock for crown rot (Utkhede and Smith, 1997). Lyophilized dry formulation of strain B8 of this antagonist proved effective. Another product Binab-1 formulated from antagonist *Trichoderma viride* have proved successful in controlling *P. cactorum* infection in apple seedlings (Orlikowski and Schmidle, 1985). Application of bacterial antagonistic *E. agglomerans* and *B. subtilis* along with arbuscular mycorrhizal fungus *Glomus intraradices* in a six year experiment significantly reduced the infection of apple plants with *P. cactorum* and increased fruit yield and tree trunk growth (Utkhede and Smith, 2000).

The application of this antagonist has also been tried in combination with fungicide metalaxyl as soil trunk drench twice in a year for three years and once in spring for seven years. The combined application reduced disease ratings of *P. cactorum* (Utkhede and Smith, 1993). Similarly the same combination (each 1g/tree) when applied once in a year resulted higher annual increase in trunk diameter of effected apple plants (Levesque *et al*., 1993). Kumar (2002) has observed that pre inoculation of apple seedlings with *Trichoderma longibrachiatum* along with simultaneous inoculation with *Bacillus subtilis* effectively controlled *P. cactorum* infection in apple seedlings under pot cultures.

Crown gall

Crown gall disease is worldwide in its occurrence. It affects almost all temperate fruits including pome and stone fruits. Its incidence is found more in nurseries as compared to mature trees in orchards. The galls are formed mostly on stem and roots near the soil line. The infected plants grow poorly and become stunted. The disease in caused by a bacterium *Agrobacterium tumefaciens* (Smith and Twon.) Conn. The most characteristic property of this bacterium is its ability to introduce part of the Ti-plasmid (T-DNA) into the plant

cells and to transform normal plant cells to tumour cells. This property of the bacterium makes it a genetic parasite (Zambryski, 1992).

The bacterium persists in soil and initiate infection form fresh wounds at the crown or on roots and rarely on trunk and limbs. The wounds may be resulted by anything during cultivation. Even the graft union can provide the entry point for the bacterium.

There is no chemical control for crown gall and hence the only management practice available is biological control. Perhaps the best documented success of biological control agent targeted for a soil borne pathogen has been the use of *Agrobacterium radiobacter* strain K-84, which has been used over 20 years to control crown gall on various plants all over the world. Excellent biological control of crown gall in obtained by soaking germinated seeds or dipping nursery seedlings or rootstocks in a suspension of *A. radiobacter* strain K-84. The antagonist controls crown gall initiation by establishing itself on plant tissues where it produces bacteriocin agrocin 84 (Moore and Warren, 1979; Kerr, 1980). However, some strains of *A. tumefaciens* later on showed resistance to agrocin 84. A strategy was then developed to construct mutant by deleting part of transfer region of the existing strain. The resultant strain was designated K-1026 and possessed all the phenotypic traits of the parental K-84. Strain K-1026 was as effective as K-84 in protecting the plants from crown gall infection (Jones and Kerr, 1989). Strain K-1026 was registered in 1988 in Australia under the name 'Nogall' as peat based formulation for the control of crown galls on stone fruits. It is the most successful commercial deployment of any specific microorganism to control soil borne diseases. Strain K-84 and its genetically modified strain K- 1026 were tested for biocontrol of crown gall on some sensitive root stock of peaches viz., bitter almond, peach x bitter almond hybrid GF 677 and quince BA29. Both K 84 and K 1026 were effective and both reduced the percentage of galled plants as well as number of galls per plants under field conditions. The best results were obtained on bitter almond tree rootstock (Ali *et al*., 2004). Similarly the commercial formulation 'Nogall" when applied @ 150 mg/l before planting to colt root stock of cherry controlled crown gall effectively, the rootstock is otherwise very susceptible to crown gall (Wazir *et al*., 2000). Johnson and Dileone (1999) found that wounded cherry plants when dipped into different concentration of *A. radiobacter* strain K 84 prior to pathogen inoculation, the amount of disease suppression per unit of antagonist decreased with increasing antagonistic dose under field condition. The strains agrocin 84 and K 1026 have also been found to inhibit development of crown gall in apple (Sun *et al*., 2000).

Other antagonists reported for biological control of crown gall in temperate fruits are *Bacillus subtilis* and *Streptomyces* spp. Gupta and Khosla (2007) observed antagonistic activity of *Bacillus subtilis* against *A. tumefaciens* and observed that this antagonist when applied 24 hours prior to pathogen inoculation as soil drench, reduced crown gall incidence on cherry root stock colt. Al-momani *et al*. (1999) have reported inhibitory activity of *Streptomyces spp.* isolated from temperate fruit orchard soil against *A. fumefaciens* infecting almond trees.

FOLIAR AND FRUIT DISEASES

Apple scab

Apple scab occurs worldwide and is the most important disease of apples. Its primary effect is reduction of the quality of fruits. It also reduces size and results in premature fruits drop, defoliation and poor fruit bud development for the next year, and it reduces the length of time infected fruit can be kept in storage. In India the disease was first noticed in Kashmir in 1930 (Nath, 1935). This disease appeared in epidemic form in 1973 and caused large scale damage to apple crop in Kashmir. In Himachal Pradesh the disease was noticed in 1977 (Gupta, 1978) and spread to epidemic form in 1983 (Gupta, 1989). The disease destroyed most of the apple crop in the state. The disease was declared as one of the five main plant diseases of national importance of Government of India.

The disease symptoms are observed on leaves, petioles, blossoms, fruits and pedicels. On emerging leaves velvety brown to olive green lesions appear first on lower surface and later on the both the surfaces. As the infected leaf ages, the tissue adjacent to the lesion thicken resulting in deformed leaves. The number of lesions may range from one to many and some times the entire surfaces become covered with scab and the term sheet scab is used to refer this type of symptoms. Infection of petioles and pedicels result in premature defoliation of leaves and fruits. On fruit similar lesions appear as the infected fruit enlarge the lesions become brown and corky.

The disease is caused by a fungus *Venturia inaequalis* (Cke.) Wint. Both the imperfect and perfect stages of the fungus are encountered in nature. The perfect stage is produced in overwintered leaves or fruits on the orchard floor. The pseudothecia formed of diseased fallen leaves in the presence of moisture continue to mature with development of asci and ascospores. The mature ascospores are discharged over a period of 5 to 9 weeks which coincides with time between pink and full bloom stages of apple bud development. Once the primary infection has taken place, the secondary infection is through the conidia produced on the primary lesions. Adequate moisture is essential for the discharge of ripe ascospores from the pseudothecia. The germination of ascospores takes place under suitable temperature and moisture (leaf wetness) condition. The conditions of temperature and leaf wetness duration for scab infection are well defined on Mills period or Tables. On the basis of weather data and amount of primary inoculum scab prediction and warning has become possible and an advantage in the monitored apple scab control programme especially in Himachal Pradesh.

For the management of the disease in India a protective fungicide spray programme is being adopted. The first spray of protective fungicide must start at silver tip stage. And this way 6-7 sprays of various fungicides are needed during the growing season which causes pollution and health hazards. Hence the work on resistance breeding and biological control of apple scab was initiated. The work on biological control is primarily focused on the control of overwintering stage of the pathogen on leaf litter. Carisse *et al*. (2000) have studied the effect of antagonistic fungi viz., *Microsphearopsis* spp. *Diplodia* spp. and *Trichoderma* spp. on ascospore production under natural conditions either as foliar postharvest sprays or as a

ground application at 90% leaf fall. They found significant reduction in ascospore production. Antagonistic fungus *Microsphaeropsis ochraceae* has been considered as potent bio-sanitation agent against apple scab as this fungus kills the resting structures of *V. inaequalis* and thereby reduces disease in the subsequent crop reason by lowering the initial inoculum (Carisse *et al*., 2007). In Canada use of biocontrol agents for the management of apple scab has become popular in organic apple production (Carisse and Dewdney, 2002). Use of antagonistic fungi to control overwintering stage of scab pathogen in fallen leaves is seen as an alternative to chemical control measures (Bengtson *et al*., 2001). Yeasts isolated from apple leaves have also been evaluated against *V. inaequalis* for their antagonistic activity (Fiss *et al*. 2003). Three strains H 10, H 15 and H 25 were found to suppress scab severity on apple seedlings under green house trials. Application of a concentration of 1.5 x 10^7 yeast cells per ml on 9 year old apple tree cv. Golden Delicious led to significant reduction in scab. Altinok and coworkers (2002) studied antagonistic effect of 30 different fungal isolates against apple scab on three cultivars of apple viz. granny Smith, Stark spur Golden and Starkrimson. Some isolates such as C*ryptococcus* spp. (white yeast), *Sporobolomyces* (pink yeast), *Alternaria* spp., *Epicoccum* spp. and *Popularia* spp. were found to produce volatile antibiotics and resulted 100% inhibition of colony development of scab pathogen.

Some biofungicides have also been evaluated against apple scab. Pleskatsevich and Berlinchick (2004) evaluated Fruitine, a biofungicide formulation of *Bacillus subtilis* strain BIMV 262 under integrated disease control system against scab and observed reduction in the disease. Other biofungicide formulations viz., Dizofungin, Biostat and Narciss of fungal and bacterial species were found to induce resistance in apple plants against scab in Russia (Nadykta, 2004).

Apple powdery mildew

Powdery mildew of apple is a major foliar disease prevalent in all apple growing countries of the world. The disease was first noticed on apple seedlings in Iova, USA in 1871 (Bessey, 1877). It is caused by the biotrophic fungus *Podosphaera leucotricha* (Ell. and Ev.) Salm. It can be a significant commercial problem in every stage of apple development, from reducing the growth of nursery stock to causing fruit russetting (Jones and Aldwinckle, 1990). In apple the pathogen overwinters in dormant buds, and infected buds may fail to develop new shoots during the subsequent growing season. Conidia developing from overwintering mycelium serve as the primary source of inoculum, and secondary spread of the pathogen is incited by inoculum that develops from infection of young leaves, blossoms and young fruits. Perfect stage of the pathogen is also reported in nature (Bharat and Bhardwaj, 2000) but is not believed to have a major role in the disease cycle. For biological control of powdery mildew all agents that have been reported to provide biocontrol have been fungal in nature. As powdery mildews are biotrophs and typically do not require exogenous nutrients for germination and initial penetration, control through competition for nutrients is not a viable strategy. Like wise as exposure of the pathogen on the leaf surface after spore germination is limited, control through antibiosis is not likely to be a suitable mechanism for disease control. As such the greatest attention has been focused on the use of mycoparasites for the control of powdery mildews. These include *Ampelomyces quisqualis* (Novitskaya and Puzanova, 1992). The mechanism of biocontrol by the fungus has been established as hyper-parasitism as this fungus posses the ability to colonize the mycelium of powdery mildew and produce reproductive structures. This fungus is naturally occurring hyper-parasite of both sexual and asexual structures of powdery mildew pathogen. It parasitizes and form pycnidia with in powdery mildews hyphae, conidiophores and cleistothecia. The parasitized colonies of powdery mildew are dull in appearance, flattened off-white to gray in colour with reduced spore production (Falk *et al*., 1995). Vaidya and Thakur (2005) have also isolated *A. quisqualis* from affected apple plants and other plant species belonging to rosaceae family. It showed the natural occurrence of this hyper-parasite in western Himalayan region for managing powdery mildew disease. Use of plant extracts along with hyper-parasite especially walnut extract has also been found to reduce the primary infection of apple foliar diseases including powdery mildew and (Meszka and Bielenin, 2006).

The mycoparasite *A. quisqualis* isolate A-10 has been released as a commercial product AQ 10 TM for biological control of powdery mildew (Grove and Boal, 1997). Another formulation Ampelomitsin form *Ampelomyces* spp. has been observed achieve 70-80 percent control of powdery mildew of temperate fruits (Smol-Yokova *et al*., 2004).

POSTHARVEST DISEASES

Amongst temperate fruits stone fruits are comparatively more perishable and prone to postharvest diseases. Postharvest diseases result in 10-50 per cent losses to temperate fruits. (Steppe, 1976). Many fungi and bacteria can cause post harvest spoilage and the major pathogens are species of *Alternaria, Aspergillus, Botyosphaeria, Botrytis, Colletotrichum, Monilinia, Mucor, Penicillium, Rhizopus and Trichothecium.* In most instances the post harvest pathogens gain entry to susceptible fruit tissues and cause infection via entry through fruits surface wounds that are generated through fruits surface wounds that are generated through the process of harvesting and postharvest handling. Some pathogens can also enter through natural opening like lenticels or decay may be initiated in the sinus between the calyx and core cavity (Spotts *et al*, 1988).

Attention to fruits handling practices in the field and during storage to reduce mechanical and physical injuries and management of controlled atmospheric condition can be effective techniques in reducing post harvest diseases. However, these methods do not ensure effective protection of stored fruits. Hence postharvest disease control has been reliant on the use of fungicides (Eckert and Ogawa, 1988). But the use of fungicides is being avoided in modern era of organic farming due to perceived or actual hazards to human health as well as environment. As the consumption of fruits can be sources of direct ingestion of fungicides, the use of biological methodologies for control of post harvest diseases has enjoyed significant attention and even an modicum of success over the past two decades. The primary sources of biological agents for the

control of post harvest pathogens have been the microbial community resident to the phyllosphere and fruit surface (Janisiewicz, 1987). Bacteria and yeasts or yeast like organisms have been the most commonly employed agents for the biological control of post harvest diseases of temperate fruits (Sharma and Kaul, 1999). Several studies have identified bacterial agents for control of post harvest diseases of apple pear and peaches. A saprophytic strain of *Pseudomonas syringe* provided biological control of gray mould, blue mould and mucor rot on pear and also on apple (Janisiewicz and Marchi, 1992; Jeffers and Wright, 1994). The strain *P. syringe* ESC-11 is currently registered for post harvest application to apples and marketed as the product Bio-save 110. An isolate of *Burkholderia cepacia* provided biological control of blue mould and gray mould of Golden delicious apples (Jamisiewicz and Roitman 1988). *Bacillus subtilis* applied to wounded apple reduced fruit rot caused by *Botrytis cineria, Penicillium expansum and P. malicorticis* (Leibinger *et al*., 1997). Biological control of postharvest brown rot of stone fruits has been reported by the application of *Bacillus subtilis, Epicoccum nigrum* and *Pseudomonas* spp. (Pusey and Wilson, 1984; Smilarick *et al*., 1993; Madrigal *et al*., 1994; Foschi *et al*., 1995). *B. Subtilis* can also be incorporated into wax which is normally used for the control of brown rot of peaches (Pusey *et al*., 1986). Successful biological control of postharvest diseases of fruits has been obtained through application of yeasts. In apple diversity of yeasts have been studies for biological controls of post harvest decays. Control of *Penicillium expansum, Botrytis cineria and Mucor spp*. has been reported using yeasts *Condida guilliermondii* (McLaughlin *et al*., 1992), *C. oleophila* (Mercier and Wilson, 1994), *Cryptococcus laurentii* (Roberts, 1990), *Kloeckera apiculata* (McLaughlin *et al.*, 1992) and *Sporobolomyces roseus* (Janisiewicz *et al*., 1994). The yeast *Candida oleophila* strain 182 has been commercialized as postharvest fungicide Aspire for conduct of *Botrytis* spp. and *Penicillium* spp.

However, Biological control of postharvest diseases has been obtained through the use of individual isolates, the application of strain mixtures has been promoted the application of multiple agents, which in concert expand the range of biocontrol activity. Janisiewicz (1988) found that combination of *Pseudomonas* spp. and *Acremonium breve* gave complete control of *B. cineria* and *P. expansum* on apple. A co-application involving the bacterial antagonist *P. syringe* and the yeast *S. roseus* applied in equal biomass provided control of blue mould that was superior to that obtained by treatment with the individual agents applied separately using a biomass equivalent to that of the mixture (Janisiewicz and Bors, 1995). Mixture of two *Aureobasidium pullulans* strains and an isolate of *Rhodotorula glutinis* were superior to any of the strains applied individually in controlling decay caused by *B. cineria, P. expansum and P. malicorticis* (Leibinger *et al*., 1997).

Mechanisms reported to contribute to biological control of postharvest diseases include antibiosis competitive exclusion, induced resistance in the host and production of hydrolytic enzymes suppression of *B. cinerea* and *P. expansum* by *Burkholderia cepacia* is reported to involve impart the production of antibiotic pyrrolnitrin (Janisiewicz *et al*., 1991). *Bacillus subtilis* produced antifungal substance i.e. iturin peptides which possess a wide spectrum of antifungal activity. This substance was determined to be primary mode of action of this antagonistic bacterium to *Monilinia fructicola* causing brown rot of stone fruits (McKeen *et al*., 1986). Utilization of carbon or nitrogen sources, nutrient competition and physical exclusion of pathogen are considered the possible mechanism in control of post harvest diseases of fruits by yeasts (Roberts, 1990). Droby and Chalutz (1994) suggested that application of yeasts to wounds on different fruit resulted in enhanced ethylene production. As ethylene is reported to have a role in induction of the resistance process, it was proposed that induction of the host resistance may operate in the disease suppression resulting from yeast application.

There has been increasing interest in determining the practicality of applying bio-control agents in the fields prior to harvest as a mean to control postharvest pathogens. This interest emanates from the fact that the infection of fruit by postharvest pathogens may occur in the field prior to harvest or as result of wound created during harvesting and handling but preceding the movement of fruit into dump tanks where bio-control agents have been applied. Biological control of postharvest diseases of fruit in a preharvest setting does appear in option on the basis of limited studies. Application of *Trichoderma harzianum* to apple in field significantly reduced symptoms of bulls eye rot in storage. Leibinger *et al*. (1997) applied antagonists to Golden Delicious apple in field to control postharvest disease and application of a combination containing two antagonists *Aurobasidium pullans* and *Rhodotorula glutinis* was as effective as chemical fungicides used against these diseases. These findings suggest that application of certain biocontrol agents that are a characteristic element of the resident microflora of fruits may be useful in reducing infection in the field and subsequent infections during fruit storage.

CONCLUSION

Biological control of certain diseases is currently a viable option in most temperate fruit production systems in both the preplant and the postharvest settings. At present, the most significant opportunities for successful use of biological control remain in the management of soil-borne diseases and postharvest diseases, as commercial biocontrol products are currently available. A lot of research work is needed in the biological control of foliar and fruit diseases. Research that is centered on the ecology of orchard ecosystems will be integral to the successful implementation of biological control as a component of a system approach to disease management. This it is apparent that extensive ecological research will be required in order to achieve optimum utilization of the biological resources resident to orchard ecosystems for managing disease of temperate fruits.

REFERENCES

Agarwala, R. K. (1961) Problems of root rot of apple in Himachal Pradesh and Prospectus of its control with antibiotics. Himachal Hortic. 2, 171-178.

Agarwala, R. K. and Sharma, V. C. (1966) White root rot disease of apple in Himachal Pradesh. Indian Phytopath. 19, 82-86.

Ali A, Amina, F., Moncef, H. and Ali, B. (2004) Efficacy of non-pathogenic *Agrobacterium* strain K84 and K1026 against crown gall in Tunisia. Phytopathol. Mediter. 43, 167-176.

Al-Momani, F., Saadoun, I. and Malkawi, H. I. (1999) *Streptomyces* spp. from Jordon soils with *in vitro* inhibitory activity against *Agrobacterium tumefaciens* Ab. 136 pti 854. African Plant Production 5, 129-130.

Altinok, H. H., Erkilik, A. and Canihos, Y. (2002) Antagonistic effect of volatile and non-volatile antibiotics produced by fungi isolated from apple phyllosphere on *Venturia inaequalis* (Cke). Wint. Bulletin-0ILB/SROP 25(10), 249-252.

Bengtson, M., Green, H., Hockenhull, J. and Pedersen, H. L. (2001) Microbiological control of cherry leaf spot and apple scab. DJE Rapport Markbrug 49, 33-38.

Bessy, C. W. (1877) On injurious fungi: The blight (*Erysiphe*): Iowa State College of Agriculture Bienn. Rep. pp 185-204.

Bharat, N. K. and Bhardwaj, L. N.(2000) Occurrence of perfect stage of powdery mildew of apple in North Western Himalaya. Plant Dis. Res. 15, 81-82.

Bharat, N. K. and Bhardwaj, L. N. (2001) Interactions between VA-mycorrhizal fungi and *Dematophora necatrix* and their effect of health of apple seedlings. Indian J. Plant Pathol. 19, 47-51.

Bingye, X. and Shengrui, Y. (1998) Studies on replant problems of apple and peach. Acta Hortic. 477, 83-88.

Caesar, A. J and Burr, T. J. (1987) Growth promotion of apple seedling rootstocks by specific strains of rhizobacteria. Phytopathology 77, 1583-1588.

Carisse, O. and Dewdbney, M. (2002) A review of non-fungicidal approaches for the control of apple scab. Phytoprotection 83, 1-29.

Carisse, O., Holloway, G. and Leggett, M. (2007) Potential and limitations of *Microsphaeropsis ochraceae*, an agent for bio-sanitation of apple scab. Biological Control-a Global Perspective 2007, 234-240.

Carisse, O., Philion, V., Rolland, D. and Bernier, J. (2000) Effect of fall application of fungal antagonists on spring ascospore production of apple scab pathogen, *Venturia inaequalis.* Phytopathology 90, 31-37.

Catska, V. (1994) Interrelationship between vesicular-arbuscular mycorrhiza and rhizosphere microflora in apple replant disease. Biologia Plantarum 36, 99-104.

Catska, V. and Hudska, G. (1990) The possibility of using biological preparations of *Agrobacterium radiobacter* for biological control of apple replant problem. Replant Newsletter 3, 3.

Catska, V. and Taube-Baab, H. (1994) Biological control of replant problems. Acta Hortic. 363, 115-119.

Droby, S. and Chalutz, E. (1994) Mode of action of biocontrol agents of post harvest diseases. **In:** Biocontrol of postharvest diseases Theory and Practice, Wilson, C.L.Q. and Wisniewski, M. E. (eds.) Boca Raton, F L: CRC Press, pp. 365-389.

Eckert, J. W. and Ogawa, J. M. (1988) The chemical control of postharvest diseases: deciduous fruit, berries, vegetables and root/tuber crops. Annu. Rev. Phytopathol. 26, 433-469.

Falk, S. P., Gadoury, D. M. and Pearson, R. C. (1995)Partial control of grape powdery mildew by the mycoparasite *Ampelomyces quisqualis.* Plant Dis. 79, 483-490.

Fiss, M., Barckhausen, O., Gherbawy, Y., Kollar, A., Hamamoto, M. and Auling, G. (2003) Characterization of apiphytotic yeasts of apple as potential biocontrol agents against apple scabs. (*Venturia inaequalis*). Zeitsch. Pflanz. Krankh. Pflanzensch. 110(6), 513-523.

Foschi, S., Roberti, R., Bremelli, A. and Flori, P. (1995) Application of antagonistic fungi against *Monilinia lexa* agent of fruit rot of peach. Bull OILB/SROP. 18, 79-82.

Freeman, S., Sztejnberg, A. and Chet, I. (1986) Evaluation of *Trichoderma* as a biocontrol agent for *Rosellinia necatrix*. Plant Soil 94, 163-170.

Grove, G. G. and Boal, R. J. (1997) Apple powdery control trials using the mycroparasite *Ampelomyces quisqualis* (AQ 10). F & N Tests 52, 7.

Gupta, A. K. and Khosla, K. (2007) Integration of soil solarization and potential native antagonist for the management of crown gall or cherry root stock colt. Scientia Hortic. 112, 51-57.

Gupta, G. K. (1978) Present status of apple scab (*Venturia inaequalis*) in Himachal Pradesh and strategy for its control. Pesticides 12, 13-14.

Gupta, G. K. (1989) Apple scab-economics and Technology. Pesticides 23, 33-39.

Gupta, V. K. and Jindal, K. K. (1989) Management of white root rot of apple (*Rosellinia necatrix*) by biological and cultural methods. Proc. Int. Hortic. Cong. Italy 2, 3229.

Gupta, V. K. and Utkhede, R. S. (1986) Factors affecting the production of antifungal compounds by *Enterobacter aerogenes* and *Bacillus subtilis*, antagonists of *Phytophthora cactorum.* J. Phytopathol. 117, 9-16.

Ieki, H., Kubomura, Y. and Koi, S. (1969) Detection and vertical distribution of white root rot fungus in forest soils. Ann. Phytopathol. Soc. Japan 35, 76-81.

Ito, S. I. and Nakamura, N. (1984) An outbreak of white root rot and its environmental conditions in the experimental arboretum. J. Japan For. Sci. 66, 262-267.

Jaffee, B. A., Abawi, G. S. and Mai, W. F. (1982) Role of soil microflora and *Pratylenchus penetrans* in the apple replant disease. Phytopathology 72, 247-251.

Janisiewicz, W. J. (1987) Postharvest biological control of blue mold on apples. Phytopathology 77, 481-485.

Janisiewicz, W. J. (1988) Biocontrol of postharvest disease of apples with antagonist mixtures. Phytopathology 78, 194-198.

Janisiewicz, W. J. and Bors, B. (1995) Development of a microbial community of bacterial and yeast antagonists to control wound invading postharvest pathogens of fruit. Appl. Environ. Microbiol. 61, 3261-3267.

Jamisiewicz, W. J. and Covey, R. P. (1983) Biological control of collar rot caused by *Phytophthora cactorum*. Phytopathology 73, 822.

Janisiewicz, W. Z. and Covey, R. B. (1983b) Biological control of *Phytophthora cactorum* Z. Pflanz. Pflanzenschutz. 90, 140-145.

Janisiewicz, W. J. and Marchi, A. (1992) Control of storage rots on various pear cultivars with a saprophytic strain of *Pseudomonas syringe.* Plant Dis. 76, 55-560.

Janisiewicz, W. J. and Roitman, J. (1988) Biological control of blue mold and gray mold on apple and pear with *Pseudomonas cepacia*. Phytopathology 78, 1697-1700.

Janisiewicz, W. J., Peterson, D. L. and Bors, R. (1994) Control of storage decay of apples with *Sporobolomyces roseus*. Plant Dis. 78, 466-470.

Janisiewicz, W. J., Yourman, L., Roitman, J. and Mahoney, N. (1991) Postharvest control of blue mold and gray mold of apple and pears by dip-treatment with pyrrolnitrin, a metabolite of *Pseudomonas cepacia*. Plant Dis. 75, 490-494.

Jeffers, S. N. and Wright, T. S. (1994) Comparison of four promising biological control agents for managing postharvest diseases of apples and pears. Phytopathology 84, 1082.

Johnson, K. B. and Dileone, J. A. (1999) Effect of antibiosis on antagonist dose plant disease response relation ships for biological control of tomato and cherry. Phytopathology 89, 974-980.

Jones, A. L. and Aldwinckle, H. S. (1990) Compendium of apple and pear diseases. APS Press. St. Paul, Minnesota 101pp.

Jones, D. A. and Kerr, A. (1989) *Agrobacterium radiobacter* strain K1026, a genetically engineered derivative of strain K-84, for biological control of crown gall. Plant Dis. 73, 15-18.

Kerr, A. (1980) Biological control of crown gall through production of agrocin 84. Plant Dis. 64, 25-30.

Kumar, B. (2002) Studies on collar rot of apple. Ph.D. Thesis, Dr. Y.S. Parmar UHF, Nauni, Solan H.P., India.

Leibinger, W., Breuker, B., Hahn, M. and Mendgen, K. (1997) Control of postharvest pathogens and colonization of the apple surface by the antagonistic microorganisms in the field. Phytopathology 87, 1103-1110.

Levesque, C. A., Holley, J. D. and Utkhede, R. S. (1993) Individual and combined effect of *Eaterobacter aerogenes* and metalaxyl on apple tree growth and Phytophthora crown and root rot development. Soil Biol. Biochem. 25, 975-979.

Madrigal, C., Pascual, S. and Melgarejo, P. (1994) Biological control of peach twig blight (*Monilinia laxa*) with *Epicoccum nigrum*. Plant Pathol. 43, 554-561.

Marchi, A. and Utkhede, R. S. (1994) Effect of *Enterobacter aerogenes* or rhizosphere microflora of apple trees. J. Phytopathol. 141, 127-132.

Mazzola, M. (1998) Elucidation of the microbial complex having a causal role in the development of apple replant disease in Washington. Phytopathology 88, 930-938.

Mazzola, M., Granastein, D. M. Elfving, D. C., Mullinix, K. and Gu, Y. H. (2002) Cultural management of microbial community structure to enhance growth of apple in replant soils. Phytopathology 92, 1363-1366.

McKeen, C. D., Reilly, C. L. and Pusey, P. L. (1986) Production and partial characterization of antifungal substances antagonistic to *Monilinia fructicola* from *Bacillus subtilis*. Phytopathology 76, 136-139.

McLaughlin, R. J., Wilson, C. L., Droby, S., Ben-Arie, R. and Chalutz, E. (1992) Biological control of postharvest diseases of grape, peach and apple with the yeasts *Kloeckera epiculata* and *Candida guilliermondii*. Plant Dis. 76, 470-473.

Mercier, J. and Wilson, C. L. (1994) Colonization of apple wounds by naturally occurring and introduced *Candida oleophila* and their effect on infection by *Botrytis cineria* during storage. Biol. Control 4, 138-144.

Meszka, B. and Biclenin, A. (2006) Non-chemical possibilities for control of apple fungal diseases. Phytopathologia Polonica 39, 63-70.

Moore, L. W., and Warren, G. (1979) *Agrobacterium radiobacter* strain 84 and biological control of crown gall. Annu. Rev. Phytopathol 17, 163-179.

Mosse, B. (1973) Advances in the study of vesicular-arbuscular mycorrhiza. Annu. Rev. Phytopathol. 11, 171-192.

Nadykta, V. D. (2004) Prospects of biological plant protection from phytopathogenic organisms. Zashchita-i-Karantin Rastenii 6, 26-28.

Nath, P. (1935) Studies in the disease of apple in Northern India. II. A short note on apple scab due to *Fusicladium dendriticum* Fuckel. J. Indian Bot. Soc. 14, 121-124.

Nemec, S. (1980) Effect of fungicides on endomycorrhizal development in sour orange. Can. J. Bot. 58, 522-526.

Novitskaya, L. N. and Puzanova, L. A. (1992) Biological protection of fruit nursery from powdery mildew. Zashch Rust. (Mosc.). 6, 25.

Orlikowski L B and Schmidle A. 1985. On the biological control of *Phytophthora cactorum* with *Trichoderma viride*. Deutsch. Pflanzensch. 37, 78-79.

Pleskatsevich, R. I. and Berlinchik, E. E. (2004) Evaluation of the efficiency of local paste like biofungicide fruitine against apple scab. Zashchita Rastenii 28, 132-138.

Pussey, P. L. and Wilson, C. L. (1984) Postharvest biological control of stone fruit brown rot by *Bacillus subtilis*. Plant Disease 68, 753-756.

Pusey, P. L., Wilson, C. L., Hotchkiss, M. W. and Franklin, J. D. (1986) Compatibility of *Bacillus subtilis* for postharvest control of peach brown rot with commercial fruit waxes, dichloran and cold storage conditions. Plant Dis. 70, 587-590.

Roberts, R. G. (1990) Postharvest biological control of gray mold of apple by *Cryptococcus laurentii*. Phytopathology 80, 526-530.

Roiger, D. J. and Jeffer, S. N. (1991) Evaluation of *Trichoderma* spp. for biological control of *Phytophthora* crown rot of apple seedlings. Phytopathology 81, 910-917.

Sharma, M. (2000) Non chemical methods for the management of white root rot of apple, Ph.D. Thesis, Dr. Y.S. Parmar UHF, Nauni, Solan (HP), India.

Sharma, R. C. and Kaul, J. L. (1999) Postharvest diseases of temperature fruits and their management. **In:** Diseases of Horticultural Crops Fruits, Verma, L. R. and Sharma, R. C. (eds.). Indus Pub. Co. New Delhi, 582-623pp.

Sharma, S. K. (1993) Studies on management of white root rot of apple. Ph.D. Thesis, Dr. Y.S. Parmar UHF, Nauni, Solan (HP), India, 146p.

Smilarick, J. L., Deris-Arrue, R., Bosch, J. R., Gonzalez, A. R., Herson, D. and Janisiewicz, W. J. (1993) Control of postharvest brown rot of nectarines and peaches by *Pseudomonas* Species. Crop Prot. 12, 313-320.

Smol-Yakova, V. M., Podogornaya, M. E., Puzanova, L. A., Cherkezova, S. R. and Yakuba, G. V. (2004) Ecologization of production of stone fruits from diseases. Sadovodstovo-i-Vinogradorstvo 5, 5-8.

Spotts, R. A., Holmes, J. R. and Washington, W. S. (1988) Factors effecting wet core rot of apples. Aus. Plant Pathol. 14, 53-57.

Steppe, H. M. (1976) Postharvest losses of agricultural products. Rep W.P./225/76 Serial No. 240 UNDP, Tehran, Iran.

Sun, Y., Wang, H., Wang, J. and Song, X. (2000) Pathogenic bacteria of apple crown gall and their biological control. Acta Phytopathol. Sinica 30, 332-336.

Sztejnberg, A., Freeman, S., Chet, I. and Katan, J. (1987) Control of *Rosellinia necatrix* in soil and in apple orchard by solarization and *Trichoderma harzianum*. Plant Dis. 71, 365-369.

Utkhede, R. S. and Li, T. S. C. (1989b) Evaluation of *Bacillus subtilis* for potential control of apple replant disease. J. Phytopathol. 126, 305-312.

Utkhede R S and Smith E M. (1991a) Biological and chemical treatment for control of *Phytophthora* crown and root rot caused by *Phytophthora cactorum* in high density apple orchard. *Can. J. Plant Pathol*. **13**: 267-270.

Utkhede, R. S. and Smith, E. M. (1991b) Phytophthora and Pythium spp. associated with root rot of young apple trees and their control. Soil Biol. Biochem. 23, 1059-1063.

Utkhede, R. S. and Smith, E. M. (1993) Long term effect of chemicals and biological treatment on crown rot and root rot of apple trees caused by *Phytophthora cactorum*. Soil Biol. Biochem. 25, 383-386.

Utkhede, R. S. and Smith, E. M. (1997) Effectiveness of dry formulation of *Enterobacter aerogenes* for control of crown and root rot of apple trees. Can. J. Plant Pathol. 19, 397-401.

Utkhede, R. S. and Smith, E. M. (2000) Impact of chemical, biological and cultural treatments on growth and yield of apple in replant disease soil. Aus. Plant Path. 29, 129-136.

Utkhede, R. S., Li, T.S.C. and Smith, E. M. (1992) The effect of *Glomus mosseae* and *Enterobacter aerogenes* on apple seedlings grown in apple replant disease soil. J. Phytopathol. 135, 281-288.

Utkhede, R. S. (1984) Effect of bacterial antagonists on *Phytophthora cactorum* and apple crown rot. Phytopathol. Z. 109, 169-176.

Utkhede, R. S. (1987) Chemical and biological control of crown and root rot of apple caused by *Phytophthora cactorum*. Can. J. Bot. 61, 3343-3348.

Utkhede, R. S. (1996) Replant disease and soil sickness. In: Management of soil-borne diseases (Eds. Utkhede RS and Gupta VK), Kalyani Publishers Ludhiana pp. 21-39.

Utkhede, R. S. and Li, T.S.C. (1989). Chemical and biological treatments for control of apple replant disease in British Columbia. *Can. J. Plant Pathol*. **11**: 143-147.

Utkhede, R S. and Smith, E. M. (1994) Development of biological control of apple replant disease. Acta Hortic. 363, 129-133.

Vaidya, S. and Thakur, V. S. (2005) *Ampelomyces quisqualis* Ces. -a mycoparasite of apple powdery mildew in western Himalayas. Indian Phytopath. 58, 250-251.

Wazir, F. K., Meladul, K., Qureshi, J. A., Barech, A. R. and Kakar, K. M. (2000) Effect of physical and biological control measures soil. Sarhad Journal Agriculture 16: 49-51.

Zambryski, P. C. (1992) Chronicles from the *Agrobacterium* plant cell DNA transfer strong. Annu. Rev. Plant Mol. Biol. 43, 465-490.