

## Biological control of arid fruit diseases

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### Abstract

Area of plantation of arid fruits is increasing every year under arid and semi-arid regions of the country. Fruit crops like ber; pomegranate, aonla, date palm and other minor fruits are mostly grown in water deficit areas. Among different production constraints in these crops, diseases are also concerned with yield and quality of fruits. For example, powdery mildew of ber can devastate whole orchards and fruit rots can trim down yield and quality of fruits. Leaf and fruit spots in pomegranate affect the foreign trade; rust in aonla results in poor quality of fruits in the absence of proper management practices in these crops. Low establishment of new plantation in date palm is mainly because of sucker rot in addition to fruit rot incited by common saprophytes. Despite, advancements in plant pathology and molecular plant pathology, much attempts have not been focused on etiology and management of arid zone fruits and therefore, presently a comprehensive information on biological control is discussed herewith.

**Key words:** Ambient temperature, physiological loss in weight, spoilage loss, economic life and respiration rate

### Introduction

The control efficacy of various fungicides for the management of most of the diseases of arid zone fruits has been worked out by many workers. Biological control is gaining importance for management of crop diseases. Particularly, bacterial antagonists are also useful for post harvest treatments for the control of fruits diseases (Pusey and Wilson, 1984). The important diseases in different fruit crops along with their control measure is discussed.

### Biological control of ber diseases

Indian jujube (*Ziziphus mauritiana* Lam) belongs to the Order Rhamnales, Family Rhamnaceae and Genus *Ziziphus*. This crop is relatively free from diseases wherein the dry, hot weather coupled with very less relative humidity prevails. However, diseases like powdery mildew and leaf spots in humid region, fruit rots in arid region and post harvest diseases due to common saprophytic fungi in all marketing locations of ber fruits are economically important and concern with yield and quality of fruits. Ber being one of the major under utilized fruits, systematic research has not been carried out even on major diseases. However, after the establishment CIAH, Bikaner, much progress has been made with regard to ber diseases and their management with reference to biological control. Verma *et al.* (2000) has conducted a survey and observed the diversity of ground flora, soil micro flora and fauna

under plantations of *Ziziphus mauritiana* in Mohan Bhatta land (Bilaspur), Madhya Pradesh, India. The populations of fungi, bacteria, nematodes and vesicular-arbuscular mycorrhizal (VAM) fungi were more diverse in soils under plantations.

Ber powdery mildew is a major disease causing great loss up to cent per cent in production and quality of ber fruits. Disease caused by *Oidium erysipoides* f.sp. *Ziziphi* was first reported from Allahabad. Kumar *et al.* (1978) noted the occurrence of powdery mildew of ber (*Ziziphus* spp.) in Indian arid zone. Nallathambi and Thakore, 2003 have worked out this method for the first time under field experiments on management of powdery mildew using the native isolates of *Trichoderma* (CIAH-240) and *Pseudomonas fluorescens* (CIAH-196) with 50% less concentration than recommended dose of fungicide (karathane). Spray of bioagents on susceptible ber cultivars (Gola and Umran) at pea stage at monthly interval results maximum of 90% control. Spraying 5% culture filtrate of *Trichoderma* (CIAH-240) followed by conidial suspension of *Trichoderma* (5 and 10%) spray resulted in 82.19 and 83.2% control, respectively. In case of cv.Gola per cent control efficacy was 95.81 by combined application of *P.fluorescens* and 0.05% karathane (Nallathambi and Umamaheswari, 2003 and Nallathambi *et al.*, 2003a and 2003b). Therefore, the bioagents are of great importance and an alternative component in integrated management formulations. The mode of action revealed mostly disruption of conidia, which is important of for the dissemination and subsequent infection. In some cases, mycelia strands were

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also disrupted after exposing to the antagonists. However, detail investigations are under progress.

Ber fruit rots are not a major problem in other regions of the country. However, in western parts particularly in arid regions of Rajasthan, it is a major problem than powdery mildew in ber. Nallathambi (2001) have studied in details about different kinds of rots due to various fungal pathogens. In almost all the major varieties of ber growing in arid regions of Rajasthan, different types of fruit rots were recorded. Diseases caused by *Alternaria* species have also been successfully managed by bio control agents like *Trichoderma harzianum* and *Bacillus* sp (Mercer and Papadopolous, 1990). Different native isolates of *Trichoderma* were evaluated for *in vitro* inhibition of *A. alternata*. Nallathambi and Thakore (2002b) has tested various isolates of bio agents against *A. alternata*. Out of 16 isolates, 9 isolates could check more than 50% growth of pathogen. Native isolate CIAH-240 (5% level) was significantly superior to rest of the isolates. Isolates viz., CIAH-142; CIAH-150, CIAH-161, CIAH-165a and CIAH-259 resulted less than 50% checks. It is well-established fact that *Trichoderma* species suppress plant pathogens by competition, antibiosis and mycoparasitism. Different native isolates of *Trichoderma* inhibit the pathogen by different mechanisms. The superior isolate CIAH-240 was mycoparasitic as well as secreted toxic metabolites. Isolates CIAH-142 and 165a can be used as mycoparasitic while rest of isolates are merely competitive. Isolate CIAH-149 and SBI-48 can secrete antifungal metabolites in growth medium to suppress *A. alternata*. Out of 14 native isolates of *P. fluorescens*, CIAH-196 inhibited the maximum mycelial growth. Production of toxic metabolites like phenazine, pyocyanin and pyrrolnitrin and lytic enzymes are the characteristic features in virulent strains of *P. fluorescens*. Interestingly, sporulation of test pathogen was suppressed by some of the native isolates. *P. fluorescens* isolates viz., CIAH-226, SBI-48 and SBI-62175 could secrete the siderophores (yellowish pink), which checked the mycelial growth of the pathogen (Nallathambi and Thakore 2002a). However, sporulation of test fungus was not arrested. Microscopic observations also revealed severe distortion in pathogen hyphae at the juncture of hyphal tip and bacterial colonies.

#### ***Alternaria* rot**

Generally post harvest infection due to microbial organisms results into severe losses particularly in fruits due to high perishability. Fruits are ready-made food with high carbohydrates and other nutrients thereby utilization is easy for fungal colonization. In order to find some post harvest treatments for combating *A. alternata* infection which also was found to spoil fruits in transit and storage experiments were conducted under laboratory conditions using biocontrol agents, fungicides and combination of these two methods to reduce the infection. Nallathambi (2001) has identified some of the effective isolates of *P.*

*fluorescens* under *in vivo*. All native isolates of *P. fluorescens* are effective with more than 60% control efficiency after 7 days of treatment. In addition, physical properties like texture and colour of ber fruits are better in bacteria treated fruits. Isolates of bioagents i.e. CIAH-240 (*Trichoderma*) and CIAH-196 (*P. fluorescens*) were found to be better than rest of the biocontrol agents tested and therefore, these two effective isolates have been taken for further experiments. Dinocap and copper oxychloride at 50 ppm resulted in more than 50% control. Whereas, at 100 ppm concentration, except mancozeb, wettable sulphur and dinocap were found to control more than 60%. Copper oxychloride has given 67.7% control. Dinocap also expressed some scorching effect on treated fruits and smell of treated fruits was also bad. Some of the systemic fungicides except carbendazim have resulted more than 50% control at both concentrations tested. Moreover, propiconazole and tridemorph can injure the fruits resulted in scorching or necrotic spots on fruits just after treatment.

Integration of more than one component can result better efficacy on management of post harvest pathogens. Effective fungicides as well as bioagents were combined for the control of fruit rot of ber so that maximum loss is reduced with minimum use of fungicides. Effective isolate of *Trichoderma* (CIAH-240) and *P. fluorescens* (CIAH-196) were chosen for combined treatment with fungicides. Fungicidal solution (50 and 100 ppm a.i.) and conidial suspension of *Trichoderma* (CIAH-240) may be prepared in concentrated form first and slowly dispensed into fungicides solution upto  $10^6$  conidia per ml. Mixing of *Trichoderma* certainly improved the efficiency of the treatment, as there was marked efficiency in disease control. More than 70% efficiency can be realized when thiophenate methyl, mancozeb and alcidine at 50 ppm whereas more than 80% PEDC was obtained when 100 ppm of thiophenate methyl, chlorothalonil, mancozeb and alcidine were mixed with *Trichoderma* isolate (CIAH-240). Per cent control efficiency was more than 60% when *P. fluorescens* isolate (CIAH-196) was mixed with thiophenate methyl, captan and alcidine at 50 ppm. Similarly mixing of *P. fluorescens* with thiophenate methyl, captan, and alcidine can be effective to control the *Alternaria* rot of ber at 100 ppm; the PEDC being 82.68, 78.33 and 77.58, respectively (Nallathambi and Thakore, 2003). Fungicides solution mixed in *Trichoderma* (isolate, CIAH-240) solution first, then with bacterial cells suspension and then pathogen inoculated fruits were treated in this mixture. Lower concentration of fungicide (50 ppm a.i.) mancozeb + metalaxyl, triadimefon, thiophenate methyl, captan, chlorothalonil, copper oxychloride, mancozeb and alcidine gave more than 50% PEDC. Nevertheless, mancozeb, thiophenate methyl and alcidine significantly controlled the disease at 50 ppm with both the bioagents. In order to ascertain the actual resistance level, some of the genotypes were evaluated under laboratory conditions using its toxin. Genotypes,



Chuhara, Bagwadi, Chuhara, Bagwadi, Reshmi and Ponda showed immune response to fruit rot pathogen.

### Biological control of pomegranate diseases

Pomegranate (*Punica granatum* L.) is a popular fruit of arid and semi arid regions. Apart from other biotic and abiotic stresses, diseases are major threat to this crop particularly in southern states. In earlier days, much attention was given on post harvest disease. However, leaf and fruit spots are the major diseases in Maharashtra, Karnataka some parts of Gujarat and Rajasthan. Wherever humid conditions prevail, much incidence of fruit and leaf spots could be seen. Leaf spots can indirectly reduce the yield and fruit spots affect the physical appearance of fruits and thereby market value of the fruit is reduced. There are various other diseases at post harvest stages but those are easily manageable. Aril necrosis or blackening of aril and pomegranate wilt are important concern in productivity and quality of fruits. There are suitable fungicides for the management of these diseases however, being a export oriented crop and considering the residual effect and quality of fruits for international trade, use of chemicals is not appropriate. In order to manage wilt disease, different approaches were attempted and it was noted that the soil application of bacterial culture, *Bacillus subtilis* was effective in field condition in reducing pomegranate wilt incidence (Somasekhara, 2002). The antagonism of 56 rhizobacteria, isolated from healthy and diseased plants of pomegranate against *F. oxysporum* was evaluated *in vitro* by dual culture technique. Of the 10 isolates antagonistic to *F. oxysporum*, PHRB-13 and PHRB-32 showed the greatest inhibition (65.39%) of mycelial growth. Based on morphological and cultural characteristics, PHRB-5, PHRB-6, PHRB-9, PDRB-20, PHRB-41, and PHRB-54 were identified as non-fluorescent *Pseudomonas*, PHRB-13 as fluorescent *Pseudomonas*, and PHRB-32 and PDRB-45 were *Bacillus* sp (Chavan and Dake, 2001). Laboratory experiments were conducted to determine the effect of airborne antagonist *B. thermophilus* (*Bacillus thermophilus*) on *A. alternata* and *Drechslera rostrata* (*Setosphaeria rostrata*) of leaf spot of pomegranate. Conidial germination and mycelial growth of the three fungi were inhibited by *B. thermophilus* at  $2.2 \times 10^9$  and  $2.2 \times 10^8$  cfu/ml respectively. Under ordinary conditions, the bacterium was able to survive for more than 20 months *in vitro*. However, its population declined after 34 days of spray on pomegranate leaves. *B. thermophilus* produced a thermo-labile metabolite toxic to *A. alternata* and *D. rostrata*. *B. thermophilus* restricted the fungal growth and invasion in host plants, and caused morphological changes in the germ tubes or mycelia of these pathogens (Mandhare and Suryawanshi, 2003). Most of the 149 strains of bacteria tested were antagonistic to *Sphaeropsis malorum* from *Phoma punicae* from pomegranate. *Bacillus mesentericus* and *B. subtilis* strains were the most active antagonists (Oganyan, 1977). Therefore, there is lot of scope for the

exploitation of biological control of pomegranate diseases and it needs more research on basic aspects before moving to field experiments and commercial formulations of specific antagonists against target pathogens.

### Biological control of date palm diseases

In India, date palm is grown in very limited areas mostly confined to water deficit areas of Gujarat and Rajasthan. There are different diseases in this hardy fruit crop but only few diseases are common in India. The Graphiloea leaf spot is major one followed by fruit rots. Recently it was observed that the failure in establishment of off shoots or newly planted suckers of date palm attributed with Diplodia rot. Sporadic incidence of wilt was also noticed in established plants. The other diseases reported in this crop are not common and therefore this text is restricted with the major diseases of India. In order to manage the soil borne diseases of date palm, the knowledge on the soil microflora and their potential use for the biological control is essentially required. In this regard, some of the basic research has been carried out in other countries. Atika et al. (1977) observed the rhizosphere of date palm cultivars of susceptible and resistant to *F. oxysporum* f.sp. *albedinis* showed that the microflora varied with seasonal changes, the nature of root exudates, plant health and genome. Nitrogen fixation, ammonification, nitrification, denitrification, starch, hemicellulose and cellulose degradation were also studied. Actinomycetes constituted a large part of the microflora of soil from the rhizospheres of susceptible and resistant cvs. of date palm. Of 271 actinomycetes tested against the pathogen, 50% were antagonistic (Sabaou et al., 1980). The qualitative and quantitative distributions of bacterial populations in the rhizosphere of 2 date-palm cultivars, 1 sensitive and 1 resistant to fusariosis, and with the antagonistic capacity of these bacteria against the pathogen *Fusarium oxysporum* f.sp. *albedinis* were reported by Lamari and Sabaou (1993). In the external zones of young roots, bacterial growth was stimulated more by the sensitive cultivar than by the resistant one. A survey of 665 isolates permitted the bacteria to be grouped in 2 main genera, *Pseudomonas* and *Bacillus*, and in a coryneform group composed of *Arthrobacter*, *Brevibacterium* and *Pimelobacter*. The generic and specific composition differed between the 2 cultivars and varied with root age (young or old) and the root zones considered (internal or external). In external zones, *Pseudomonas caryophylli* and *Pseudomonas gladioli* were the most abundant for the sensitive cultivar. In the endorhizosphere, *Bacillus firmus* was more abundant in the resistant cultivar, and *Pseudomonas fluorescens* biovar II in the sensitive one. Differences were also noted in the percentages of antagonistic bacteria against *F. o. f. sp. albedinis*. It was concluded that plants can influence their own rhizosphere bacterial populations. Benjama (1994) observed bacterial contaminants on date palm in culture test tubes and the



investigations revealed that the contaminants were introduced with date palm hearts selected in the field for seedling multiplication. A Gram negative, flagellate type of *Bacillus*, represented by *B. pumilus* and *B. sphaericus* was identified from date palm, and a Gram positive type, represented by *B. brevis*, *B. laterosporus* and *B. circulans* was identified from date palm. The effect of filtrates from 9 microorganisms on *in vitro* germination and mycelial growth of *F. oxysporum* f.sp. *albedinis* was investigated by Sedra and Maslouhy (1995). Of the microorganisms tested, 6 were antagonists isolated from Marrakech date palm grove soils, 4 *Pseudomonas fluorescens* isolates, *Stachybotrys* sp. and an unidentified actinomycete. In the presence of the filtrates from the 6 antagonistic microorganisms, inhibition rate varied with the antagonists from 26.6 to 69% for spore germination and from 43.6 to 80.7% for mycelial growth. The inhibitory action of the filtrates was related to the antagonistic capacity of the microorganisms and to the chemical nature of antibiotic substances they produce.

### Biological control of aonla diseases

Indian gooseberry (*Emblica officinalis* Gaertn.) is used as fruit and medicine. Area under cultivation is increasing rapidly not only in tropical and subtropical areas of India but also under arid region. In India, though there is limited effect of diseases on its production. However, aonla rust and post harvest diseases are major constraints. In other countries like China, the brown spot (*Phyllosticta emblica*), false anthracnose (*Kabatiella emblica*), Pestalotiopsis leaf spot (*P. heterocornis*) and powdery mildew (*Oidium* sp) have been reported. It is imperative to know about these diseases and nature of pathogen so that appropriate strategies could be formulated for the better management. Not much attempts have been made on the biological management of the diseases. Mamatha et al. (2000) have screened Seeds of *E. officinalis* for mycoflora incidence. Dominant mycoflora were isolated and their effects were studied on quality aspects like germination and vigour. Seeds inoculated with dominant fungi showed significant decrease in germination and seedling vigour. Seed samples were subjected to four different pre-treatments. Treatment with *Trichoderma* spp. was most effective both in reducing the incidence of mycoflora and enhancing the germination and vigor. However, this method can be well exploited for post harvest diseases since majority of the pathogens are saprophytic on injured fruits. In rare cases, colonization of antagonistic fungi like *Trichoderma* could be seen.

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