CHAPTER FIVE

DISCUSSION AND SUMMARY

DISCUSSION

The present investigation on rhizosphere fungi in tomato ('Rupali') and brinjal ('Mahyco-56') plants grown at two localities in Kolhapur District were undertaken with a view to study the floristic composition of fungi in the rhizosphere, to compare the fluctuations in population and species composition during various stages of growth and at the two localities, to compare the fungal population in the rhizosphere of these two crops in the respective fields and to evaluate clinically the importance of mycoflora around the root region of these commonly cultivated varieties with respect to the healthy growth.

The fungi from rhizosphere and non-rhizosphere soils were studied by using the dilution-plate method. This method was used by Timonin and Thexton (1951) for study of rhizosphere microflora of onion and garlic plants, Mishra and Srivastava (1969) for legume plants, Mishra et al., (1971) for healthy and infected plants of Cucurbita maxima, Ranga Rao (1972) for the different varieties of rice and Ursekar (1975) for different varieties of onion.

There is general agreement that in the rhizosphere more microorganisms thrive than in soil free from influence of roots. The observations in the present investigations are generally in consonance with the earlier findings and show that more fungal species exist in the rhizosphere soil than in the non-rhizosphere soil, except in the rhizosphere of tomato plants grown at Chinchwad. The remarkable feature of rhizosphere mycoflora at this locality was the prevalence of Trichoderma viride. Vagherova et al., (1940) and Lugauska (1961) have reported that antagonistic fungus Trichoderma viride is encouraged in the rhizosphere of many plants.

The present study also reveals that the rhizosphere fungal population shows variations according to the age of plant. Maximum population was generally recorded at the time of maximum vegetative growth. This, probably, was due to high rate of root exudates recorded during this time. Starkey (1929), Parkinson and Clarke (1964), Mishra and Srivastava (1969), Mujumdar (1968), Ranga Rao (1972), Ursekar (1975) etc. have obtained similar results.

During this work 16 genera of fungi were isolated from the rhizosphere and about 13 from the non-rhizosphere soil. At 30 day stage in the rhizosphere the fungal population was considerably higher as compared to the non-rhizosphere soil. Timonin (1940) has reported the establishment of rhizosphere microflora within 3 days of seed germination. Further development of rhizosphere population depends on normal growth of the plant. The rhizosphere effect increases with age of the plant and normally reaches its maximum at the stage of greatest vegetative growth.

At 60 day stage the fungal population in the rhizosphere of both the crops was found to be minimum as compared to other stages of growth. According to Clarke and Parkinson (1960) and Parkinson and Clarke (1961) the fluctuations in the fungal population in the rhizosphere might be due to the utilization of root exudates by rhizosphere fungi which positively affect and increase the microflora of rhizosphere, however, at the same time antagonistic substances from roots may be released which inhibit the fungal growth.

Notwithstanding the discrepancy observed in the period of maturity of the tomato crops grown at the two localities, it was observed that the maximum fungal population was existing during the intense vegetative growth of the plants. The fungal population was highest at 90 day stage when the plants had started producing flowers at Chinchwad locality but at the S.U.C., Kolhapur the plants matured late due to poorer soil fertility and therefore, the maximum population was observed at 120 day stage.

In case of fungal population in the rhizosphere of brinjal it was observed that the population was higher at 90 day stage at both the localities indicating the rhizosphere effect but later on there was observed a decline in the fungal population during the post-flowering stages at S.U.C., Kolhapur which can be attributed to the proper soil condition prevailing. On contrary, the fungal population during these stages in Chinchwad locality exhibited the sustainence of higher population on account of its rich soil condition.

In general, it was observed that the fungal population in rhizosphere and non-rhizosphere soil at S.U.C., Kolhapur was more heterogenous as compared to the Chinchwad locality, probably due to heterogenous nature of soil. Since the soil at the former locality was seldom under cultivation while the latter was continuously under cultivation and received heavy doses of chemical fertilizers, probably some essential elements might have been used up by previous crops making possible the growth of only those fungal forms which can adapt to a soil which lacks or has less amounts of some essential elements and excess or toxic amounts of others.

Chesters and Parkinson (1959) reported definite fungal succession on the roots as the plant matures. They isolated Mucorales and sterile forms in the early stage of growth of oat plants. In the present investigation, in the rhizosphere of brinjal sterile forms and Mucorales have been recorded during early stage of growth.

The dominance of fungal species varies from plant to plant and also according to the nature of soil. It was observed that the two crops show great fluctuations with respect to dominance of fungal species during various stages of growth. The variations in dominance of fungal species during various stages of growth are attributable to life cycle and maturity of plants.

In the rhizosphere of tomato, at 30 day stage *Cladosporium herbarum* was dominant, at 60 and 150 day stages species of *Volutella* were dominant at S.U.C., Kolhapur while at Chinchwad at 90 day stage *Trichoderma* was dominant which was relegated to subdominance by species of *Aspergillus* at 120 day stage. The striking feature of the tomato rhizosphere was the presence of yeast (budding) at 60 and 90 day stages in Chinchwad and S.U.C., Kolhapur localities respectively, which might probably be due to some sugary root exudates.

In the rhizosphere of brinjal, at 30 day stage sterile mycelia and at 60 and 90 day stages species of *Volutella* occurred as dominant forms while species of *Coniothyrium* and *Circinella* were recorded at 120 and 150 day stages respectively at S.U.C., Kolhapur. At the Chinchwad locality at 60 day stage sterile mycelia, at 90 day stage *Cladosporium* and at 120 and 150 day stages *Paecilomyces varioti* were dominant.

There is general conformity that the occurrence and dominance of fungal species in the rhizosphere varies from the plant to plant in the given locality. In the present investigations it was observed that at S.U.C., Kolhapur, at 30 day stage *Cladosporium* and sterile mycelia were dominant in rhizosphere of tomato and brinjal respectively. At 60 day stage species of Volutella occurred as dominant in rhizosphere of both plants but at 90 day stage yeast (budding) and Volutella were dominant in rhizosphere of tomato and brinjal respectively. At 120 day stage Coniothyrium and species of fungi like Rhizoctonia and Pyrenochaeta were dominant in rhizosphere of brinjal and tomato respectively. At 150 day stage Volutella and Circinella were dominant forms in rhizosphere of tomato and brinjal respectively.

Despite the difference in dominant fungal species, the occurrence of species of Volutella in rhizosphere of both the crops at a couple of stages of growth speaks volumes about the importance of nature of soil in determinating the occurrence and dominance of fungal forms to a large extent.

At the Chinchwad locality, at 60 day stage yeast (budding) and sterile mycelium were dominant in rhizosphere of tomato and brinjal respectively. At 90 day stage in rhizosphere of tomato Trichoderma and Coniothyrium were dominant and in brinjal Cladosporium herbarum was dominant. At 120 day stage in rhizosphere of tomato species of Aspergillus dominated while in brinjal Paecilomyces varioti was dominant at 120 and 150 day stages.

The fact that dominance of fungal species varies from plant to plant and also according to nature of soil has been emphasized by the work of Thornton (1956) on wheat and clove.

Parkinson and Clarke (1961) isolated species of Fusarium, Aspergillus fumigatus and species of Cylindrocarpon in wheat and clove, whereas species of Mortierella and Penicillium in Allium porrum. Mishra et al., (1969) isolated Rhizopus nigricans and species of Cladosporium as dominant types at pre-flowering and post-flowering stages respectively. He also reported that the species of Fusarium and Cladosporium herbarum were dominant in healthy plants of Cucurbita maxima. In 1972, Ranga Rao working on some varieties of rice noted the dominance of A.niger, A. nidulans, A.terreus and Fusarium oxysporum. However, Mills and Vlitas (1967) while working on rhizosphere fungi of sugarcane (B 50112) got the remarkable results. They isolated same numbers of fungi from rhizosphere and non-rhizosphere soil. They did not notice any distinct rhizosphere effect. Ursekar (1975) noted the dominance of species of Fusarium and Penicillium at 120 and 150 day stages respectively in onion varieties and species of Aspergillus at 60 and 90 day stages.

In the present study the rhizosphere fungal population was well established at 30 day stage, it declined at 60 day stage and from there onwards it reached to its maximum at flowering stage. These changes are probably due to root exudates. Chesters and Parkinson (1959) have also suggested that root exudates play important role in the establishment of rhizosphere population and also that change in mycoflora from initial stage to final stage of growth of plant is also due to same reasons. Results obtained by Rovira (1965), Mishra and

Srivasatava (1969) support this opinion. The present investigation is also in conformity with these observations.

Nevertheless, it is believed that the root exudates play a minor role as plant becomes mature. This is apparent from a sudden change in rhizosphere population at the fag-end stage of growth. Chesters and Parkinson (1959) working on oat and Mishra and Srivastava (1969) on some legumes have also reported similar changes in rhizosphere population. Similar observations are obtained in these investigations.

In this investigation, about 16 genera from rhizosphere and 13 genera from non-rhizosphere soil have been isolated. About 20 species belonging to 13 genera from rhizosphere of tomato, about 20 species belonging to 14 different genera from rhizosphere of brinjal and about 18 species belonging to 13 different genera from non-rhizosphere soil have been isolated. From S.U.C., Kolhapur, about 19 species belonging to 13 different genera in rhizosphere of tomato; about 15 species belonging to 12 different genera in rhizosphere of brinjal and about 11 species belonging to 11 different genera from non-rhizosphere soil have been isolated. From Chinchwad locality about 5 species belonging to 4 genera from rhizosphere of tomato; 10 species belonging to 7 genera from rhizosphere of brinjal and about 8 species belonging to 5 different genera from non-rhizosphere soil have been isolated. These findings reveal that the number of species recorded were slightly higher in rhizosphere soil.

The diagnostic features of this rhizosphere study include the prevalence of Trichoderma viride at Chinchwad locality; it was not recorded in the rhizosphere soil at S.U.C., Kolhapur except in saplings raising plots. Another distinguishing feature was the dominance of Cladosporium herbarum at the early stages of growth in rhizosphere of brinjal at both localities. The dominance exercised by species of Volutella at S.U.C., Kolhapur in the rhizosphere of both the crops in general and tomato in particular, is of great significance. It was conspicuously not recorded from the Chinchwad locality.

At the Chinchwad locality Aspergillus nidulans and A.niger were dominant during post-flowering (150 day) stage of growth in the rhizosphere of tomato while A. nidulans was co-dominant in the rhizosphere of brinjal. Another striking feature of the mycoflora of rhizosphere of brinjal at this locality was the dominance of Paecilomyces varioti at the two consecutive stages (120 and 150 day) of growth.

It should be emphasized that nine different species of Aspergillus were isolated during this investigation while only 4 species of Penicillium were isolated in rhizosphere and non-rhizosphere soils, moreover, the species of Penicillium never occurred as dominants and were always relegated to subdominance or co-dominance. Penicillium was not recorded from rhizosphere of tomato and non-rhizosphere soil at Chinchwad. These findings are in consonance with the general observation that species of

Penicillium appear commonly in temperate soils and species of Aspergillus are more abundent in tropical soils.

Peterson (1961) reported that species of Fusarium were dominant in acid soils. In the present investigations, except in the saplings raising plots, the occurrence of Fusarium was not recorded and also no wilting of plants was observed, implying the alkaline nature of soil. There was no record of other diseases such as damping off, rots, root galls implying that the soil was non-diseased.

In conclusion, it should be noted that several factors might be involved to give such a picture of mycoflora of rhizosphere and non-rhizosphere soils of both the plants at the two localities. Most of them are common soil hyphomycetes.

Alongwith the study of rhizosphere, in the present investigations a comprehensive study of some post-harvest diseases of fruits and vegetables from various markets of Kolhapur District with an intention of knowing causal organisms, extent of damage caused and the cultural features of causal organisms was undertaken.

Some temperate fruits like apples and peach arrive in the various markets from far away places like Kashmir and Simla. Some tropical fruits like Citrus, Mango, Sapodilla, banana, pomegranate are received from different places like Nagpur, Ratnagiri, Pune, Jalgaon, Gholvad (Near Mumbai), Nim, Pandharpur etc. Other fruits like custard apple, guava, papaya and lemon are arriving from nearby villages or neighbouring districts.

In this work about 35 diseases on 10 fruits and 9 vegetables have been studied and the causal organisms of most of these diseases were isolated. These include various types of rots, decays, cankers, fruit spots, cottony growth and moulds of pathogenic as well as non-pathogenic nature.

Most of the diseases of fruits and vegetables are incited by common fungi like *Rhizopus*, *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Ulocladium*, *Fusarium* etc. Some of the common diseases previously reported from this state have been collected during these investigations such as rots of banana incited by *Fusarium* and *Gloeosporium*, blue and green moulds of apple, *Botryodiplodia* rots of custard apple and bullock's heart (Ramphal), *Rhizopus* rot of sweet potato, *Cladosporium* rot of fruits of *Coccinia* and the common black mould rots of onion and garlic incited by *Aspergillus niger*.

It may also be noted that some of these market diseases such as fruit rots of grape, leaf spots of coriander, *Trigonella* and *Hibiscus* were found to be of field origin. Certain diseases were particularly predominant during only certain seasons and absent in others and are probably determined by the temperature conditions and humidity factor.

In the present investigations some distinct diseases of fruits and vegetables were recorded and studied. For instance, the Pestalotia rot of fruits of sapodilla incited by Pestalotia sapotae; Botryodiplodia rot of Anona fruits; Phoma rot of custard apple incited by Phoma psidii; waxy rot of Citrus fruits incited Geotrichum candidum; green mould of Citrus reticulata; rots of grape berries incited by Phoma sp., Phoma psidii and Pestalotia menezesiana; anthracnose and black spots of guava fruits incited by Colleototrichum and Aspergillus; rots of banana incited by Fusarium, Gloeosporium and Thielaviopsis; rots of fruits of Coccinia incited by Fusarium and Cladosporium; fruit rot of Zizipus fruits incited by Ulocladium chartarum; blue mould of apple incited by Penicillium expansum and Alternaria rot incited by A. alternata etc.

Investigations in post-harvest diseases made by several workers have revealed that main causes of spoilage of fruits and vegetables in transit and storage are high temperature, high humidity, high moisture content and rich nutrients of fruits and injuries sustained by the fruits during the process of marketing. Many post-harvest disease causing pathogens enter in these fruits and vegetables at the time of harvesting or just before harvest. The rots of apple caused by Aspergillus niger, Penicillium expansum and Trichothecium roseum can be avoided by careful handling of fruits to avoid the injuries and wrapping them in tissue paper (Dey and Nigam, 1933 and Keshwalla, 1936). Jamaluddin et al., (1974) reported maximum rotting of Citrus fruits at 25°C due to waxy rots incited by Geotrichum candidum, this organism has a high temperature optima and can be inhibited almost indefinitely by storage at 0°C. Pathogens such as Penicillium and Cladosporium grow slowly at near freezing temperature and limit the storage life of the produce if they are not controlled by some other treatment.

The injury created by severing the fruits from the plant is a frequent point of initiation of post-harvest diseases by wound pathogens. Penicillium, Rhizopus and Thielaviopsis are not capable of penetrating the intact surface of the host but they readily enter through injuries and natural openings and cause devastating damages.

Studies of pectolytic and cellulolytic enzymes secreted by the primary pathogens during invasion and subsequent colonization has been done by many workers and these are of great importance in adopting the control measures to minimize the heavy losses incurred due to post-harvest diseases. The cell wall degrading enzymes of pathogenic fungi such as Alternaria citri, Clathridium corticola, Aspergillus niger, A.awamori and Phoma destructiva have been studied by different workers. Chandra and Khanna (1977) have reviewed this aspect.

Fruits contain a high percentage of water and therefore should be stored in the cellars having high humidity, otherwise the product will lose weight rapidly because of evaporation.

For most fruits a relative humidity of 85 to 90 percent is desirable.

Fruits and vegetables continue to respire after they are harvested and as a result heat is produced. This is known as vital heat. The fruits which have high rate of respiration perish most rapidly. Anaerobic condition and high temperature accelerate respiration and ageing. CO_2 has an increasing effect on the process of ripening and hence it is essential to understand the phenonmenon of Pasteur effect and role of ethylene in ripening in order to increase the storage life of fruits. Low temperature delays the development of disease in two different ways : i)It delays ripening of fruits and thereby the period of resistance to the pathogens is prolonged. ii) the growth of the pathogen is inhibited at low temperature. Therefore, after harvesting the fruits must be cooled quickly to remove the field heat.

The lots of diseased fruits and vegetables can be salvaged partly or the further spread of the disease can be checked by use of fungicides and antibiotics either as dips or treatments such as Ferbam, Benlate (Benomyl), Plantvax, Aureofungin etc. (Jamaluddin and Tandon, 1975; Prasad and Bilgrami, 1977).

The principal sources of inoculation of fruits and vegetables are : i) Containers used to transport or store the harvested fruits and vegetables. ii) Water used to clean, cool and to apply chemical treatment after harvest iii) Brushes and conveyer beds which come in contact with the product as it flows through the packing house.

The fruits and vegetables must be carefully packed. Rigid containers such as cases or crates should be used. The containers must be sufficiently open to permit reasonable air circulation to remove respiratory heat given out by these products.

A survey in 1965 in U.S.A. had estimated that losses of about 200 million dollars are incurred due to post-harvest diseases of fruits and vegetables. In India more severe losses are inflicted on these perishable plant products due to its sub-tropical climate. It has been estimated that there is an average loss of 20-30% due to post-harvest diseases of fruits and vegetables in India (Mehta, 1975).

SUMMARY

A. RHIZOSPHERE STUDY :

1. The present investigation was undertaken with a view to understand the importance and significance of fungi in rhizosphere soils of tomato and brinjal varieties and to compare them.

2. Rhizosphere mycoflora was studied by dilution plate method. The fungi were grown and maintained in M2 agar slants to obtain their pure cultures.

3. During this work fungi belonging to 16 different genera were isolated from rhizosphere and 13 from Non-rhizosphere soil.

4. The composition of mycoflora of rhizosphere and non-rhizosphere soil was observed from 30 day stage to harvesting stage.

5. At S.U.C., Kolhapur locality, in rhizosphere of both the plants, species of *Volutella* were dominant while in rhizosphere of brinjal sterile forms and Mucorales were recorded in early stages.

6. At Chinchwad locality, in rhizosphere of tomato *Trichoderma viride* and species of *Aspergillus* were dominant while in rhizosphere of brinjal sterile forms, *Cladosporium* and *Paecilomyces* were dominant.

7. The rhizosphere population was more heterogenous at S.U.C., Kolhapur locality.

8. About 9 species of Aspergillus and 4 species of Penicillium were recorded during this work. Fusarium spp. were conspicuous by their absence.

B. POST-HARVEST DISEASES OF FRUITS AND VEGETABLES :

1. The study of diseases of fruits and vegetables received in the various markets of Kolhapur District (M.S.) was undertaken during the year 1994-1995.

2. In the present work about 35 diseases of 10 fruits and 9 vegetables were collected and studied including 4 new host records from Maharashtra.

3. Diseases encountered during this work were various types of rots, cankers, fruit spots, cottony growth, moulds, leaf spots etc.

4. Most of the pathogens causing these diseases are common fungi like Rhizopus, Aspergillus, Penicillium, Cladosporium, Alternaria, Fusarium etc.

5. Many pathogens were isolated on P.D.A. nutrient medium and their characteristics were studied.

6. The prominent diseases collected and studied include Botryodiplodia rots of Anonaceous fruits, green and

blue moulds of *Citrus* fruits, waxy rot of lemon, *Phoma* rots of custard apple and grapes, *Ulocladium* rot of *Zizipus* fruits, *Rhizopus* rot of sweet potato, *Cladosporium* rot of *Coccinia* fruits etc.

7. Rots of *Anona squamosa* and *Vitis vinifera* incited by *Phoma psidii*, fruit rot of *Zizipus jujuba* caused by *Ulocladium chartarum* and black rot of banana incited by *Thielaviopsis paradoxa* are new host records from the Maharashtra State.

8. It was observed that certain diseases were particularly common in certain seasons only, co-existent with the fruits occurring in those seasons and absent in other seasons.

9. Most of the diseases are contracted through injuries or natural openings while some are carried from field which then proliferate in transit and storage.

10. The principles of prevention of post-harvest diseases comprise the entire gamut of prophylactic and control measures including proper handling, appropriate sanitation, cold storage, air circulation, proper packaging, suitable humidity and use of fungicides and antibiotics to avoid the spoilage, in future, by the growers and vendors.