PART: VI
General Discussion and Conclusions
The state of Maharashtra has done an admirable work in the field of 'Pomiculture'. The government has decided to enhance the horticultural production by increasing the land under cultivation by about five times.

Post-harvest diseases or 'Market-Pathology' is an important branch in plant pathology, which deals with various types of pathogens attacking different fruits and vegetables during transit, storage and marketing processes. Because, of inadequate storage facilities and other related aspects, the damage of perishables, drastically cuts the quantum of export etc.. It is an important study of great economic significance which needs thorough investigations at every stage. This study was carried out a decade before in Maharashtra. The markets of Pune needed an attention on this topic to investigate it in detail for up-dating the information of research and its related aspects. Extensive survey of Pune markets was carried out during 1987-92 to assess the degree of spoilage by different fungal pathogens. After surveying each market at least for about ten times in a month it was observed that maximum spoilage was recorded in case of vegetables like: tomato, little-gourd and cucumber. While in case of fruits, jack, muskmelon, apple, citrus, ber, sapota and watermelon, showed severe storage which rots were attributed to various fungal pathogens.

Post-harvest disease not only reduce the market value of the fruits resulting in economic loss but also affect the economy of the industries engaged in products (food) of such fruits. Such losses are much higher in perishable fruits than in other crops. Repeated observations showed that spoilage of vegetables was
about 2-10% and of fruits was around 5-20%. Maximum spoilage of vegetables and fruits was during June to August i.e. in monsoon season. During this season, commonly occurring fungi were spp. of like: Fusarium, Colletotrichum, Cladosporium, Aspergillus, Penicillium and Geotrichum. The spoilage exceeded to 50-60% in case of certain vegetables e.g. little-gourd. Stalk-end rot of little-gourd was noted in all local markets of Pune but was absent in Bombay market. On the contrary, Fusarial rots of a number of other vegetables were commonly observed at Bombay markets. The number of vegetables infected by spp. of this genus were frequently met-with in Bombay as compared to Pune and Ahmednagar. A total of sixty-nine fungi belonging to 39 genera of Phycomycetes and Deuteromycetes were reported from various markets. Out of these, 11 genera were found frequently in almost all markets and the rest were detected rarely. Such, were the spp. like: Epicoccum nigrum on watermelon, Sclerotium rolfsii on beet root, Dorsatomyces stemonitis on potato and Rhizoctonia bataticola on watermelon etc.

Repeatedly encountered genera in all the markets of three selected sampling areas were: Alternaria, Aspergillus, Botryodiplodia, Cladosporium, Colletotrichum, Geotrichum, Gloeosporium, Mucor, Penicillium, Rhizopus and Trichothecium. Amongst these B. theobromae, G. candidum and T. roseum showed wide host range and occurred repeatedly. It is interesting to note that B. theobromae has been reported here for the first time on bottle-gourd, G. candidum on carrot and watermelon and T. roseum on papaya. Further, B. theobromae and G. candidum occurred repeatedly on ten
and T. roseum on six other fruit-hosts. Hence, these three fungi were selected for detailed study.

In Ceylon, a fruit rot disease leading to premature ripening as in case of jack-fruit in 1936, was attributed to B. theobromae. In India, Rao (1968) reported that approximately 30-40% fruits rotted due to this disease. After about twenty-seven years this disease was again observed in Pune markets and showed more or less similar aggressiveness.

Fruit rot diseases of watermelon have been reported from all parts of India by different workers including Singh & Chohan (1978, 1980). These workers from North-India have reported a number of diseases due to Geotrichum candidum, Macrophomina phaseolina, Alternaria alternata, Colletotrichum lagenarium on this fruit, which were also reported in Pune markets but, no detailed study was undertaken especially of the disease due to B. theobromae i.e. gray rot and also the waxy rot due to G. candidum.

In case of muskmelon, a "leak disease" due to Pythium butleri, was studied in detail by Singh (1977). Suhag and Duhan (1983) worked on gummy collar rot of muskmelon caused by Rhizoctonia bataticola in 1983. It was apparent from the available literature that the pink rot disease was never studied in detail so far. The disease incidence was more in Pune and Bombay markets but however, surprisingly this disease could not be observed at Ahmednagar markets. This indicates that the disease might have appeared during transit or storage for a short-while. Muskmelon is mostly cultivated in districts of Ahmednagar and Pune. The transport period is more for Pune and Bombay markets as such the disease develops subsequently in transit resulting in
rotting at market yards. Ceponis (1986) studied disorders in muskmelon shipments to the New York market and reported maximum load of shipments (53.3%) was affected by *Fusarium* from Arizona, and 2.7 percent of shipments were affected by *Rhizopus* soft rot. No detailed study has been undertaken on diseases of muskmelon in India so far.

Work on control aspect of jack-fruit infected by *Rhizopus nigricans* was undertaken by Pandey *et al.* (1979) and also by Roy (1983) on *Rhizopus artocarpi*. Barkai-Golan *et al.* (1970) suggested gamma irradiation for the control of jack-fruits rot due to *Trichothecium roseum* and *Penicillium viridicatum*. Since scanty attempts were made in the past to control post-harvest diseases of watermelon, muskmelon and jack-fruit, this aspect was therefore, undertaken in the present research program.

The following important diseases prevailed in various markets of Pune *viz.* gray rots of lemon, custard-apple, mango and wood-apple caused by *B. theobromae* and anthracnose of little-gourd and watermelon caused by *Colletotrichum lagenarium*, *Phomopsis* rot of grape caused by *Phomopsis viticola* which was reported by Bihari lal & Arya (1982) from North-India. *Phytophthora* rot of pomegranate was reported from Akola district of Maharashtra by Utikar (1986) and an year early by Abou-Heilah (1985) from Saudi-Arabia, this was surprisingly detected at pune markets in a severe form during 1989-90. *Phytophthora* rot of brinjal was reported by Jain & Bhatnagar (1978) from Rajasthan and Soft rot of pomegranate caused by *Coleophoma empetri* by Sherkar *et al.* (1980) from Maharashtra. These two diseases were also again
detected at various markets in the present study. Singh & Chohan (1980) investigated anthracnose of cucurbits by *Colletotrichum lagenarium* which was also found severe during the present survey.

Among the fungal rots, species of the genus *Fusarium* were found to be the most dominant infecting a variety of fruits and vegetables in the markets surveyed. It was observed on 22 vegetables and 15 fruits. Fusarial diseases were severe and alarming and found to be consisting of species. In Bombay, diseases were quite common on variety of fruits and vegetables during storage. As compared to Pune and Bombay, the incidence and prevalence was less in markets of Ahmednagar. Out of the three markets surveyed, in Bombay *i.e.* Crawford, Byculla and Sion, again maximum incidence of diseases, was noted in Byculla market. This may be attributed to marketing and storage conditions by and large and the factors like, light, temperature and moisture content of the sellers.

As regards to the main four storage diseases under study:
Four fungi *i.e.* *Botryodiplodia theobromae* - Isolate 1 (isolated from watermelon) and *Botryodiplodia theobromae* - Isolate 2 (isolated from jack-fruit), *Trichotheceum roseum* isolated from muskmelon and *Geotrichum candidum* isolated from watermelon were taken-up for their detailed physiology study. This included factors like: effect of temperature, hydrogen-ion concentration (pH 3-9) and other nutritional factors (use of different media, eleven carbon and nine nitrogen sources etc.) on the growth and sporulation of these four important storage pathogens. The study in respect of effect of temperature (*in vivo* and *in vitro*) especially in the control of storage rots, indicated a paramount
temperature at which fruits could be stored safely. For watermelon and jack-fruit a temperature below 20°C and for muskmelon preferably below 15°C were found to be ideal for storage i.e. for long holding of the produce. The study on the effect of temperature in vitro on the rots of watermelon, muskmelon and jack-fruit was particularly not investigated so far, has been carried out here. It was also further observed that when the temperature was raised to 30°C and above, the rotting percentage also amplified. At 35°C though there occurred scanty growth of these three fungi externally, majority of the fruits exhibited rotting internally. Sharma and Dharam Vir (1985) reported that when the temperature was raised above 30°C, rotting percentage decreased. In this respect the present observation differed. In the present work at, 25°C by and large, infection also steadily increased. As the storage temp. enhanced above 30° and 35°C, spoilage of the three fruits was maximum. T. roseum caused 50 percent rotting in muskmelons even at 20°C. Only at temp. of 10°C the infection was found zero.

Results in respect of effect of pH on B. theobromae isolates 1 & 2 showed maximum growth at pH 6. G. candidum and T. roseum tolerated acidic (pH 3-4) as well as alkaline (pH 8-9) ranges. Ragab et al. (1971) showed that the initial growth of B. theobromae was stimulated by pH 8 but according to the present observations growth was maximum at pH 6 for isolate 1 and pH 7 for isolate 2. Finally, pH 6/7 was optimum for all four fungi tested. In general a pH between 6-7 was found to be optimum for all the four pathogens.
A study on the effect of media (Synthetic & Non-synthetic) yielded some useful results in respect of growth and sporulation. Studies on Carbon and Nitrogen nutrition on growth and sporulation of the four fungi revealed that the two isolates of \textit{B. theobromae} utilized almost all disaccharides and sugar alcohols indicating their distinctiveness as compared to the isolates studied by the previous workers. \textit{T. roseum} utilized mono and disaccharides efficiently. In general, organic nitrogen sources were efficiently better utilized than the rest by all the four fungi studied.

Post-infection changes under pathogenesis in respect of sugars, total phenols, pectinolytic and cellulolytic enzymes was carried out in fruits of watermelon (\textit{B. theobromae} and \textit{G. candidum} infections), jack-fruit (infection by \textit{B. theobromae}) and muskmelon (pink rot infection by \textit{T. roseum}) by various standard technique. In general, sugars were the most affected, as they were easily utilizable. Total phenols increased in the host as a result of the host-parasite metabolism resulting in the formation of aliphatic and aromatic compounds. Pectinolytic and cellulolytic enzymes play a definite role in disintegration of cell walls of fruits and vegetables. Hence, assay of these enzymes were carried out. Analysis and estimation of sugars carried by these techniques exhibited that all sugars decreased after four days. Estimation of sugars by H.P.L.C. technique was carried out after four days. By this technique it was observed that in the infected tissue of jack-fruit 88.24 mg/100g of galactose increased but a loss in total sugar was 1.61g/100g.

In watermelon, infection due to \textit{G. candidum}, 6.99 mg/100g of
galactose decreased and total sugars exhausted were 6.26g/100g. In the tissues infected by *B. theobromae*, 7.34 mg/100g of galactose and 4.43 g/100g of total sugars depleted as compared to healthy tissue.

In watermelon, by paper chromatography method, it was observed that, after four days, the quantity of glucose, fructose, sucrose and galactose reduced considerably and further after eight days the first three exhausted. Taneja *et al.* (1980) obtained similar results when they analyzed tissue of jack-fruit infected by *Fusarium moniliforme* and *Rhizopus artocarpi*. They also investigated sugars quantitatively in this fruit and noted an increase in reducing sugars *viz.* 77.81% and 46.67% respectively. While due to *Aspergillus niger* 16.53% of sugars decreased. In the present study, reducing sugars increased upto the second day of infection by *B. theobromae* (16.15%) which, however, declined further to 35.03%. From these results it is apparent that *B. theobromae* is a weak pathogen as compared to *Rhizopus artocarpi* and *Fusarium moniliforme*. It was further observed that it attacked oligosaccharides (i.e. starch) present in fruits and degraded into non-reducing sugars and the same was this was examined by chemical as well as H.P.L.C. techniques. A study into the physiology revealed that *B. theobromae* showed utilization of oligosaccharides efficiently and this established as a distinct isolate (jack-fruit) than the other isolates studied by early workers obtained from various other fruit sources etc. (Tandon and Srivastava, 1971) utilization of sugars was faster in case of *G. candidum* than *B. theobromae*. In water-
melon, the infected tissue (by *G. candidum*) showed accumulation of phenols to a maximum. Thus, these results establish that in fruits, utilization of sugars and formation of phenols subsequently were maximum during the same, by the pathogens studied. In general, pectinolytic activity was maximum in the three pathogens than the cellulolytic activity.

The study on pectinolytic and cellulolytic enzymes was also undertaken *in vitro* as no such work of this nature was carried out previously in respect of these three particular isolates of *T. roseum*, *G. candidum* and *B. theobromae*. The study on specific viscosity vs. fluidity showed that maximum specific viscosity of pectinolytic enzyme was observed in case of PMG of *B. theobromae* Isolate-2. In case of *G. candidum*, very slow activity was observed initially which, however, turned faster after six days of incubation. Effect of three carbon sources namely glucose, fructose and sucrose was studied to investigate the correlation between carbon sources and the enzyme activity. None of these sugars could show any specific effect on enzyme activity. Similar results were obtained by Akinrefon (1968) who concluded that there was no correlation between the nitrogen sources and the enzyme activity.

'Disc-gel electrophoresis' revealed that pectinolytic enzymes of *B. theobromae* Isolate-1 and Isolate-2 had one band of the same molecular weight fraction i.e. 24,500 besides, having two other bands of different molecular weights. The pectinolytic enzymes of *G. candidum* and *T. roseum* also showed more or less the same molecular weight fractions. These results enabled to find out molecular weights of pectinolytic enzymes (PMGTE) of these
The study dealing with the control of post-harvest diseases with nine fungicides and one antibiotic *in vitro* and *in vivo* yielded some interesting results. It was revealed that Dith-M-45 was superior amongst all these. The growth of both *T. roseum* and *B. theobromae* was arrested at 200 ppm but that of *G. candidum* at 400 ppm. Dithane-Z-76 inhibited the growth of *B. theobromae* (Isolates-1 and 2) at 1200 ppm and 2000 ppm respectively. It inhibited the growth of *G. candidum* and *T. roseum* at 400 ppm. Ziride showed effectiveness against *G. candidum* but failed to check the growth of other fungi below 2000 ppm. Foltaf was effective against *B. theobromae* isolate 1 and 2 but was not so for *G. candidum* and *T. roseum*, as was evident by their arrested growth of 400 ppm and 1000 ppm respectively.

*In vivo* studies, the fungicides which were tested *in vitro* were further screened for their efficacy *in vivo* (*i.e.* on fruits). Amongst the nine fungicides screened, Dithane-M-45, Dithane-Z-76, Foltaf and Ziride effectively inhibited the growth of the three pathogens, at lowest concentrations. These were tested for control of fruit rots of watermelon, jack-fruit, muskmelon, fig, and custard-apple. The results pertaining to *in vivo* studies were promising. Application of Dithane-M-45 at 400 ppm conc. was found to be most effective in checking gray rot of watermelon. Waxy rot of watermelon was checked by Ziride at 400 ppm. Successful control of Pink rot of muskmelon was obtained by Dithane-M-45 and also by Foltaf (at 400 ppm). Datar and Mayee (1985) also recommended Dithane-M-45 (at 0.2 % conc.) as it was superior to
Ziram and Zineb. Effectiveness of Dithane-M-45 and Dithane-2-78 was positive while testing them in the field by Singh (1986) to control *Drechslera graminea* on barley cv. Amber. Kalra and Sohi (1985) also concluded that the same fungicide (i.e. Dithane-M-45) could check the fruit rot of tomato incited by *Alternaria tenuis* efficiently.

These results conclusively proved that four fungicides (Dithane-M-45, Dithane-2-78, Foltaf, Ziride) were useful in controlling storage rots of five fruits and Dithane-M-45 amongst these, was found promising as a pre-storage 'fruit-dip.'