PART - III

MISCELLANEOUS
CONTROL OF DISEASE

Post harvest diseases of fruits account for huge losses in this esteemed edible commodity. A number of physical and chemical treatments have been investigated and suggested to reduce fruit spoilage due to rottings in shipment and storage. Both the physical and chemical measures of control are either fungistatic or fungicidal.

The chief physical measures practised include treatment of harvested fruits to radiation, cold or heat. The chemical control measures practised are fumigation with sulfurdioxide, ethylene, chlorine, ammonia, and ozone; dry or liquid sprays of borates, sulfur, salts of zinc, nickel and other metals; and organic substances like carbamates, aminobutane, o-phenylphenols, pentachloronitrobenzene (PCNB), 2,6-dichloro-4-nitroaniline (DCNA), and 2,4-D and 2,4,5-T etc. The subject has been comprehensively detailed in a review by Eckert and Sommer, 1967.

In the present studies some of the commercial fungicides were used to determine their controlling effect upon the Rhizoctonia fruit-rot of musambi.
Method

To study the effect of fungicides musambi fruits were dipped in 0.2% solutions of the fungicides for about 5 minutes. After the fungicide treatment the fruits were then removed and allowed to dry at room temperature. These treated fruits were inoculated with Rhizoctonia sp. and were placed in moist chambers and incubated at room temperature. The inoculations were done by agar-plug method already described in chapter - II, sec. -B. (Part - I).

The fungicides taken for treatment were:

i) "Elitene" (Tata-Fison Ltd.):
   Copper oxychloride and Zineb.

ii) "Brassicol" (Hoechst):
    Pentachloronitrobenzene.

iii) "Cuman" (CIBA):
    Zinc-dimethyl-dithiocarbamate 80%.

iv) "Casan" (CIBA):
    Wettable sulfur.

v) "Thiram":
    Tetramethyl thiuram disulfide.

The observations were recorded when the diseased symptoms developed in the control.
Observations

It was found that the disease developed in the control fruits in three to four days after inoculations. Of the treated fruits only the "Elitane" treated fruits developed infection. Infection did not take place in fruits treated with Brassicol, Cuman, and Cosan. In thiram treated fruits, the infection did occur but the rotting was comparatively very much reduced.

Conclusion

The observations recorded here indicated that mostly the organic fungicides were effective in controlling the Rhizoctonia fruit-rot in musambi. However, since most of these fungicides are known to be systemic in their action, their use in this form of control need investigations about their effects upon the consumer. And if found suitable, it will be worthwhile to practise fungicidal sprays of brassicol, cosan, and cuman on the harvested musambi fruits prior to their shipment and storage. The musambi fruits have a longer physiological life after their harvest, and can be preserved in good condition for quite a long time for later use, if the post harvest infections could be controlled. A treatment, with a 0.2% solution of 'cosan' for five minutes, to the harvested musambi fruits
should prove useful in controlling the Rhizoctonia fruit-rot. Since the 'cosan' is only a wettable sulfur, its use might not involve other considerations of their harmful effects upon consumers.
II

OTHER ORGANISMS INFECTING MUSAAMI AND OTHER CITRUS FRUITS

During the isolations of the causal organisms for the storage rots of fruits, the following pathogens of the common citrus fruits were also recorded:

I. Musaami (Citrus sinensis)
   1. Penicillium digitatum
   2. Aspergillus niger

II. Mandarin orange (Citrus reticulata)
   1. Penicillium digitatum
   2. Torula sp.
   3. Aspergillus flavus
   4. Fusarium jevanticum

III. Lemon (Citrus medica)
   1. Rhizopus stolonifer
   2. Mucor sp.
   3. Fusarium sp.

No detailed work was done on the above organisms. Rhizoctonia fruit-rot which was doing the maximum damage was selected for detailed study.

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III

SUMMARY

_Rhizoctonia_ sp. was found to cause a post-harvest brown-rot of _musambi_ (Citrus sinensis (Linn.) Osbeck) fruits. The supplies of _musambi_ from Nasik, Poona, and Warud had a high incidence of this disease.

The pathogenicity tests performed indicated that the pathogen was capable of infecting fruits when the inoculum contained an initial nutrition sustaining potential, in the absence of any injury on the fruit. It was observed that the pathogen obtained entry in the host tissues biochemically by macerating the surface cells. Mechanical modes of penetration could not be observed.

The enzyme complex of _Rhizoctonia_ sp. for maceration and pathogenesis was found to be consisting of protopectinase (PP), pectinmethylesterase (PME), polygalacturonase (PG), and cellulases (C₁ and Cₓ). All of these enzymes were produced both _in vivo_ and _in vitro_. Negligible amounts of pectin methylgalacturonase (PMG) were detected _in vivo_, and it was not produced _in vitro_ except on induction - that too in insignificant quantities.
Pectinmethylesterase and polygalacturonase were more active in pathogenesis. In vivo production of PME was higher, in the initial stages of pathogenesis, than that of PG. However, both the PME and the PG production increased along with the development of the rot up to the 8-day stage. There appeared a definite synergistic relation between PME and PG. The liberation of free methoxyl groups from pectins by the PME lowered the pH of the substratum and rendered it more favourable for PG action. Polygalacturonase was found responsible for bringing about maceration of tissues, and might be the 'macerating factor' in this case. Cellulase was also found to be active in pathogenesis.

In vitro studies indicated that the pathogen obtained good growth on Richard's and host-extract media. Richard's medium also favoured macerating enzyme production, whereas the autoclaved host-extract medium did not favour the pectolytic and cellulolytic enzyme secretion. Sucrose and fructose gave better results than other carbohydrates for the same. Cellulase (C₅) production on glucose-containing media in larger amounts than other media, excepting sucrose containing, was significant. A more acidic range of pH viz., 3 to 4.5 and the temperature between 20°C to 30°C. for incubation were found to be
optimum both for mycelial growth and enzyme secretion.

Presence of native pectic or cellulosic substances in the culture medium induced larger secretion of PME and cellulases. The activity of both the C₁ and the Cₓ cellulases were demonstrated in the culture filtrates. These studies showed that the pathogen *Rhizoctonia* sp. produced pectolytic enzymes constitutively, and their production might increase inductively. Cellulases C₁ and Cₓ were inductive in production, however, there seemed every possibility for concluding about the Cₓ cellulase being constitutive in view of its constant presence in a large number of different culture filtrates.

The pectolytic and cellulolytic enzyme moiety of the pathogen that were mainly responsible in this disease were found to be thermolabile. The thermal inactivation taking place between 60° and 70° C. Only PME and cellulase were stable up to 70° C., whereas PP and PG tolerated temperature up to 60° C. The pH of the substrate for the pectolytic enzyme action was found to lie in the more acidic range. Cellulase activities required a near neutral to neutral pH range of 6 to pH 8.

Chromatographic analysis of the healthy and diseased fruit extracts showed that arginine, sucrose,
and a phenolic compound ($R_f$ 0.32) were utilised from the host substrate in pathogenesis. The free metabolites of the host-pathogen interactions were $\alpha$-aminocaprylic acid, methionine and valine; fructose, rhamnose, xylose and an unknown sugar; and a high resolving phenolic substance ($R_f$ 0.90). There was a general quantitative increase in the amino acids $\alpha$-alanine, glutamic acid, and nor-leucine; and a decrease in the quantities of sucrose.

Total sugar and reducing sugar content of the fruit got depleted on infection. Total nitrogen was also found to decrease in diseased fruits, which could not be correlated with the increase in free amino acid content in diseased tissues.

The treatment of fungicides for control of Rhizoctonia fruit-rot showed that the fungicides, brassicol, cuman, and cosan were effective in preventing infection. Blitane, one of the inorganic fungicides containing copper, was totally ineffective. This indicated that use of organic fungicides like pentachloronitrobenzene (brassicol), carbamates (cuman) and wettable sulfur (cosan) as a post-harvest treatment could be advanced as a suitable mode of control of Rhizoctonia fruit-rot of musambi.