CHAPTER VII

CONTROL OF POST-HARVEST SPOILAGE OF MANGOES
The world's population is expected to increase by around 50% between 1980 and the end of this century (1). An increase in food productivity is thus necessary to cope with this rise in population. In a developing country like India, food shortages can be reduced by controlling post-harvest food losses, resulting in increased food availability. Though this area has been given little consideration, it first received attention at the World Food Conference in Rome in 1974 wherein a resolution was passed to reduce world postharvest losses by 50% by the end of 1985.

Perishable foods deserve greater attention because, as the name suggests, these commodities cannot be kept for a very long time and in certain cases, only a few weeks. The reason for this is that they have a high moisture content (between 50-90%), possess a large unit size, their respiratory rate is high and hence heat production is higher. Also, they are generally soft textured and can be damaged easily. Most important, they are known to be susceptible to infection by bacteria and fungi.

In 1978, a report of the National Academy of Science, Washington, indicated that about 50% losses occurred in vegetable crops. Fruits were reported to be even more susceptible to spoilage. In the case of papayas, spoilage has even resulted in a 100% loss of the fruit crop (2).
Several methods have been devised to control such losses due to microbial spoilage. These methods aim at either preventing pre-or post-harvest decay or eradicating established infections. They include treatment with chemicals (3-5), heat (6-8), irradiation (8-10) and low temperature storage (11-13).

The present study aims at examining whether $\gamma$-radiation, Bavistin (2(Methoxy-carbamoyl)-benzimidazole, a fungicide) and aureofungin, (an antifungal antibiotic) are able to effectively control post-harvest spoilage of two varieties of mangoes by a mold, **Penicillium cyclopium**.

Extensive studies have been conducted to determine the effect of $\gamma$-radiation on the extension of shelf life of fruits (14-17). One of the roles of $\gamma$-radiation in prolonging the shelf life of fruits has been suggested to be the destruction of spoilage microorganisms, fruit flies and seed weevils. A significant reduction in anthracnose infection of mangoes was obtained by irradiating fruits at a dose of 105 krad followed by hot water dip treatment (18). However, Phatak and Khandelwal (19) were unable to control **Diplodia** stem end rot of mango fruit by the same treatment.

*In vitro* studies were performed with the spore suspension of **Penicillium cyclopium** to examine its inactivation pattern by $\gamma$-radiation. As can be seen from Fig.1, the $D_{10}$ value (dose required for 90% inactivation)
Fig. 1: The inactivation pattern of spores of *Penicillium cyclopium* exposed to various doses of gamma-radiation.
for the spores of this isolate is 50 krad. Thus a 25 krad exposure, which is the optimum known to be required to delay ripening, is not sufficient to kill the spores and control spoilage.

The effect of $\gamma$-radiation on the development of infection in ripe alphonso mangoes inoculated with different spore densities was examined (Table 1).

Table 1: The effect of $\gamma$-radiation on the development of infection in ripe alphonso mangoes inoculated with different spore densities of Penicillium cyclopium. 5 fruits were kept in each set. After inoculation the fruits were incubated at 35$\pm$1°C for 4 days.

<table>
<thead>
<tr>
<th>No. of spores inoculated</th>
<th>Degree of infection</th>
<th>Unirradiated mangoes</th>
<th>Irradiated (25 krad) mangoes</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2 \times 10^7$</td>
<td>++++</td>
<td>++++</td>
<td></td>
</tr>
<tr>
<td>$2 \times 10^6$</td>
<td>++++</td>
<td>++++</td>
<td></td>
</tr>
<tr>
<td>$2 \times 10^5$</td>
<td>++++</td>
<td>++++</td>
<td></td>
</tr>
<tr>
<td>$2 \times 10^4$</td>
<td>++++</td>
<td>++++</td>
<td></td>
</tr>
<tr>
<td>$2 \times 10^3$</td>
<td>+++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>$2 \times 10^2$</td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
</tbody>
</table>

++++ severe infection
+++ moderate infection
++ mild infection

It can be seen that a spore density as low as $2 \times 10^2$ was capable of producing infection. In all cases, the degree of infection in $\gamma$-irradiated mangoes was more or less
similar to that of unirradiated mangoes, suggesting that low doses were not able to protect the fruits against infection.

Plate 1 shows the effect of $\gamma$-radiation on the development of infection in artificially infected unripe alphonso mangoes. The degree of infection increased upon irradiation upto 50 krad. The enhancement of spoilage in fruits irradiated at 25 krad may be a result of the degradation of starch and cellulose to reducing sugars by irradiation (Fig. 2 a & b). A decrease in the levels of starch immediately after exposure of unripe bananas to doses of 35 krad has been reported by Surendranathan and Nair (16). A 50 krad dose enhanced the infection process in mangoes as indicated in Plate 1. This phenomenon has also been reported earlier by Padwal-Desai et al (8) in the spoilage of grapes by Aspergillus species. The results thus indicate, that a low irradiation dose (25 krads) is not effective in protecting mangoes against infection. Similar observations made by Maxie et al (20) in the case of certain fresh fruits and vegetables and also by Phatak and Khandelwal (19) who studied the control of Diplodia stem end rots of mango fruits, further support this conclusion.
Plate 1: The effect of gamma-radiation on the development of infection by *Penicillium cyclopium* in unripe alphonso mangoes. Mangoes were given (A) no dose, (B) a 25 krad dose and (C) a 50 krad dose after artificial inoculation and incubated at 35±1°C for 3 days.
Fig. 2: The release of reducing sugars in 1% aqueous solutions of (a) starch and (b) carboxymethyl cellulose exposed to various doses of gamma-radiation.
As mentioned earlier, antimicrobial agents have been used extensively to limit the spread of pathogenic microorganisms. Some of the strategies so far used for controlling spoilage of fresh fruits and vegetables are outlined in Table 2.

Table 2: Strategies for the control of pathogenic microorganisms in fresh fruits and vegetables

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Spray developing crop with fungicides to prevent preharvest infection.</td>
<td>Protective fungicides - captan, manebe etc.</td>
</tr>
<tr>
<td>2. Inactivate pathogens in the post harvest environment.</td>
<td>Broad spectrum sanitizing agents, formaldehyde, hypo-chlorite, quaternary ammonium compounds.</td>
</tr>
<tr>
<td>5. Protect surface of product against infection through wounds created after application of fungicide.</td>
<td>Benomyl.</td>
</tr>
<tr>
<td>6. Inhibit fungus sporulation and the spread of hyphae to adjacent fruits.</td>
<td>Biphenyl, benomyl, ( \text{SO}_2 ) dicloran, metalaxyl.</td>
</tr>
</tbody>
</table>

Source: Eckert, J.W., 1983 (Ref. 21)
The application of a protective fungicide has been used extensively in the tropics to control anthracnose on mangoes and papayas and in Europe to control lenticel rots of apples (22-25). For the present study, an antifungal antibiotic viz. aureofungin and a systemic fungicide viz. Bavistin were selected for studying the control of post-harvest spoilage of mangoes by Penicillium cyclopium. Systemic fungicides have an advantage over other fungicides in the sense that they possess a greater penetrating power and have the ability to inactivate the pathogen situated deep inside the tissue. Thus, such a fungicide is preferred for post-harvest treatments.

In vitro studies were performed to examine the effect of Bavistin and aureofungin on the growth of Penicillium cyclopium (Fig.3). It can be seen that Bavistin exerted a much greater inhibitory effect on the growth of the isolate than aureofungin. Bavistin (at a concentration of 2 mg%) completely inhibited spore germination, while aureofungin (at a concentration of 10 mg%) inhibited growth by about 40%. Earlier, Ogawa (26) and Long (27) have reported that 2-substituted benzimidazole fungicides (thiabendazole and benomyl) were able to prevent latent infections of Gloeosporium in apples and bananas.

Unripe langra mangoes were dip treated for 15 minutes in aqueous solutions containing various concentrations of Bavistin. After this, the fruits were infected naturally.
The growth of Penicillium cyclopium in Sabarud's broth medium in the presence of various concentrations of aureofungin (●—●) and Bavistin (O—O). Values expressed are the mean of the results obtained with 3 independent sets of experiments.
Fruits treated with the fungicide were less susceptible to spoilage than the untreated ones (Table 3). A concentration of 100 mg/lit of the fungicide was most effective in preventing spoilage. However, those fruits treated with this concentration developed an abnormal flavour, probably due to the penetration of the fungicide into the tissues. Hence, the dip treatment time was reduced from 15 mins to 1 min. This resulted in fruits which retained their natural flavour. At the same time, spoilage was also controlled as effectively (Fig.4).

Table 3: Development of spoilage by *Penicillium cyclopium* after natural infection in langra mangoes treated with various concentrations of Bavistin. (Results of the first two days of incubation are not depicted as there was no visible sign of spoilage during this period.)

<table>
<thead>
<tr>
<th>Concentration of Bavistin</th>
<th>% Spoilage* on 3rd day</th>
<th>5th day</th>
<th>8th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>40</td>
<td>70</td>
</tr>
<tr>
<td>1 mg/lit</td>
<td>10</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>10 mg/lit</td>
<td>10</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>100 mg/lit</td>
<td>N.D.†</td>
<td>N.D.†</td>
<td>20</td>
</tr>
</tbody>
</table>

† N.D. = Not detectable

* % Spoilage is expressed as

\[
\text{the no. of fruits spoiled} \times 100
\]

\[
\text{the no. of fruits incubated}
\]

Results expressed are a mean of 3 independent experiments taken to the nearest whole number.
Similar studies were conducted with aureofungin, restricting the dip treatment time to 1 min. Results of such an experiment are shown in Table 4. 1 mg/lit and 10 mg/lit of aureofungin did not exert any protective effect. A 100 mg/lit concentration however, afforded protection against infection up to the 5th day of incubation.

Table 4: The effect of aureofungin on the development of natural infection by *Penicillium cyclopium* in langra mangoes.

<table>
<thead>
<tr>
<th>Concentration of aureofungin</th>
<th>% Spoilage* on</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3rd day</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
</tr>
<tr>
<td>1 mg/lit.</td>
<td>20</td>
</tr>
<tr>
<td>10 mg/lit.</td>
<td>20</td>
</tr>
<tr>
<td>100 mg/lit.</td>
<td>10</td>
</tr>
</tbody>
</table>

*Values expressed and % Spoilage were calculated as explained under Table 3.*

A combination of 100 mg/lit of aureofungin and 10 mg/lit of Bavistin also failed to exhibit significant
protection after the 5th day of incubation (Table 5). Earlier, Chhatpar et al (28) reported that aureofungin at a 1 mg% concentration completely inhibited the growth of Rhizoctonia bataticola, which is responsible for the formation of black spots on mangoes. Secondly, Fusarium semitectum infection in tomato seeds was effectively controlled by Difolaton and Bavistin (29).

Table 5: Development of Spoilage by Penicillium cyclopium after natural infection in langra mangoes treated with Bavistin, aureofungin and a combination of both.

<table>
<thead>
<tr>
<th>Concentration of fungicide/antibiotic</th>
<th>% Spoilage* on 3rd day</th>
<th>5th day</th>
<th>8th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>10 mg/lit Bavistin</td>
<td>20</td>
<td>40</td>
<td>70</td>
</tr>
<tr>
<td>100 mg/lit aureofungin</td>
<td>20</td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>10 mg/lit Bavistin + 100 mg/lit aureofungin</td>
<td>10</td>
<td>20</td>
<td>50</td>
</tr>
</tbody>
</table>

*Values expressed and % spoilage were calculated as explained under Table 3.

Benzimidazole fungicides, thiabendazole, benomyl, carbendazim and thiophanate-methyl were introduced as anti-spoilage agents in the late 60's and since then they have revolutionized the field of post-harvest pathology as they
are able to prevent the development of latent infections of Diplodia, Phomopsis and Colletotrichum on certain tropical fruits. All benzimidazole fungicides and their derivatives share a common antifungal spectrum although they differ quantitatively in activity (30). These fungicides have been used extensively to control Penicillium mediated decay and stem end rot of citrus fruits, Penicillium blue mold and Gloeosporium lentical rots of apples (22,31); Colletotrichum and Ceratocystis infections in pineapples (32), papayas (33), bananas (34), citrus fruits (34), mangoes (35) and pears (36).

The success of the benzimidazole fungicides in the control of post-harvest diseases has been attributed to two factors (a) a high intrinsic activity of these compounds against many fungi responsible for post-harvest diseases and (b) the ability to penetrate, at least to some degree, into the host to reach the site of infection (36-38).

The results with antifungal agents indicate that Bavistin was able to control spoilage more effectively than aureofungin when used at the same concentration. This could be due to the fact that the former has a greater penetrating power than the latter. Although benzimidazole fungicides are known to control several
diseases of potato tubers caused by *Fusarium phoma* (39,40), they have two serious drawbacks. Firstly, these fungicides are not active against *Rhizopus*, *Alternaria*, *Geotrichum*, *Phytophthora* and all bacteria (21), which represent some of the most important post-harvest spoilage organisms. Secondly, sensitive pathogens generally build up benzimidazole resistance subsequent to excessive use of the fungicide. Citrus fruits attacked by *Penicillium* can no longer be treated with this fungicide as the fungus develops resistance to it. Our studies with mangoes revealed that the organism did not develop resistance towards the fungicide, but it is feared that the problem faced in the case of citrus fruits might spread to other fruits.

Several new fungicides have been developed in order to overcome benzimidazole resistance. These include Guazatine, a diguanadine broad spectrum fungicide (41), Imazalil, phenoxyphoril and procioraz-imidazole fungicides (42-44). These fungicides have assisted in controlling spoilage due to *Penicillium* and *Alternaria* rot of tomatoes.

Further experiments were conducted to examine whether $\gamma$-radiation in combination with Bavistin could control spoilage in alphonso and langra varieties of mangoes.
Fig. 4 shows the results obtained with the langra variety of mangoes. It can be seen, that \( \gamma \)-radiation alone is not able to protect the fruits against spoilage by *Penicillium cyclopium*. However, radiation in combination with the fungicide did lower the extent of spoilage, although the degree was higher than that of fungicide treatment alone. Fig. 5 depicts the effect of radiation in combination with fungicide treatment on spoilage in the alphonso variety of mangoes. This variety was also found to be more susceptible to infection after a low dose of radiation (25 krads). A combination of radiation and fungicide did result in a lower number of fruits getting spoiled. However, the degree of infection, once again, was greater as compared to fruits which were treated with fungicide alone. As expected, control or untreated fruits showed the maximum degree of spoilage. In the case of langra mangoes, the \( \gamma \)-irradiated fruits showed a greater degree of spoilage than untreated ones. This could be due to fact that some fruits become more prone to physical damage after low dose irradiation (8). Our results differ from those obtained earlier by Kojima and Buddenhagen (45). They found that although low dose \( \gamma \)-radiation could not control post-harvest diseases in the papaya fruit, a combination of radiation and fungicide treatment was more effective for decay control than either treatment alone. Anthracnose infection of mangoes was also
Fig. 4 i- The development of natural infection by P. cyclosporum in langra mangoes given various treatments as: -(O—O ), no treatment, (Δ—Δ ) 25 krad of gamma-radiation, (●—● ) 100 mg/lit of Bavistin and (▲—▲ ) a combination of 100 mg/lit of Bavistin + 25 krad dose of gamma-radiation.
Fig. 5: The development of natural infection by *P. cyclopium* in alphonso mangoes given various treatments as: (O—O) no treatment, (Δ—Δ) 25 krad of gamma-radiation, (●—●) 100 mg/lit of Bavistin and (▲—▲) a combination of 100 mg/lit of Bavistin + 25 krad dose of gamma-radiation.
significantly reduced by a combination of radiation followed by hot water treatment (18). However, the dose employed for such studies was 105 krad and a 25 krad dose treatment has been shown to be optimum for delaying the process of ripening in mangoes (46). Doses greater than 25 krad hasten the infection process and might even damage the tissues leading to surface discoloration, abnormal ripening and softening. The papaya fruit seems to be the only promising subject for practical treatment with $\gamma$-radiation (24).

Low temperature storage is considered to be one of the most effective and practical methods of delaying the onset of decay in post-harvest fruits. Though this method does not eradicate the pathogen, it is much safer as compared to the fungicides since it does not leave any toxic residues in the fruits. There are two ways in which low temperature delays the onset of post-harvest diseases. Firstly, low temperature is known to impair the process of ripening and therefore indirectly prolongs the appearance of disease symptoms. Secondly, it may also by itself, inhibit the growth of the spoilage organism. One limitation to the application of low temperature storage for controlling spoilage in tropical fruits is the susceptibility of these fruits to be inflicted by "chilling-injury" (47-49).
Fig. 6: (a) The growth of Penicillium cyclopium and (b) the % of inhibition in growth of Penicillium cyclopium at various incubation temperatures.
In the earlier section it has been shown that unripe fruits are more resistant to spoilage by *Penicillium cyclopium* as compared to partly ripe and ripe fruits. The ripening process was stimulated as the temperature of incubation increased between 25-35°C. An increased degree of spoilage could be correlated with increased ripening of the two types of fruits. The langra variety was found to be more susceptible to attack by *Penicillium cyclopium* as compared to the alphonso variety. Since mangoes develop chilling injury at 5°C (49), fruits stored at temperatures between 10-20°C will remain free from this physiological disorder. At the same time, spoilage can be effectively curtailed.

Another strategy applied for controlling post-harvest decay is to maintain the host resistance by retarding the ripening and senescence process via the application of plant growth regulators. 2,4-dichlorophenoxy acetic acid treatment to lemons has been reported to delay the senescence of the button which is the usual point of attack by the fungus *Alternaria citri* (50,51). Similarly, gibberellic acid treatment to navel organes was found to delay the senescence of the peel (52). This has been thought to reduce the decay of oranges by *Penicillium* species.
**In vitro** studies were carried out to examine the effect of plant hormones on the growth of *Penicillium cyclopium*. As can be seen from Table 7, abscisic acid and indole acetic acid supported the growth of the isolate, while kinetin exerted an inhibitory effect. A previous report from our laboratory has established that kinetin delays the process of ripening in mangoes (53). Since it also retards the growth of this fungus, it has a potential for use in controlling post harvest spoilage of mangoes.

Table 7: The effect of some plant growth regulators on the growth of *Penicillium cyclopium* in Sabarad's medium.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Growth* (mg dry wt) in the presence of ABA KIN IAA GA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>51  51  51  187</td>
</tr>
<tr>
<td>$10^{-9}$ M</td>
<td>70  53  51  185</td>
</tr>
<tr>
<td>$10^{-8}$ M</td>
<td>73  58  54  185</td>
</tr>
<tr>
<td>$10^{-7}$ M</td>
<td>77  41  62  179</td>
</tr>
<tr>
<td>$10^{-6}$ M</td>
<td>68  37  68  181</td>
</tr>
<tr>
<td>$10^{-5}$ M</td>
<td>81  33  73  180</td>
</tr>
<tr>
<td>$10^{-4}$ M</td>
<td>N.D. 33 N.D. 175</td>
</tr>
</tbody>
</table>

ABA = Abscisic acid, KIN = Kinetin, IAA = Indole acetic acid.
N.D. = Not determined.
*Values expressed are a mean of the results obtained with three independent sets of experiments.
In order to ascertain whether plant hormones such as abscisic acid and kinetin have any role to play in the increased decay observed after $\gamma$-radiation, aqueous solutions of the hormones were irradiated at 30 krads. Fig. 7 shows the absorption spectrum of abscisic acid. Maximum absorption is observed at 245 nm. It has been reported earlier that an alkaline solution of abscisic acid absorbs maximally at 245 nm (54). It can be seen that after a low dose of radiation the peak completely vanishes, indicating that the molecule is destroyed. On the other hand kinetin, after irradiation, shows only a 10% decrease in absorbance at its absorption maximum (Fig. 8). Since kinetin is observed to inhibit and abscisic acid is found to support the growth of *Penicillium cyclopium*, it is unlikely that these hormones might be responsible for the increased microbial spoilage observed after $\gamma$-radiation. It is possible that $\gamma$-radiation might have a similar effect on hormones in intact fruits as that observed in aqueous solutions of the pure hormones. Contrarily, $\gamma$-radiation may not elicit the same effects *in vivo*. A detailed study needs to be performed before concluding the effect of $\gamma$-radiation on kinetin and abscisic acid in intact fruits.
Fig. 7: The effect of gamma-radiation on the absorption spectrum of an alkaline solution of abscisic acid. (●—●) unirradiated and (○—○) 30 krad irradiated solutions.
Fig. 8: The effect of gamma-radiation on the absorption spectrum of an alkaline solution of kinetin. (●—●) unirradiated and (○—○) 30 krad irradiated solution.
In conclusion, it can be said that treatment of mangoes with the fungicide, Bavistin, storage of mango fruits at low temperature and treatment of fruits with plant growth regulators like kinetin, could be exploited for controlling post-harvest spoilage of mangoes by *Penicillium cyclopium*. It would be worth examining the effect of a combination of these three different methods on the decay control of mango fruits.
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