CHAPTER VI

MICROBIAL SPOILAGE OF MANGOES BY PENICILLIUM PURPUROGENUM STOLL.
Fruits being perishable in nature undergo heavy losses during storage, transport and handling, before it reaches the consumer. This lead to wastage and reduction in the market value. The origin of these losses and the microbiological deterioration to be the major factor responsible for it was considered by Coursey (1).

The knowledge regarding the mode of infection is essential in developing an effective eradication programme. Fruits attached to the plant may be infected by direct penetration of pathogenic fungus through the intact cuticle, by wounds, or by natural openings on the surface of the host. Many post-harvest diseases are initiated through injuries to the produce during and subsequent to harvest, such as cut stems and mechanical damage to the surface cells in the course of handling and transportation. Major damage is caused to flowers and fruits, besides infecting leaves and twigs. Disease may cause drying of branches, leaf spots, destruction of flowers, shedding of tender fruits and rotting of ripening fruits on the tree or in storage.

Mango is the most important fruit in India, its production reaching about 13 million metric tonnes annually. The estimate of loss is 20-33% of the fresh produce annually and the major factor responsible for it is microbial decay (2). One of the most serious disease of mango is, anthracnose, caused by Glomerella cingulata, Collectotrichum gloeosporioides and Gloeosporium mangiferae (3,4).

Stem-end rot is another serious disease of mango caused by Diplodia natalensis (5). The black spot formed on the surface
of mango is caused by *Rhizoctonia bataticola* (6) *Aspergillus*, *Botrydiploidia* and *Colletotricum* spp. are frequently associated with mango rot (7). There are several other fungi which infect mangoes either on the tree or during storage viz. *Fusarium* spp. (8), *Rhizopus arrhizus* (8), *Phomopsis* spp. (3), *Penicillium cyclopium* (9).

Bacteria have also been implicated as the causative organism of several mango diseases. The formation of small necrotic spots on the leaves, fruits and tender stems of mango is due to *Pseudomonas mangifera indica* (10). *Erwinia mangifera* is known to be associated with black spot formation (11).

Alphonso mangoes develop a characteristic soft flesh termed as 'spongy tissue'. There is a slight desiccation in the centre of the tissue, surrounded by a soft halo. Chhatpar et al. (12,13) have isolated various species of *Bacillus* from these tissues viz. *B. marcerans*, *B. cereus*, *B. subtilis* and *B. megaterium*.

In order to device an efficient eradication programme, it is essential to understand various physico-chemical changes taking place in the fruit upon infection and the effect of various components of the host in sustaining growth of the organism. For the present study, *Penicillium purpurogenum* Stoll was isolated from a naturally infected ripe alphonso mango and some factors responsible for rendering fruits susceptible to infection were studied.

The degree of infection, as observed by the artificial infection of mango by spore suspension of *P. purpurogenum*,...
increases as the fruit ripens. This may be because fruits at the ripe stage are rich in carbohydrates and nitrogenous compounds which support the growth of the organism. Similar observations were made during artificial infection of mango by \textit{P. cyclopium} (14). The susceptibility of apples to blue mold \textit{Penicillium expansum}, increases with both maturity and ripeness. Likewise, naval oranges become more susceptible to \textit{Penicillium} infection with the advance in maturity (15).

The unripe fruits are less susceptible to infection by \textit{P. purpurogenum}, suggesting that there are some factors present in the fruit tissue at this stage of ripening which render unripe fruits resistant to microbial attack. Unripe fruits are rich in their acid content. As ripening proceeds, there is an increase in the levels of sugar with a concomitant decrease in that of acids. The pH of the fruit also shifts from 2.0 at the unripe stage to 5.5 at the ripe one (16). The low pH values of the fruit tissues inhibit the growth of the bacteria capable of degrading plant tissues and this makes fungus a favourable etiological agent for infection in fruits. Vegetables, on the other hand, have pH values ranging from pH 5 to 7 and this makes them more susceptible for bacterial rot (17,18). The effect of pH on the growth of \textit{P. purpurogenum} was checked.

As shown in Fig.1, the optimum growth of the isolate was observed at pH 4.0, while at pH 6.0 and 2.0, the growth was about 1.5 and 2.3 times less, respectively. The pH of the unripe fruit falls between pH 2-2.5, while as that of partly ripe mango is between pH 4-4.5 (16), which supports optimum growth of the
Legend to Fig. 1: The effect of pH on the growth of *P. purpurogenum*. Values expressed are a mean of three independent determinations.
isolate. Therefore, pH of the fruit tissue could be one of the factors responsible for rendering unripe mango resistant towards P. purpurogenum infection.

The low pH values of the unripe fruit suggest higher acid content which is an important constituent governing resistance to microbial attack. A decrease in acidity of the apple tissue during storage was found to be responsible for decrease in resistance to Nectria galligena (19, 20). Since citric and malic acids are the predominant acids of the mangoes, their effect on the growth of the isolate was checked (Fig. 2 A&B). The concentration of citric and malic acids used were in the range with those observed during ripening of mangoes (16). Fig. 2 A&B, show that with the increase in the acid content, the growth of the isolate decreases. Citric acid at 4g% concentration inhibits growth of P. purpurogenum by 50%, whereas malic acid brings about 37% inhibition at 1g% concentration. The maximum concentrations of the acids used were in line with those present in the unripe mangoes (16).

Beside acids, sugars too are important constituents of the fruits influencing the growth of the microorganisms. Studies were carried out to see the effect of various sugars on the growth of P. purpurogenum. It was observed that increasing concentrations of starch, glucose and sucrose (upto 4g%) stimulate the growth of the isolate, whereas fructose stimulates the growth only upto 1g% concentration (Fig. 3 A-D). The isolate was not capable of utilizing higher concentration of fructose (Fig. 3 D). On the other hand, cellulose supports the growth only
Fig. 2: Growth of *P. purpurogenum* in the presence of various concentrations of A) Citric acid and B) Malic acid as sole carbon sources. Values expressed are a mean of results obtained from three independent sets of experiments.
Fig. 3: Growth of *P. purpurogenum* in presence of various concentrations of A) glucose, B) starch, C) sucrose, D) fructose and E) cellulose. Values expressed are a mean of results obtained from three independent sets of experiments.
upto 20 mg% concentration, increasing the concentration (upto 100 mg%) inhibits the growth of the isolate (Fig. 3 E). The maximum concentration of cellulose employed was 10 times less than what was observed in the ripe fruits (16), suggesting that the isolate does not posses higher cellulolytic activity. Fruits are generally rich in sugars. In most fruits, amount of sugars increases with maturity. Post-harvest spoilage causing organisms grow at the expense of these sugars. During infection, a decrease in the levels of complex carbohydrates occurs which is mainly due to extracellular enzyme produced by the pathogen. The sugars released are in turn utilized by the organism for proliferation (9).

Biochemical analyses of the infected tissues were carried out in order to correlate results obtained by in vitro experiments with the changes occuring in in vivo conditions. The level of total carbohydrates and sucrose decrease by 14% and 49% respectively, upon infection, whereas that of fructose increases by 18% and that of glucose increases by 2.5 times during infection as compared to the healthy counterpart (Table I). The increase in the concentration of glucose and fructose during infection could be due to the enhanced degradation of sucrose. The decrease in the sucrose level may be attributed to higher invertase activity. Considerable loss of sucrose was observed in orange fruit infected with Curvularia lunata (21). Similarly, Cladosporium oxysporum and Drechslera rostrata infecting loquat and cape gooseberry respectively, utilized their total sugar content within five days (21). A decrease in total carbohydrates and an increase in total reducing sugar in mangoes infected with
Cellulose Growth (gm dry wt.) per 100 ml.

Conc. of carbohydrate (mg %)
Table 1: The levels of various sugars in healthy and *Penicillium purpurogenum* infected mangoes.

<table>
<thead>
<tr>
<th>Sugars (g/100gm.fr.wt.)</th>
<th>Healthy tissue</th>
<th>Infected tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Carbohydrates</td>
<td>15.0</td>
<td>12.9</td>
</tr>
<tr>
<td>Sucrose</td>
<td>9.8</td>
<td>4.0</td>
</tr>
<tr>
<td>Fructose</td>
<td>4.4</td>
<td>5.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.8</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Values expressed are a mean of three independent sets of experiments.

*Penicillium cyclopium* have also been observed (14). A comparative utilization of glucose, sucrose and fructose by different pathogens was observed by Ghosh *et al* (22). *Pestalotia psidii* and *Phoma psidii* were found to utilize the sucrose in guava fruits rapidly, while *Gloeosporium psidii* and *Diploidia natalensis* were also hydrolyser of these sugars. In the same study, all organisms except *D. natalensis* were found to consume glucose earlier than fructose. In the case of papaya, *Gloeosporium papaya* caused rapid breakdown of sucrose.

The development of post-harvest disease in fruit tissues depends on the ability of the pathogen to induce or secrete enzymes (23). The levels of various enzymes viz. cellulase, amylase, invertase, sucrose synthetase, malate dehydrogenase and malic enzyme were checked from the healthy and
*P. purpurogenum* infected mango tissues (Table II). The cellulase and amylase activities increased by about 12% and 22% upon infection. Such low activities of cellulase during infection correlated with the inability of the isolate to utilize cellulose in vitro. This indicates that fungus has very low cellulolytic activity and thereby cannot penetrate well into the fruit tissue by dissolving cell wall. A 2.5 fold increase in the activity of cellulase was observed in the mangoes infected with *P. cyclopium* (9). The higher activity of amylase indicates the capability of the isolate to utilize starch and thereby producing high amount of reducing sugars. The activities of invertase and sucrose synthetase were increased upon infection.

**Table II: The specific activities of various enzymes from healthy and *Penicillium purpurogenum* infected mango tissues.**

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Healthy tissue</th>
<th>Infected tissue</th>
</tr>
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<tbody>
<tr>
<td>Invertase</td>
<td>0.15</td>
<td>2.4</td>
</tr>
<tr>
<td>Sucrose synthetase</td>
<td>0.08</td>
<td>1.3</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Malic enzyme</td>
<td>0.017</td>
<td>0.008</td>
</tr>
<tr>
<td>Amylase</td>
<td>6.8</td>
<td>7.6</td>
</tr>
<tr>
<td>Cellulase</td>
<td>8.0</td>
<td>9.8</td>
</tr>
</tbody>
</table>

Specific activity is expressed as enzyme units mg⁻¹ protein. Values expressed are mean of three independent sets of experiments.
sucrose synthetase, on the other hand, increase by 16 times upon infection of mango with *P. purpurogenum*. The decrease in the level of sucrose observed correlated with the increase in invertase activity. The activity of malate dehydrogenase (which channelizes malate into gluconeogenic pathway) also increased by 1.3 times whereas that of malic enzyme was lowered by 2.2 times during infection as compared to the healthy counterpart, suggesting accumulation of acids during infection. Low levels of sugars in the infected portion of mango fruit were correlated with a higher acid content (Table III), suggesting that sugars may be converted to acids. The pH of the infected tissue was found to be 4.5 which was close to the optimum pH required for the growth of the isolate, while that of healthy counterpart was pH 5.9.

Table III: The change in pH and the level of total acids upon infection of alphonso mangoes by *Penicillium purpurogenum*.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>pH</th>
<th>Total acids (g%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>5.9</td>
<td>0.029</td>
</tr>
<tr>
<td>Infected</td>
<td>4.5</td>
<td>0.087</td>
</tr>
</tbody>
</table>

New organic acids have been reported to be synthesized during infection of fruits by spoilage organisms. Production of succinic acid during pathogenesis in fruits is of special interest (24). Number of fungi are reported to synthesize succinic acid from carbohydrates present in the fruits (25, 26). It is thought
that the increase or sudden appearance of a particular organic acid may be the result of interaction between the host and the pathogen (27).

Fruit tissues are generally rich in phenolic compounds (28). These are either cinnamic acid derivatives, flavans, anthocyanidins and anthocyanins, flavonols or condensed polyphenols (28). The concentrations in which they are present in ripe fruits range from 0.1 gm%, as observed in the case of grapes (29) to 5.7 gm%, as found in plums (30). These compounds have been reported to possess antimicrobial activity (31,32). Benzoic acid present in the immature apples confers resistance against Nectria galligena (19,20). To check the effect of phenolics on the growth of Penicillium purpurogenum, two marker phenols were selected—benzoic acid and ferulic acid (a cinnamic acid derivative). As can be seen from Fig. 4 A & B, both the compounds were inhibitory to the growth of Penicillium purpurogenum. Ferulic acid proved to be a more potent inhibitor than benzoic acid.

These results suggest that the presence of high acidic content and unavailability of sugars at the unripe stage makes the fruits less susceptible to infection by Penicillium purpurogenum. It is likely that phenolics may also contribute to the same. The higher level of enzymes in the infected tissue may be due to either induction or secretion by the fungal isolate. Such enzymes are reported to be of fungal origin (33,34), but the possibility that fruit tissues are induced to produce such enzymes cannot be ruled out.
Fig. 4: Growth of *P. purpurogenum* in presence of various concentrations of A) Benzoic acid and B) Ferulic acid. Values expressed are a mean of results obtained from three independent sets of experiments.
Penicillium purpurogenum Stoll has been reported to produce rubratoxin A (35), which can cause death accompanied by hemorrhages and hepatotoxicity when given to guinea pigs, rabbits, mice and dogs (36). It was essential to device a programme to control the post-harvest spoilage of mangoes caused by \textit{P. purpurogenum}.

Large number of antimicrobial agents have been used to control spoilage of fruits by microorganisms (15). The application of protective fungicides has been used in tropics to control anthracnose of mangoes and papaya and lenticles of apples in Europe (37,38). 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 \textit{Penicillium purpurogenum}. Systemic fungicides have an advantage over other fungicides in the sense that they posses 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 treatment.

\textit{In vitro} studies were performed to examine the effect of bavistin and aureofungin in the growth of \textit{P. purpurogenum} (Fig.5 A&B). Bavistin exerted a much greater inhibitory effect on the growth of the isolate than aureofungin. Bavistin at a concentration of less than 1 mg% completely inhibits spore germination while aureofungin at 30 mg% inhibits the spore germination. Earlier Ogawa (39) and Long (40) have reported that 2-substituted benzimidazole fungicides (thiabendazol and benomyl) were able to prevent latent infections of \textit{Gloeosporium} in apples.
Fig. 5: Growth of *P. purpureoenum* in presence of various concentrations of A) Bavistin and B) Aureofungin. Values expressed are a mean of results obtained from three independent sets of experiments.
and bananas. Chhatpar et al (41) have successfully used aureofungin to inhibit black spot formed by *Rhizoctonia bataticola* in mangoes.

Unripe alphonso mangoes were dip treated in an aqueous solution containing varying concentrations of bavistin and aureofungin respectively. These fruits were then artificially infected with a spore suspension of *P. purpurogenum* and appearance of infection was observed till the ripe stage (i.e. 8th day of ripening). As can be seen from the Table IV, no infection was observed in any case till the ripe stage, suggesting that the fungicides penetrated in the fruit tissue and completely inhibits spore germination.

### Table IV: The effect of Bavistin and Aureofungin on the spoilage of mango by *Penicillium purpurogenum*.

<table>
<thead>
<tr>
<th>Antibiotic used</th>
<th>Conc. of Antibiotic mg/lit.</th>
<th>3rd day infection</th>
<th>5th day infection</th>
<th>8th day infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Bavistin</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aureofungin</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

++ : infection present, - : infection absent
These results showed that complete eradication of *P. purpurogenum* could be achieved when unripe mangoes were treated with either bavistin or aureofungin. Since the isolate does not possess higher penetrating power with the help of cellulolytic activity, it will be easier to control the infection with the help of systemic fungicides. As the concentration of bavistin required to inhibit fungal growth is very less, it will be advantageous to use it even for human consumption and such low concentration do not deteriorate any fruit quality.
REFERENCES:


