CHAPTER VI

SOME FACTORS RESPONSIBLE FOR THE SPOILAGE OF MANGOES BY PENICILLIUM CYCLOPIUM
All fresh fruits go through various stages of ripening. This phenomenon of ripening is a normal physiological response, which may result in many physiological disorders affecting the quality of the product. However, the greatest single cause of reduced quality and quantity of fruits and vegetables is microbial spoilage, which can occur during any stage of development up to final harvesting. Food processors are more concerned with microbial spoilage during harvesting, transportation and storage of fruits than during the growing of these products.

Though a fruit is exposed to a number of species of microorganisms during its lifetime, only a few of these can be termed as true pathogens in the sense that they are able to invade perfectly healthy tissues in order to develop at the expense of the host. Approximately 23 species of fungi and bacteria are known to be responsible for the major decays of plant products after harvest (1). Most of these microorganisms have the potential for attacking only a few different products from related species of fruits. For example, *P. digitatum* causes green mold on citrus fruits but does not cause any disease in apples and pears. On the other hand, *P. expansum* attacks apples and pears but not citrus fruits. An important factor in determining the types of organisms which can grow and cause losses of fruits and vegetables is the pH of the
particular product. In most fruits the pH of the tissues is quite low, ranging from about 2-3 for lemons to around 5 for bananas (2). This fact reduces the probability of bacteria being the spoilage agents of fruits and increases the probability that molds will be the etiological agents of decay. The pH range of vegetables is somewhat higher and is normally between 5.0 and 7.0. Therefore, vegetables are more susceptible to bacterial spoilage than fruits.

Other factors that play a role in host parasite interaction are the ability of the pathogen to overcome host defences and to initiate growth under conditions of water potential and nutrients present in host tissues.

The mango fruit can be considered to be one of the most important fruits grown in India. The preservation of this fruit is thus a challenging problem. Due to prolonged storage and ripening, mangoes become susceptible to microbial infections. Such infections tend to reduce the market value of these fruits (3,4,5).

Spoilage in harvested tropical fruits and vegetables is mainly of fungal or bacterial origin. The mango, being a tropical fruit, is susceptible to infection by species such as Gloeosporium mangiferae which is known to cause anthracnose, Aspergillus niger, Aspergillus flavus, Fusarium species, Rhizopus arrhizus and Rhizoctonia bataticola which are frequently associated with fruit rots (5,6,7,8). Among the bacteria, Pseudomonas mangiferae...
indica has been shown to form necrotic spots on the fruit and stem of mangoes (9). Erwinia mangiferae is associated with black spots (10), and various species of Bacillus have been identified as the causative agents for "spongy-tissue" formation (11).

In order to develop methods to effectively control post-harvest spoilage, it is necessary to first understand the various inherent biochemical properties of a fruit, which may influence the invasion of its tissues by a pathogen. Keeping this in mind, the present study was undertaken to examine some factors which might confer resistance to mango fruits against infection by a mold Penicillium cyclopium isolated from a spoiled, ripe alphonso mango. Artificial infection studies in alphonso and langra varieties of mangoes revealed that the degree of infection, as indicated by the development of black spots and softening of the tissues at the site of infection, increased with the process of ripening (Plates 1-5). This could be due to the fact that the level of acidity decreases with ripening, while that of sugars and nitrogenous compounds increases with the process of ripening. There are reports which indicate that carbon and nitrogenous compounds are released on to the surface of fruits and leaves where they stimulate the growth of the pathogens (12,13,14). The susceptibility of apples to blue mold viz. Penicillium
Plates 1-5: The degree of infection by *Penicillium cyclopium* during various stages of ripening in alphonso and langra varieties of mangoes. (1) unripe (2) partly ripe and (3) ripe alphonso mangoes. (4) unripe and (5) ripe langra mangoes. The fruits were artificially infected as explained under Materials and Methods and incubated at 35±1°C for 5 days.
expansum, increases with both maturity and ripeness and appears to result from an increase in susceptibility of the flesh to bruise damage. Likewise, navel oranges become more susceptible to invasion by Penicillium molds with advance in maturity during the season (1).

Since the unripe mango fruits were found to be more resistant to infection by Penicillium cyclopium, it was of interest to examine some factors that could render the unripe mangoes less susceptible to infection by this mold. One of the first studies performed, was to examine the effect of pH on the growth of the isolate. The acid content of fruit tissues has been regarded as one of the most important factors for their general resistance to microbial infections, especially those caused by bacterial pathogens (15). Tomatoes, peppers, cucumber and pear fruits are seriously affected by bacterial soft rot. The pH of fruit tissues is generally below 5, because of which the growth of most bacteria which are capable of degrading plant tissues is inhibited, while vegetable tissues are generally less acidic (15,16).

Fig.1 shows the effect of pH on the growth of Penicillium cyclopium. It can be seen that the optimum growth of the mold occurred at pH 5.0, while at pH 2 and pH 3, the growth was about three times less as compared to this optimum value. Since the pH of the unripe mango
Fig. 1: The effect of pH on the growth of *Penicillium cyclopium* in Sabaraud's broth medium. Values of growth are expressed as a mean of 3 independent determinations.
falls between 2-2.5 (17), it is quite likely that pH may be one of the factors involved in the resistance of the unripe fruit towards infection. The pH of the mango fruit tissues increases with the process of ripening, being 5.5 in the case of ripe mangoes (17). Immature apples are reported to be resistant to infection by *Nectria galligena* due to the ability of the fruit to produce benzoic acid (18,19). As the pH of immature apples is acidic (pH 3), benzoic acid is highly fungitoxic. The decrease in acidity during storage of apples leads to dissociation of benzoic acid, as a result of which the susceptibility to infection increases.

Since citric and malic acids are the predominant organic acids present in mangoes, their effect on the growth of the isolate was checked. The concentrations selected were in keeping with those observed to occur in mangoes during various stages of ripening (Table 1).

**Table 1**: Changes in some major chemical constituents of mangoes (var. Alphonso) during ripening.

<table>
<thead>
<tr>
<th>Constituent (gm%)</th>
<th>Stage of Ripening</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unripe</td>
</tr>
<tr>
<td>Sucrose</td>
<td>2.4±1.6</td>
</tr>
<tr>
<td>Fructose</td>
<td>1.97±1.4</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.6±0.66</td>
</tr>
<tr>
<td>Citric acid</td>
<td>3.2±0.95</td>
</tr>
<tr>
<td>Malic acid</td>
<td>0.894±0.4</td>
</tr>
</tbody>
</table>

Source: Mattoo *et al* 1975 (Ref.17)
Fig. 2: Growth of *Penicillium cyclopium* 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 3 independent sets.
Results of such an experiment are presented in Fig. 2 (a&b). It can be seen that the growth of the mold decreased as the concentrations of these acids approached those known to be present in the unripe fruits. Citric acid at a 4 gm % concentration inhibited the growth of the mold by 45%, while malic acid at a 1% concentration brought about only 15% inhibition in growth.

The second factor which can influence the infection process is the availability of sugars and other carbohydrates. Experiments were conducted to examine the effect of starch on the growth of this isolate. It was observed that increasing starch concentrations between 1 gm% to 5 gm% had a stimulating effect on the growth of this isolate as shown in Fig. 3. This indicated that the organism had the ability to degrade starch and utilize the sugars released for its growth. This was further confirmed by growing the isolate on Sabaraud's agar containing 1% starch. A dilute iodine solution was added to the plates after 48 hrs of incubation. It is obvious from Plate 6 that starch in the vicinity of the individual colonies is hydrolysed, probably due to the production of extra-cellular amylase by the organism. Another feature observed in the case of the starch grown culture, was that the isolate failed to sporulate even after seven days of incubation, while in glucose medium
Fig. 3: The effect of starch as the sole source of carbon on the growth of *Penicillium cyclopium*. Values expressed are a mean of the results obtained from three independent sets.
Plate 6: Colonies of *P. cyclopium* grown on Sabaraud's agar containing 1% starch as a sole source of carbon. A dilute iodine solution was added to the plates to check for starch hydrolysis.
the organism initiated spoliation after five days. This could be due to the slow release of sugars as a result of starch hydrolysis which maintained the organism in its growth phase.

Glucose and fructose, when taken in specified concentrations, were also found to support the growth of the isolate (Fig. 4 a and b). The organism could utilize fructose more efficiently than glucose. 3 gm% fructose was found to be optimum for growth beyond which concentration it decreased. However, at all concentrations the growth was higher as compared to the control.

An interesting observation made, was that the mold was incapable of utilizing sucrose as the sole carbon source. Concentrations between 1 gm% and 10 gm% did not influence growth significantly (Table 2). Fruits are generally rich

Table 2: The effect of varying concentrations of sucrose on the growth of *Penicillium cyclopium*.

<table>
<thead>
<tr>
<th>Concentration (gm%)</th>
<th>Growth* (mg, dry wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>115</td>
</tr>
<tr>
<td>1</td>
<td>111</td>
</tr>
<tr>
<td>2</td>
<td>118</td>
</tr>
<tr>
<td>3</td>
<td>121</td>
</tr>
<tr>
<td>4</td>
<td>103</td>
</tr>
<tr>
<td>5</td>
<td>106</td>
</tr>
<tr>
<td>6</td>
<td>114</td>
</tr>
<tr>
<td>7</td>
<td>114</td>
</tr>
<tr>
<td>8</td>
<td>116</td>
</tr>
<tr>
<td>9</td>
<td>117</td>
</tr>
<tr>
<td>10</td>
<td>117</td>
</tr>
</tbody>
</table>

*Values expressed are a mean of the results obtained with three independent sets of experiments.*
Fig. 4: - Growth of *Penicillium cyclopium* in the presence of various concentrations of (a) Glucose and (b) Fructose. Values expressed are a mean of the results obtained from 3 independent sets.
in sugars and their market value depends on the quality and concentration of sugars. In most fruits, the amount of sugars increases with maturity. Post harvest spoilage causing microorganisms generally grow at the expense of sugars. During pathogenesis, usually a decrease in the levels of complex carbohydrate occurs which is mainly due to extracellular enzymes produced by the pathogen. The sugars released are in turn utilized by the organism for proliferation.

Biochemical analyses of the infected portion of the fruits was performed in an attempt to correlate the results obtained from *in vitro* experiments with events probably occurring *in vivo*. It was observed that the level of total sugars was lower in the infected portions of the fruit as compared to the healthy portions. However, the levels of total reducing sugars (including glucose) increased upon infection. Sucrose and fructose levels on the other hand were decreased (Table 3). It was surprising

Table 3: The levels of various sugars in healthy and *Penicillium cyclopium* infected alphonso mango tissues.

<table>
<thead>
<tr>
<th>Sugar(s)</th>
<th>Sugar content (gm%) of Healthy*</th>
<th>Infected*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tissues</td>
<td>tissues</td>
</tr>
<tr>
<td>Total Sugars</td>
<td>13.9</td>
<td>8.91</td>
</tr>
<tr>
<td>Total Reducing Sugars</td>
<td>5.4</td>
<td>7.7</td>
</tr>
<tr>
<td>Sucrose</td>
<td>8.0</td>
<td>0.42</td>
</tr>
<tr>
<td>Fructose</td>
<td>4.1</td>
<td>3.5</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.66</td>
<td>3.84</td>
</tr>
</tbody>
</table>

*Values expressed are a mean of the results obtained with healthy and infected portions of three fruits artificially infected in the unripe stage and incubated for 8 days at 35±1°C.
to note that the levels of sucrose fell upon infection,
as under in vitro conditions, the organism failed to
utilize sucrose as the sole carbon source. It is likely
that some factor may be either present in the fruit or
is produced by the organism, which leads to increased
hydrolysis of sucrose upon infection. A proteinaceous
factor has been isolated from Aspergillus japonicus,
which stimulates tissue maceration by pectolytic enzymes.
However, this factor is not active alone, or in in vitro
assays with pure enzymes (20). The drop in the levels
of fructose in infected tissues confirms the observation
that the organism is able to utilize fructose more
efficiently than glucose. Sucrose and maltose have been
reported to be untraceable in pineapple fruits infected
with Botryodiplodia ananassae (21). Instead, only glucose
and fructose could be detected in the diseased tissues.
Ghosh et al observed a marked difference in the
comparative utilization of sucrose, glucose and fructose
by different pathogens (22). Pestalotia psidii and
Phoma psidii were found to utilize the sucrose in guava
fruits rapidly, while Gloeosporium psidii and Diplodia
natalensis were slow hydrolysers of this sugar. In the
same study, all organisms except D. natalensis were found
to consume glucose earlier than fructose. In the case of
papaya, Gloeosporium papayae caused rapid breakdown of
sucrose.
Orange fruits, upon infection with *Curvularia lunata*, exhibited lower levels of sucrose (23). Similarly, *Cladosporium oxysporum* and *Drechslera rostrata* infecting loquat and cape gooseberry respectively, utilized the entire available sugar within 5 days (24). Again, the response of these two pathogens towards glucose and fructose was found to differ. The former organism utilized fructose earlier than glucose, while the latter consumed both these sugars simultaneously. Preferential utilization of fructose was also observed in the case of *Phomopsis psidii* infecting guava and *P. viticola* infecting grape berries (25). A decrease in total carbohydrates and an increase in total reducing sugars has been observed in apples infected with *Pestalotia anonicola*, *Stachytarsus* species and *Trichoderma viride* (26).

Spoilage of mangoes by *Penicillium* differs from spoilage by *Aspergillus* species; in that, *Aspergillus* was not able to utilize sucrose even under in vivo conditions as observed by Majmudar and Modi (6). Moreover, it could utilize glucose more efficiently than fructose and the levels of these sugars decreased in infected mangoes.

Development of a post-harvest disease in fruits depends upon the capability of the pathogen to secrete
enzymes which depolymerize insoluble pectic polymers found in plant cell walls. Polygalacturonase, endopectinlyases, cellulases and hemicellulases are some of the better studied enzymes known to be involved in cell wall degradation.

*Penicillium cyclopium* infected mangoes were found to have increased levels of pectin lyase and cellulase, suggesting that this organism was capable of digesting the cell walls quite efficiently (Table 4). Pectin lyases have been implicated in maceration of citrus fruit peels attacked by *Penicillium digitatum* and *Penicillium italicum* (27). Considerable depletion of soluble and insoluble pectic compounds has been observed in pome fruits infected with *Sclerotinia fructigena* (28).

Table 4: The activity of some hydrolytic enzymes in healthy and *Penicillium cyclopium* infected alphonso mango tissues.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Specific Activity* of</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Invertase</td>
<td>Amylase</td>
<td>Cellulase</td>
<td>Pectinlyase</td>
</tr>
<tr>
<td>Healthy</td>
<td>6.3</td>
<td>16.7</td>
<td>3.1</td>
<td>0.24</td>
</tr>
<tr>
<td>Infected</td>
<td>28.8</td>
<td>24.8</td>
<td>7.5</td>
<td>0.37</td>
</tr>
</tbody>
</table>

*Specific activity is expressed as enzyme units (as described under materials and methods) per mg protein. Values expressed and the conditions of the experiment are as mentioned under Table 3.*

Galacturonic acid was found to increase during pathogenesis by various fungi in grapes, bananas, Indian plums, sapota
and two varieties of apples (29,30). This increase has been attributed to increased levels of pectic enzymes (31). The levels of amylase and invertase were also increased by about 2 to 4 fold in *Penicillium cyclopium* infected mango tissues (also depicted in Table 4). This indicates the ability of the mold to degrade starch, thereby accounting for the increased levels of reducing sugars. Similarly, a higher level of invertase correlated well with the decrease observed in sucrose concentrations of infected fruits. Concomitantly, low levels of sugars in the infected portion of mango fruits were associated with a higher acid content, suggesting that sugars may be getting converted to acids by the fungus. The pH of the infected tissues was found to be 4.8 which was close to the pH optimum for growth of the mold, while that of the healthy counterparts was around 5.8 (Table 5).

Table 5: The change in pH and the levels of total acids upon infection of alphonso mangoes by *Penicillium cyclopium*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>pH*</th>
<th>Total Acids* (gm%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>5.8</td>
<td>0.033</td>
</tr>
<tr>
<td>Infected</td>
<td>4.8</td>
<td>0.077</td>
</tr>
</tbody>
</table>

*Values expressed and the conditions of the experiment are as mentioned under Table 3.*
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 (32). A number of fungi are reported to synthesize succinic acid from carbohydrates present in the fruits (33,34). 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 (35).

Fruit tissues are generally rich in phenolic compounds (36). These are either cinnamic acid derivatives, flavans, antocyanidins and anthocyanins, flavonols and flavonol glycosides or condensed polyphenols (36). The concentrations in which they are present in ripe fruits range from 0.1 gm%, as observed in the case of grapes (37), to 5.7 gm% as found in plums (38). These compounds have been reported to possess antimicrobial activity (39,40). Benzoic acid present in immature apples confers resistance against attack by Nectria galligena (18,19). In an attempt to examine whether phenolics have any effect on the growth of Penicillium cyclopium, two representatives were selected namely benzoic acid and ferulic acid (a cinnamic acid derivative). As can be seen from Fig.5 (a&b) both the compounds were inhibitory to the growth of Penicillium cyclopium. Ferulic acid proved to be a more potent inhibitor than benzoic acid.
### Effect of Various Concentrations of Phenolic Compounds on the Growth of Penicillium cyclopium

<table>
<thead>
<tr>
<th>Concentration of Phenol (gm %)</th>
<th>Growth (mg dry wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>300</td>
</tr>
<tr>
<td>0.2</td>
<td>200</td>
</tr>
<tr>
<td>0.3</td>
<td>100</td>
</tr>
<tr>
<td>0.4</td>
<td>50</td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
</tr>
</tbody>
</table>

**Figure 5:** The effect of various concentrations of (a) benzoic acid and (b) ferulic acid on the growth of *Penicillium cyclopium* in Sabaraud's broth medium. Values expressed are a mean of 3 independent sets of experiments.
This study thus indicates that the low susceptibility of unripe fruits towards spoilage by *Penicillium cyclopium* is probably due to the high acid and low sugar content present during this stage of ripening. It is likely that some other phenolics may also contribute to the same. Further, the levels of the cell wall degrading enzymes are higher in fungus spoiled tissues. It remains to be seen whether these enzymes are, in fact, produced by the fungus itself. Other studies have revealed that such enzymes are commonly of fungal origin (41,42). The possibility that the fruit is induced to produce such enzymes due to the presence of the fungus cannot be ruled out.

Another observation made during the course of this study was that there was a drastic decrease in the cytosolic protein content in the infected mango tissues as compared to the healthy ones. The decrease in proteins was accompanied by increased activity of acidic, neutral and alkaline proteases (Table 6). The quantitative difference was also

Table 6: The levels of total cytosolic proteins and proteases in healthy and *Penicillium cyclopium* infected alphonso mango tissues.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Protein* (gm%)</th>
<th>Specific Activity*+ of Proteases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Acidic</td>
</tr>
<tr>
<td>Healthy</td>
<td>0.235</td>
<td>1.35</td>
</tr>
<tr>
<td>Infected</td>
<td>0.0253</td>
<td>2.96</td>
</tr>
</tbody>
</table>

*Values expressed and the conditions of the experiment are as mentioned in Table 3.
+Specific activity is expressed as enzyme units/mg protein.
evident when proteins were separated on 7% polyacrylamide gels which were subsequently scanned (Fig. 6 a & b). The major peaks observed in the case of healthy tissues diminished drastically in the infected fruits. It must be noted that equal proportions of tissues were loaded on the gels.

Microbial proteases have been reported to be involved in the invasion of plant tissues (43, 44, 45), probably by hydrolysing proteins in and between the cell walls (46, 47). Bilgrami et al (48) have observed significant changes in the quality and concentrations of amino acids and a gradual depletion in the protein content of maize seeds infested by aflatoxin producing strains of Aspergillus parasiticus. Monilinia fructigena (which infects apple fruits) was found to produce an acid protease in a liquid medium containing proteins. This protease was found to be similar to the one produced in the fruit upon infection (49) and healthy fruits were devoid of the enzyme. Furthermore, no natural protease inhibitors were detected either in the healthy or infected apples. It has been suggested that the protease might be involved in the nutrition of the pathogen. Although we have not looked for changes in amino acid content after infection of mangoes with Penicillium cyclopium, it has been reported that generally, during pathogenesis, the levels of most free amino acids increase in the infected tissues of the fruits (50).
Fig. 6: Densitometric scan pattern of protein bands after polyacrylamide gel electrophoresis of extracts from (a) healthy and (b) *P. cyclopium* infected portions of alphonso mangoes.
In addition to a decrease in the levels of proteins, the levels of RNA were also lowered upon infection of mangoes by *Penicillium cyclopium* with a corresponding increase in the levels of RNase (Table 7). Lower levels of RNA and proteins and a corresponding increase in activity of the respective hydrolytic enzymes in mangoes infected with *Penicillium cyclopium*, indicate that the fungus is probably utilizing the degraded products for synthesizing its own macromolecules. Another possibility could be that the fungus is trying to ward off host resistance.

Hadwiger & Adams (51) examined the role of RNA and protein syntheses in the resistance response of plants. They observed that if RNA synthesis was not inhibited within 4 hrs, and protein synthesis within 6 hrs, of infection of *F. solani* & *F. phaseoli* in pea seedlings, a decreased resistance response was instigated.

Table 7: The levels of RNA and Ribonuclease in healthy and *Penicillium cyclopium* infected alphonso mango tissues.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>RNA* (gm%)</th>
<th>Specific Activity of Ribonuclease*+ (RNase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>0.191</td>
<td>2.9</td>
</tr>
<tr>
<td>Infected</td>
<td>0.132</td>
<td>42.3</td>
</tr>
</tbody>
</table>

* Values expressed and the conditions of the experiment are as mentioned under Table 3.
+ Specific activity is expressed as enzyme units/mg protein.
The organism *Penicillium cyclopium* has been isolated from a variety of food stuffs such as maize, groundnuts, wheat, sorghum, various legumes, maize meal and groundnut meal (52). Holzapfel was the first to report the isolation of a toxic metabolite from *Penicillium cyclopium* (53). This compound was identified to be cyclopiazonic acid and was found to be toxic to ducklings, mice and rats (54).

The presence of phytotoxic and mycotoxic compounds in fruits infected with plant pathogens is documented in the literature (55,56,57). Hence, it was of interest to examine whether the strain isolated from spoiled mangoes was toxigenic or not. Experiments conducted revealed that the strain did not produce cyclopiazonic acid but was probably producing another metabolite called cyclopiazonic acid imine (Fig. 7). Cyclopiazonic acid reacts with Ehrlich's reagent (10% 4-dimethyl amino benzaldehyde) to give a blue-violet colour. The compound isolated gave a negative Ehrlich colour reaction but the absorption maxima at 245 and 270 nm were found to be close to those reported for cyclopiazonic acid imine (58).

The toxin has been reported to be synthesized from acetate, mevalonate and tryptophan (59). Since the concentration of mevalonate increases with the process of ripening (60), resulting into increased carotenogenesis, it was pertinent to examine the effect of β-carotene on the
Fig. 7: Absorption spectrum of the toxin extracted from the extracellular broth after the growth of *Penicillium cyclopium*.
growth of the isolate and also to check the levels of 
β carotene in healthy and *Penicillium cyclopium* infected 
mangoes. Tables 8 and 9 depict the results of such experiments.

Table 8: The effect of β-carotene on the growth of 
*Penicillium cyclopium*.

<table>
<thead>
<tr>
<th>Concentration of β-carotene (mg%)</th>
<th>Growth* (mg dry wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>236</td>
</tr>
<tr>
<td>0.5</td>
<td>263.5</td>
</tr>
<tr>
<td>1.0</td>
<td>261.5</td>
</tr>
<tr>
<td>2.0</td>
<td>295</td>
</tr>
<tr>
<td>2.5</td>
<td>337.5</td>
</tr>
<tr>
<td>3.0</td>
<td>281</td>
</tr>
<tr>
<td>4.0</td>
<td>285.5</td>
</tr>
<tr>
<td>5.0</td>
<td>218.5</td>
</tr>
</tbody>
</table>

*0.1 to 1 ml of pure β-carotene solution made in acetone 
(5mg/mL) was added to 100ml of Sabarau's medium to 
achieve the desired concentrations. The pH of the medium 
was adjusted to 5.0. After inoculation, the flasks were 
incubated for 8 days at 35±1°C. 

Values expressed are a mean of the results obtained 
with three independent sets of experiments.

Table 9: The levels of β-carotene in healthy and 
*Penicillium cyclopium* infected alphonso mangoes.

<table>
<thead>
<tr>
<th>Portion of the fruit</th>
<th>Carotene content* (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>9.1</td>
</tr>
<tr>
<td>Infected</td>
<td>5.3 (-42%)</td>
</tr>
</tbody>
</table>

*Values expressed and the condition of the experiment is 
mentioned under Table 3. Value in parentheses indicates 
% reduction in carotene content in the infected portions 
of the fruit as compared to the healthy ones.
It can be seen that increasing the carotene concentration from 0.5 to 2.5 mg %, resulted in a 43% increase in the growth of the isolate. The level of carotene in the infected portions of the fruit was 42% less than their healthy counterparts. A decrease in the carotene content has also been observed by Chhatpar et al (11,61) during infection of mangoes by certain 'spongy-tissue' causing bacteria.

The decrease in the β-carotene content observed in the present case, can be explained on the basis that mevalonate produced during ripening might be channelized for the production of the metabolite cyclopiazonic acid imine. It is also likely, that the mold might be degrading the β-carotene during its growth as shown by in vitro experiments.

Although the market value of an infected fruit is reduced, it can also be a source of economic loss. Infected fruits have been reported to produce more ethylene than their healthy counterparts (62,63). Ethylene evolved leads to premature ripening of other fruits in the vicinity of the spoiled ones. In order to examine whether the organism under study evolved ethylene or not, fungal spores were inoculated into modified Pratt's medium (64) and ethylene evolution was checked at hourly intervals for a period of 12 hrs using a gas chromatograph. The organism failed to evolve detectable amounts of ethylene. Thus, it appears that the pathogenicity of P. cyclopium is not associated with ethylene evolution.
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