SECTION B

PATHOGENICITY
INTRODUCTION

The capacity of a pathogen in the invasion and expression of the diseased state in the host is known as pathogenicity. The interactions between host and parasite generally result into a number of sequential events. Mere physical contact of the pathogen to the host surface triggers several reactions in both the living entities. The pathogen, by virtue of its ability, gradually invades deep into the host tissues and simultaneously starts obliterating the normal metabolic pattern of the host. Finally, with the complete establishment of the parasite, the disease symptoms appear on the host. These symptoms often include hypertrophy, hypotrophy, necrosis, curling, chlorosis, mottling etc. The physiological state of the host thus, rendered weaken by the pathogen, many a times leads into its death. All these sequential events during pathogenesis have been lucidly discussed by Brown (1936) and Agrimo (1969).

The prerequisite of a pathogen in the pathogenesis is to come into contact with the host surface. This process is described as 'inoculation' or 'pre-penetration stage'. The next step in the pathogenesis is the actual entry of the pathogen into the host tissue, which is termed as 'penetration stage' and is generally followed by 'post penetration stage', when pathogen establishes itself within the host.
tissues and starts obtaining nutrition from it. After a lapse of some time the host expresses a number of reactions which are known as symptoms. The time lapse between inoculation and expression of symptoms by the host constitutes' incubation period'.

The physical state of the host including the thickness of the cuticle, presence or absence of the hairs, wax, stomata, lenticels etc. and the existing environmental conditions like relative humidity, temperature etc. determine the mode penetration of pathogenic organisms. The direct penetration by a pathogen some times requires supply of external nutrition (de-Bary, 1886). But, White and Baker (1954) found that Erysiphe graminis var. hordei could penetrate even without the supply of external nutrients. Inocula of certain organisms like Alternaria tenuis, Botryodiplodia theobromae and B. ananassae invariably require injury as a prerequisite (Tandon and Ghosh, 1962; Tandon and Bhargava, 1962; and Williamson and Tandon, 1966). Baken and Heald (1932) and Ramsey (1951) recorded that the lenticular regions on the apple surfaces are the major sites of entry by Penicillium expansum. In the same context the studies carried out by Tandon and Tandon (1948), Tandon and Bhargava (1958) and Agrawal and Sharma (1968) established that certain pathogenic forms can penetrate only through specific sites
like lenticels, stomata, calyx end, stalk portion or intact surface of the host. In the same reference the reviews of Brown (1936), Gaumann (1950), Dickanson (1960) and Wood (1967) are quote-worthy.

Various environmental factors like temperature, relative humidity and the age of the inoculum, not only influence the mode of penetration but also play important role in the disease development. The investigations carried out by the following workers amply support this contention.

Chauhary (1957) observed maximum disease development in chillies at 90 per cent relative humidity coupled with a temperature of 28°C. Mishra and Singh (1962) studied the influence of relative humidity and temperature on the development of anthracnose disease in banana fruits. The investigations made by Tandon and Ghosh (1962), on the disease development in pear fruits by Alternaria tenuis, established a definite role of temperature. Similar inferences were drawn by Shrivastava and Tandon (1968) on the Botryodiplodia rots of Citrus and Sapodila fruits. Chand et al., (1968) studied diseases of apples incited by Gloeosporium fructigenum and arrived at similar conclusions. While working with the pathological details of Rhizopus stolonifer rot of papaya fruits, Tandon and Mishra (1969) found that temperature and relative humidity play important
role in the disease development. Similar results were obtained by Kanwar et al., (1973) while working with the rot of pomegranate caused by Rhizopus arrhizus. Mehta et al., (1975) made detailed investigations on the pathogenicity of Alternaria tenuis and A. solani responsible for the rot of tomato fruits, and were led to conclude that relative humidity, temperature and the age of the inoculum influenced disease development. Third (1977) got similar results, while investigating Clathridium rot of apple fruits.

Since the present fruit rot is being reported for the first time and has not been investigated for any of the pathological aspects so far, hence, it was thought necessary to study the pathogenicity, the mode of infection and the range of hosts of the pathogen.
MATERIALS AND METHODS

Collection of diseased and healthy fruits and isolation of the pathogen:

An extensive survey was made in local cucurbit growing fields and store-houses to estimate intensity of the disease incidence. Diseased fruits were separated from the plants after enclosing them in polythene bags. Fresh fruits were also collected in the same manner for experimental purpose. The survey was also conducted in store-houses so as to assess post-harvest losses due to diseases. The samples of diseased material (fruits) were brought to the laboratory taking suitable precautionary measures to exclude secondary pathogenic forms. The diseased symptoms on the fruits were recorded and with the help of crude smears a rough idea of existing pathogenic forms in it was obtained.

Isolation of the pathogen was done using tissue-transfer and mycelial tip method. The diseased tissue was cut into small bits and surface sterilized in 0.01 per cent aqueous mercuric chloride solution for about 30 to 60 seconds. These bits were thoroughly washed in sterilized distilled water and plated on sterilized potato-dextrose-agar plates. These plates were then incubated at room temperature for 24 to 36
hours. The tips of the pathogen appearing on the P D A plates were transferred on fresh P D A plates. The process was repeated several times till completely pure cultures were obtained. The fungal tip method was followed to secure bacteria free cultures. The pure cultures were maintained in healthy conditions by frequently transferring the pathogen on fresh P D A slants. Cultures were stored at 28°C.

Pathogenicity test and methods of inoculation:

Pathogenicity tests were performed on healthy fruits. Healthy fruits of about 30 cm in length were surface sterilized with 0.01 per cent aqueous mercuric chloride solution for about 2 to 2½ minutes and then washed thoroughly to remove traces of sterilizing agent. Inoculum discs of 6 mm diameter were cut with the help of a sterilized cork borer from the margins of the young colonies growing on P D A plates.

The healthy fruits were inoculated in four different ways:

(1) **Inoculation after injury:**

Three separate areas on full length of the fruits were selected viz., near the stalk end, in the middle portion and
at the apical portion. With the help of a sterilized needle these areas (about 5 mm in diameter) were pricked. Thereafter, the inoculum discs were placed on these injured spots.

(ii) *Inoculation on uninjured fruits:*

Inoculations were made on three different areas as mentioned in earlier paragraph.

Each test was performed in duplicate. The inocula placed on the fruits were covered with sterilized moist cotton so as to provide humidity to it. Further, they were transferred to sterilized moist chambers having 100 per cent relative humidity and kept at 30°C for an incubation period of 72 hours. The aseptic conditions were maintained throughout, in order to exclude external contamination.

The symptoms appearing after incubation were compared with the original ones. The causal organism was reisolated from these artificially diseased fruits and confirmed for its identity with the original isolate. Thus, the test for pathogenicity was established (Plate 6 b).

(iii) *Well method for inoculation* (Granger and Horne, 1924):

A cavity was made on a healthy sterilized fruit surface using sterilized cork borer of 6 mm diameter.
The inoculum was then placed in it.

(iv) Inoculation using mycelial suspension:

Five inoculum discs with mycelial tips were shaken in 5 ml of sterilized distilled water in a electric shaker. The resultant mycelial suspension thus obtained was used for surface inoculation on sterilized uninjured fruits.

In each of the above inoculation experiments (excepting where mycelial suspension was used) the inoculated spots were covered with moist sterilized cotton. The use of moist chamber and the period allowed for are discussed earlier. After 48 hours of incubation, the symptoms and disease severity was recorded on the basis of visual observation.

Mode of infection and histopathological studies:

To determine the mode of infection, healthy and uninjured fruits were inoculated at the middle portion and incubated at 28°C for 36 hours. After incubation the diseased part was cut and preserved in a mixture of formalin (13.0 ml), glacial acetic acid (5.0 ml) and 50 per cent ethyl alcohol (200 ml). The bits were decolourized by placing them in 60 per cent aqueous chloral-hydrate solution. Free hand sections of the bits mounted in
lectophenol-cotton blue mixture were observed under microscope for histopathological details.

**Host range of the pathogen:**

Observations in the markets suggested that the fungus was active against other cucurbitaceous and also green fruits of other families.

To determine the capacity of the pathogen as to its capacity to infect hosts, the fruits enlisted in the following paragraph were used:


These fruits and vegetables were surface sterilized in the usual way inoculated on (i) injured, and (ii) uninjured surfaces. The relative humidity and temperature were maintained at the levels mentioned earlier. The incubation period, however, was kept constant for 4 days.
After completion of the said incubation period, observations were made. The size of the lesion in mm was recorded. The pathogenicity was established as per Koch's postulates.
RESULTS

Pathogenicity test:

The pathogenicity experiments established that the pathogen could easily invade both the injured and uninjured host surface. The pathogen has also exhibited its capacity to successfully invade the host tissue from any part of the fruit surface. The conclusions arrived at here were supported by following the Koch’s postulates (Plate 6 b).

Different methods of inoculation:

From the results shown in Table 1, it is fairly clear that out of four inoculation methods used the 'Well method' (Granger and Horne, 1924), proved to be the most effective one. The aerial mycelium (mycelial turf) produced on the host surface was much less in amount. The whole fruit turned water-soaked, loosing its lusture and grace and also became wrinkled. The extent of disease was the most severe here.

The injured fruits, inoculated with hyphal tip discs (inoculum discs of 6 mm diameter cut with sterilized cork borer from the margin of young colony growing on PDA plate) showed a higher degree of disease severity than those which were inoculated on uninjured surface.
<table>
<thead>
<tr>
<th>Type of inoculum used</th>
<th>Method of inoculation</th>
<th>Appearance of symptoms</th>
<th>Severity of disease*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyphal tips (Inoculum discs of 6 mm diameter)</td>
<td>On non-injured host</td>
<td>Water soaked areas</td>
<td>++++</td>
</tr>
<tr>
<td>-do-</td>
<td>On injured host</td>
<td>Cottony growth</td>
<td>++++</td>
</tr>
<tr>
<td>-do-</td>
<td>By Well method</td>
<td>Less cottony growth +</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water soaked area</td>
<td></td>
</tr>
<tr>
<td>Mycelial suspension</td>
<td>On non-injured host</td>
<td>Discrete patches of water soaked areas on full length of the host</td>
<td>+++</td>
</tr>
</tbody>
</table>

* ++++, less severe; ++++, moderate; ++++, high; and ++++, most severe state of disease.

Each test was carried out in duplicate.
The inoculum used as mycelial suspension on uninjured host surface could cause lesser degree of disease severity. The complete fruit body became infected and showed discrete distribution of the mycelial tufts with a mosaic pattern of water soaked areas.

**Mode of infection and histopathological studies:**

From the studies made on the fruit surface and that of the free hand sections of the diseased tissues, it is clear that the pathogen forms asexual spores (zoospores) which encyst and germinate on the host epidermis. The mycelial threads were seen growing both aerially and internally penetrating the host tissues. The mycelium was found to penetrate directly through the host surface (Plate 4 a).

The encysted zoospores often developed peg-like structures for penetrating into the epidermal cells. The invasion of the mycelium was found to be both inter and intracellular. The pathogen was never found to produce haustoria (Plate 4 b).

**Host range of the pathogen:**

The pathogen, *F. deliense*, causing fruit-rot of *C. utilissimus* was tested for its pathogenesis against fifteen other hosts (fruits) belonging to cucurbitaceae,
<table>
<thead>
<tr>
<th>Host</th>
<th>Diameter of lesion (mm)*</th>
<th>Susceptible (s) or Resistant (r)</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cucumis utilissimus Roxb.</td>
<td>110</td>
<td>95</td>
<td>White cottony growth</td>
</tr>
<tr>
<td>C. sativa Linn.</td>
<td>100</td>
<td>10</td>
<td>-do-</td>
</tr>
<tr>
<td>Luffa acutangula Roxb.</td>
<td>80</td>
<td>10</td>
<td>-do-</td>
</tr>
<tr>
<td>L. acutissima Mill.</td>
<td>105</td>
<td>17</td>
<td>-do-</td>
</tr>
<tr>
<td>Lagenaria vulgaris Ser.</td>
<td>20</td>
<td>-</td>
<td>-do-</td>
</tr>
<tr>
<td>Lycopersicum esculentum Mill.</td>
<td>40</td>
<td>-</td>
<td>-do-</td>
</tr>
<tr>
<td>Capsicum annuum Linn.</td>
<td>55</td>
<td>16</td>
<td>Water soaked black area</td>
</tr>
<tr>
<td>Citrullus vulgaris var. fistulosus (Stocks) Raffie &amp; Fuller.</td>
<td>80</td>
<td>-</td>
<td>White cottony growth</td>
</tr>
<tr>
<td>Komordica charantia Linn.</td>
<td>35</td>
<td>20</td>
<td>-do-</td>
</tr>
<tr>
<td>Cocoonia indica Wight &amp; Arn.</td>
<td>55</td>
<td>13</td>
<td>-do-</td>
</tr>
<tr>
<td>Abelmoschus esculentus (Linn.) Moench.</td>
<td>91</td>
<td>65</td>
<td>-do-</td>
</tr>
<tr>
<td>Mangifera indica Linn.</td>
<td>-</td>
<td>-</td>
<td>No diseased appeared</td>
</tr>
<tr>
<td>Trichosanthes dioica Roxb.</td>
<td>70</td>
<td>13</td>
<td>White cottony growth</td>
</tr>
<tr>
<td>Solanum tuberosum Linn.</td>
<td>50</td>
<td>-</td>
<td>Water soaked area</td>
</tr>
<tr>
<td>S. melangena Linn.</td>
<td>20</td>
<td>-</td>
<td>s</td>
</tr>
</tbody>
</table>
solanaceae, malvaceae, and anacardiaceae. Out of the fifteen hosts tested *Luffa aegyptiaca* proved to be next to the *Cucumis utilissimus* (present host) followed by *C. sativus* and *Abelmoschus esculentus*, while *Solanum melongena* and *Lagenaria vulgaris* proved to be the least susceptible. No disease symptoms were seen produced on green *Mangifera indica* fruits even under injured condition. (Table 2 and Plate 8).
DISCUSSION AND CONCLUSIONS

The conclusions drawn from these studies are discussed in the following lines:

The method of inoculation suggested by Granger and Horne (1924) was found to be the most effective one, out of four techniques used for the purpose. The present pathogen (*P. deliense*) deeply invaded the host tissues and could luxuriously grow when this method was employed. The aqueous suspension of the mycelium was not able to bring about a severe internal rotting in *C. utilissimus* fruits, but a luxuriant growth of the test fungus covered almost entire surface of the host. This type of inoculum seemed to be very near to the one that occurs in nature. The entire surface of the fruit appeared to be equally susceptible to the pathogenic attack as the severity of the disease incidence was almost the same when different portions on the fruit body were inoculated.

After primary infection, the further spread of the pathogen was found to take place through asexual spores i.e., zoospores. These zoospores encysted on the healthy portions of the host, penetrated directly into the epidermal cells and developed aerial mycelia at the same time. The mycelium was found to grow both in inter as well as intracellular way without developing any haustoria into the host cells.
This indicates fairly towards the haustorial nature of the mycelium.

Finally, the results obtained from the host range experiments established that the pathogen was multiphagous in its host range, as it could grow well on fruits belonging to completely unrelated families like solanaceae and malvaceae. These experiments have shown that the present pathogen was fairly able to grow on succulent fruits containing simple carbohydrates as storage food materials. The higher levels of organic acids in the host tissues appeared to suppress the growth of the test fungus and its capacity to incite diseased state. This point was made amply clear by the failure of the pathogen to grow on the green fruits of Mangifera indica in which the organic acid contents were higher in level.

The polyphagous nature of the disease fungus would suggest that the total losses accrued in the market as far as green vegetables are concerned must be considerable due to this facultative parasite.