CHAPTER II

MATERIAL AND METHOD
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Isolation of Fungi:

The fungi *Penicillium expansum, Penicillium digitatum, Collectotrichum psidii, Collectotricum circinans, Venturia ineqralis, Fusarium moniliforme, Collectotrichum falcatum, Thioioviopsis paradoxa* and *Fusarium oxysporum*, were used in the various experiments conducted in course of the investigation. They were isolated from infected plant tissues on Czapex medium at 25° - 27°C and maintained in pure cultures for inoculations.

Extracts of *P. expansum*:

From an apple infected by *P. expansum* 25 gms of the tissue was taken and then treated with 100 ml of distilled water and thus extract was prepared (pH = 5,0).

Preparation of Tissue for Bioassay:

Cylinders of apple tissues were prepared as described by Mussel and Morre (1969). Sections of the fruit were sliced transversely to the long axis and cylinders of tissue removed from about 1 cm beneath the skin and interior to the vascular zone with a 5 mm diameter cork borer. 5 mm long sections were taken from these cylinders.

Reaction Mixture in Viscometry:

0.5% pectin solution was prepared by dissolving
0.5 gm of sodium pectate in 100 ml of pure water. 5 ml of this solution and 1 ml of enzyme extract at a final pH of 8.0 constituted the reaction mixture for viscometric measurements.

Culture Filtrate for P. expansum:

250 ml of Czapek's solution containing 62.5 ml of healthy apple extract obtained by crushing and boiling 25 gms of the latter in 100 ml of water for 45 minutes, was prepared. Four conical flasks (each of 100 ml capacity) were washed, cleaned and sterilised at 20 lbs/sq. inch. pressure for 15 minutes. To each of them 50 ml of the above solution was poured. P. expansum was introduced into each flask and kept inside an incubator at 27°C. Two of them were taken out after 5 days and used for viscometric experiments. Each observation was replicated twice. The contents of the remaining flasks were used for the same operations after 11 days.

Viscometer:

An Ostwald viscometer made of pyrex of volume capacity 5 ml was used for measuring viscosity changes of the reaction mixture; percentage reduction of relative viscosity due to enzymic activity was calculated. The viscometer was used at 25°C in a constant temperature bath.
Mannitol solution:

Pure mannitol (BDH AnalaR) was used to prepare solution of concentration 0.5 (M); in the solution rectangular sized peels of apple and tomato were placed for three hours to obtain osmotic equilibrium.

Sucrose solution:

Pure sucrose (BDH AnalaR) was used for preparing solutions of concentrations ranging from 0.1 (M) to 1.0 (M).

Weights and Balance:

A number of small weights ranging from 1 gm to 200 gms were further corrected by comparing them on an accurate balance correct to 10^-5 gm. The balance used was Mettler's single pan electrical balance (Manufacturer: E. Mettler, Zurich, Switzerland).

Potentiometer:

A potentiometer of uniform wire of length 10 metres and resistance (28.72 ± 0.05) ohms at 25°C was used. For good results preliminary calibration of the potentiometer under experimental conditions was also done.

Thermo - couple:

Manganin and constantan wires were used to form
a sensitive thermo-couple arrangement for measuring osmotic pressure differences between the healthy and infected cell saps of apple.

**Standard cell**:

A standard cadmium cell of e.m.f. $1.0184 - 4.06 \times 10^{-5} (t - 20)$ V at $t^\circ C$ was used in the thermo-couple arrangement.

**KCl solution**:

Pure KCl (BDH AnalaR) was used to prepare solutions of concentrations 0.1 (M), 0.5 (M), 0.02 (M) and 0.01 (M) for use in the electro-osmotic measurement.

**Ag/AqCl Electrodes**:

Each of the two coils of platinum was first coated with silver by electrolysis of an argentocyanide solution and then was partly converted into silver chloride by further using it as an anode in the electrolysis of a dilute HCl solution. The chemicals used were silver nitrate, potassium cyanide and hydrochloric acid solutions of pre-determined purity.

The other methods used are described in the text.