STORAGE ROT OF VEGETABLES AND FRUITS

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INTRODUCTION AND REVIEW OF LITERATURE
INTRODUCTION AND REVIEW OF LITERATURE

Fruits and vegetables are indispensable for human health. They not only provide food but also minerals and vitamins, so necessary for good living.

The total area under fruit and vegetable cultivation in India is 28,00,000 and 50,00,000 acres, respectively. Despite this, fruits and vegetables that we produce fall short of the steadily increasing requirements of our country. This is partly due to excessive shrinkage resulting from decay caused by microorganisms during packing, transit and storage.

The importance of this aspect was for the first time emphasised by Powell (1906) who named this premise of plant pathology as "Market Pathology". Since then, there have been a number of reports enlarging upon the role of post-harvest diseases. Pennington (1913) stated that New York Board of Health rejected nearly 12,000,000 lbs of fruits and over 7,000,000 lbs of vegetables because of their poor quality. Adam (1916) asserted that at least 25% perishable goods sent to wholesale market was hauled to dump piles due to deterioration. Leelerg (1965) has estimated that losses of fruits and vegetables due to plant diseases was to the tune of $3,251 millions. Out of this, 200 millions was attributed to the fungi during transit and storage. Heavy losses are also reported from West Germany (Gran & Weber, 1965).
In India, in a survey conducted by Chenulu & Thakur (1965) an average loss of 10.8% of apples, 13.5% of banana, 17.7% of mango, 24.0% of potato and 19.3% of tomato has been estimated. Recently, Mehta (1974) suggested that an average loss of fruits and vegetables during storage ranges from 20 to 30%.

A variety of fungi cause severe damage to fruits and vegetables during transit, storage and marketing. The literature pertaining to post-harvest diseases of some of the fruits and vegetables has been briefly reviewed below:

**Post-Harvest Diseases Of Fruits**

**Avverhoa carambola** L. (Kamrakh):

*Trichothecium roseum* Link. and *Colletotrichum gloeosporioides* Penz. have been found to cause decay of fruits of this plant as reported by Tandon & Kakkar (1964) and Srivastava & Tandon (1968).

**Carica papaya** L. (Papaya):


*Citrus* Spp.:

Litchi chinensis Sonner (Litchi):


Mangifera indica L. (Mango):

Musa paradisiaca L. (Banana):

Serrano (1925) gave a brief description of the occurrence, symptoms and causes of banana diseases common in Philippine islands, as heart-rot caused by Fusarium Sp. and anthracnose by Gleosporium musarum Cke & Mass. Ward (1930) investigated banana diseases in Malaya while Chona (1933) in Punjab. Hoette (1935) observed black end rot caused by Gleosporium musarum Cke & Mass. and Microspora musarum, stem end rot due to Thieliopsis paradoxa (Desdy)Bouch. and Phytophthora Sp. during transportation in Australia. Several other fungal diseases caused by Deightonella torulosa (Syd)Sawada, Thoma Sp., Fusarium oxysporum (Schl ex Fr)Snyd et Hans, Botryodiplodia theobromae Pat., Fusarium roseum Link, Alternaria tenuis Nees ex Pers, Pestalotia leprolegna Speg., Trichothesium roseum Link, Geotrichum candidum Link, Pestalotia psidii Pat, Verticillium Sp., Cephalosporium Sp., Colletotrichum psidii Curz, Rhizopus stolonifer (Fr)Link, Alternaria alternata (Fr)Keissler and Cochliobolus spicifer Nelson have also been observed on the fruit (Shreemalu, 1962; Chandra & Tandon, 1963; Srivastava et al., 1964; Rao, 1964, 1965, 1966; Ratnam & Nema, 1967; Mishra & Tandon, 1969; Graham & Navratnam, 1970; Allen, 1971; Stover, 1975; Prasad, 1974).

Psidium guajava L. (Guava):

Alternaria chartarum Prouss (Siddiqui, 1963); Colletotrichum gloeosporioides Pensa (Srivastava et al., 1964); Curvularia lunata (Walker)Boedij, Phytophthora parasitica Dastur (Rao, 1964);
Cystospora Sp., *Alternaria tenuis* Nees ex Pers, *Micromyces oxysepae* Berk. & Br. (Srivastava et al., 1966); *Diplodia natalensis* Evans (Rao, 1966); *Botryodiplodia theobromae* Pat., *Oleosporium paudios* P. Henn., *Rhizopus nigricans* Ehrenb., *Phoma paudios* P. Henn (Srivastava & Tandon, 1969); *Macrophoma allabadensis* Kapoor & Tandon (Kapoor & Tandon, 1970a); *Cuvularia tuberculata* Jain (Kapoor & Tandon, 1970b) were observed on guava fruits.

**Punica granatum** L. (Pomegranate):


**Pyrus malus** L. (Apple):

Apple fruits in storage and transit often get infected by *Venturia inaequalis* (Cooke)Wint (Sater, 1922). Other common fungi on the fruit are *Penicillium expansum* Link (Fischer, 1922);

Post-Harvest Diseases Of Vegetables

Allium cepa L. (Onion):

Sclerotium cepivorum Berk., Aspergillus niger van Tiegh, Alternaria panadii Ayyanger, A. tenuis Nees ex Pers, Sclerotium rolfsii Sacc., Fusarium solani (Mart)App & Sali have been found to cause damage of onion during storage (Walker, 1924; Venkatkaryyan & Delvi, 1951; Chandra & Tandon, 1964; Mukerjee & Tewari, 1969; Dhong & Thakur, 1972). Recently Monde et al. (1976) studied neck rot of onion caused by Botrytis alli Sacc. in relation to spread of fungus under storage.
Allium sativum L. (Garlic):

Severe to moderate infection of garlic bulbs under storage and marketing by *Sclerotium cepivorum* Berk, *Rhizopus oxyssae* Went. & Gerlings, *Macrophomina phaseoli* (Maubi)Ashby, *Asperillus niger* van Tiegh and white rot of garlic by *Sclerotium rolfsii* Sacc. have been reported by Thakur et al. (1962), Mathur & Shukla (1964), Chandra & Tandon (1965), Rao (1966), Patil & Kane (1972-73).

Capsicum Spp. (Chilli):

Losses ranging from 45 to 50% have been reported to be caused by *Alternaria* Sp. and *Phytophthora* Sp. (Melhores & Dals, 1917; Leonion, 1922). Other fungi which cause damage are *Gloeosporium circulata* Spauld & Schenk (Dastur, 1920,1921); *Gloeosporium piperatum* Ell & Ever, *Colletotrichum nigrum* Ell & Holst (Bertus, 1925); *Alternaria solani* (Ellis & Mart)Jones & Grout. (Subramanian, 1954); *Colletotrichum capsici* (Syd)But. & Bis. (Misra & Dutta, 1963; Ratnam & Nema, 1967); *Fusarium semitectum* Berk & Rav. (Srivastava et al., 1964); *Helminthosporium* Sp. (Rao, 1964); *Helminthosporium dammense* (Sacc)Lind. (Tandon & Verma, 1964), *Curvularia lunata* (Walker)Boedij, *Diplodia* Sp. (Rao, 1965), *Helminthosporium capsicola* Rao, *Phoma capsici* Wagnaghi (Rao, 1966); *Bipolaris tetramera* (McKinney)Shoemaker (Rao, 1967); *Botryodiplodia theobromae* Pat. (Shreemali & Bilgrami, 1966), *Colletotrichum piperatum* Petch (Grover & Bansal, 1968), *Pestalotiopsis palmarum* (Cooke)Stey. (Sivaprakasham et al., 1969) and *Gladosporium oxyssporum* Berk & Curt (Panwar & Vyas, 1974).
Lycopersicon lycopersicum (L.) Karsten. (Tomato):

Macrophomia solani Ell & Mart (Samuel, 1932); Pullularia pullulans Berkman (Taylor & Shammer, 1945); Helminthosporium Sp. (McColloch & Pollock, 1946); Colletotrichum Sp. (Kendrick & Walker, 1948); Alternaria tenuis Nees ex Pers (Chandra & Tandon, 1964); Colletotrichum dematium (Pers ex Fr)Grove, Phythophthora parasitica Dastur (Srivastava et al., 1964); Colletotrichum capsici (Syd)But & Bis, Geotrichum candidum Link (Nao, 1965); Phoma destructiva Flower, Alternaria solani (Ellis & Mart) Jones & Grout, Trichothecium roseum Link (Nao, 1966); Gladosporium fulvum Cooke, Fusarium roseum Link, Kyrothecium roridum Tode ex Fr., Phoma Sp., Rhizopus arrhigus Fischer (Srivastava et al., 1966); Curvularia Sp., Colletotrichum Sp., Rhizopus nigricans Ehrenb., Gliocladium Sp., Helminthosporium Sp., Monilia Sp. (Ratnam & Nema, 1967); Colletotrichum capsici But & Bis (Goyal et al., 1971); Fusarium nivale (Fr) Cesati (Thakur & Yadav, 1971); et al., Cylindrocarpon scoparium Morgan (Jamaluddin/ 1974) have been observed as pathogens on tomato fruit.

Solanum melongena L. (Eggplant):

It's fruits have been found to be damaged by Alternaria solani (Ellis & Mart) Jones & Grout (Siddiqui, 1963); Curvularia lunata (Walker)Boedij (Srivastava et al., 1964); Epichocum nigrum Link (Tandon & Verma, 1964); Phomopsis verranae Sacc, Phythophthora parasitica Dastur, Pythium aphanidermatum (Eda) Fitz, Trichothecium
**Physical Factors**

The role of environmental factors specially temperature and relative humidity hardly need to be emphasised. They not only influence the germination of the inoculum but also penetration, spread and establishment of the pathogen in host. These factors also profoundly influence the development of pathogen during transit and storage (Wood, 1967). These factors have been briefly reviewed below:

**Temperature:**

Harter & Weimer (1922) isolated eleven species of **Rhizopus** as pathogen on fruits and vegetables which required different temperatures for their infection. **Rhizopus chinensis** Ehrenb. developed at 35°C; **R. oryzae** Ehrenb. ex Corda., **R. maydis** Ehrenb., **R. tritici** Fischer, **R. delemar** Fischer, **R. nodusus** Namysolwski and **R. arrhigos** Fischer required 30°C as optimum temperature; while 20–25°C were found suitable for the development of **R. artocarpi** Racib. and **R. nigricans** Ehrenb. Adam (1923) concluded that **Penicillium glaucum** Link, **Penicillium** Sp., **Venturia inaequalis** (Cooke)Wint, **Gloeosporium** Sp., **Cladosporium** Sp., **Pleospora** Sp. and
A variety of compounds have been tested. Despite the fact that large number of compounds have been tried, yet plant pathologists have not been able to discover an "ideal compound" for the control of post-harvest diseases.

**Borax and other alkaline salts:**

a) **Borax:** It was the first post-harvest fungicide used on commercial scale for the control of citrus fruit decay by *Penicillium expansum* (Link), Thom and *P. italicum* Wehmer (Fulton & Bowman, 1924; Swanson & Klotz, 1938; Moreau, 1961); of melons infected by *B. cinerea* Pers. (Barger et al., 1946) and sweet potatoes by *Phicococcus* spp. (Person et al., 1946; Martin et al., 1949). A mixture of 4% borax with 2% boric acid in water solution was more effective in controlling citrus fruit rots (Winston, 1959, 1948). Borax also successfully controlled *Hymenosphaera caricae* papayae Petrak & Cif. on papaya fruits (Khare & Dhingra, 1975).

b) **Sodium metaborate:** This salt was also effective against many phytopathogenic fungi of fruits and vegetables such as *Penicillium* Sp. on lemons (Godfrey & Hyall, 1948; Lauriel, 1954; Guille, 1956).

c) **Sodium carbonate:** Sodium carbonate solution (4%) was successfully used for the control of rot of lemons caused by *Penicillium* Sp. and *Phytophthora* Sp. (Barger, 1928; Doidge, 1929). For the control of *Phoma citricarpa* Syd. on citrus, 5% solution was more effective than 4% solution (Christ, 1959).

d) **Sodium hydroxide:** Tomkins (1935), Pilder & Tomkins (1939) and
Filder et al. (1949) used 1-3% sodium hydroxide solution, however, the fruits were also damaged.

e) Sodium-O-phenylphenate: Tomkins (1937) reported that decay of several varieties of fruits could be reduced by wrapping them in tissue paper impregnated with sodium-O-phenylphenate. The growth of Alternaria Sp. on melons (Hopkins & Macronak, 1959; Lipton & Stewart, 1961; Harding & Savage, 1965); black rot caused by Endoconidiophora Sp. and soft rot caused by Rhizopus Sp. (Kushman, 1961; Martin, 1964); Penicillium italicum Wehmer and P. digitatum Sacc. on citrus fruits (Latter & Gutter, 1962; Wild & Rippon, 1975) were controlled by dipping in 0.4-0.5% solutions.

Sulphur compounds:

a) Inorganic: Sulphur in the form of dust was used to reduce infection of Monilinia Sp. (Smith, 1930; Poulos, 1949; Haller, 1952; Smith et al., 1956) and in the form of sulphur di oxide as a fumigant on grapes infected by Botrytis Sp. (Winkler & Jacob, 1925; Jacob, 1929).

The antimicrobial property of sulphur di oxide has been attributed to the formation of sulphurous acid. It has also been observed that this compound inhibited the germination of spores of Alternaria Sp. and Botrytis cinerea Pers. (Couey & Uota, 1961; Nelson et al., 1963; Couey, 1965) and checked the growth of Botrytis Sp., Alternaria Sp. and Cladosporium Sp. on raspberries (Cappellini et al., 1961; Cappellini & Eyal, 1964).
b) Organic: Thiocturea and thioacetamide as 5% aqueous solutions provided effective control of *Penicillium* Sp., *Diplodia* Sp. and *Phomopsis* Sp. causing rots of orange (Childs & Siegler, 1946).

Mixture of sodium dimethyl dithiocarbamate and sodium-2-merceptobenothiazole in synthetic polyethylene polysulphide latex has been used as a fungicide to prevent entry of pathogens inside banana (Wardlaw, 1961; Meredith, 1961a). Suspension (0.4%) of maneob (magnesium ethylene bisdithiocarbamate) or 0.16% solution of thiram (tetrathiomethylthiuramdisulfide) successfully control banana fruit rotting caused by *Monilinia* Sp. Thiram (0.3%) was used as post-harvest dip to control decay on strawberries (Moore & Tow, 1955; Edney, 1964) and inhibits the growth of *Didymella cruciata* Sacc. and *Botrytis cinerea* Pers. (Rabandal, 1975).

Ammonia and amines:

Bottini as early as 1927 provided evidence for preventing decay of citrus fruits by using ammonia in the gaseous form. Subsequently Tomkins & Trout (1932), Grosovsky & Swiff (1934) and Barks *et al.* (1958) successfully used it for the treatment of orange decay by *Penicillium* Sp. and *Diplodia* Sp., and *Rhizopus* rot of peaches.

Efficacy of volatile aliphatic amines for reducing the decay of various fruits was observed by Eckert & Kolbensen (1963).

It was observed that 2-amino-butane salts are effective
against *Penicillium* Sp. on citrus, apple and peach and *Botrytis cinerea* Pers. on grape, *Diplodia natalensis* Evans and *Phomopsis* Sp. on citrus as a post-harvest dip (Eckert & Kolbezen, 1962, 1964; Masornak & Hopkins, 1965). Furthermore, fumigation with 100 ppm gaseous 2-aminobutane for 4 hrs was effective for the control of *Penicillium* Sp. on citrus and apple fruits (Eckert & Kolbezen, 1966). Pyrrolidine was more effective in controlling *Penicillium* Sp. on citrus (Eckert & Kolbezen, 1964).

Halogen compounds and other oxidising substances:

a) *Hypochlorous acid*: McCallan & Seeden (1940) provided evidence for the antifungal properties of hypochlorous acid. They, however, stated that gaseous chlorine was injurious to the fruits vegetables at dosages which prevented decay. Solutions of hypochlorous acid exhibit rapid fungicidal action against *Monilinia* Sp. on peaches (Daines, 1965), *Rhizopus* Sp. on peaches and melons (Lipton & Stewart, 1961), superficial infection of *Alternaria* Sp. and *Cladosporium* Sp. on melons (Lipton & Stewart, 1961) and sooty blotch caused by *Stemipeltis* Sp. on oranges (van der Plank, 1945).

b) *Chloramines and iodine*: Organic and inorganic compounds containing active chlorine bounded to a nitrogen atom are able to penetrate the site of injury and inhibit the growth of pathogens. Nitrogen trichloride has been extensively used as a fumigant to control decay of citrus fruits (Klotz, 1936; Ryall & Godfrey,
1949); melons (Barger et al., 1948) and certain vegetables infected by Penicillium Sp., Alternaria Sp. and Gladosporium Sp. (Pryor, 1950). Certain volatile alkyl chloramines as melanine (2,4,6 triamino-1,3,5-triazine) and chloramine-T (N-chloro-p-toluene sulfonamide) have been used to control decay of oranges and grapes (Klotz, 1936).

Similar property of iodine was reported by Tomkins (1935) and Satrass (1936) for inhibition of fungi on grapes and tomato fruits; however, like chlorine it damaged the fruits.

c) Ozone: It has been advocated for many years as a fumigant for the control of Botrytis Sp. and Alternaria Sp. (Somer & McColloch, 1946; Migue, 1961).

Halogenated hydrocarbons:

Van der Mark & Shervelle (1952) used polyhalogenated ethylene and ethanes for controlling decay of stone fruits, strawberries, citrus, grapes and peaches. Trichloroethylene and tetrachloroethylene were highly effective for the control of Rhisopus Sp. and Monilinia Sp. (Smith et al., 1956).

Berry (1958) found that a mixture of syn-dichloroethane and trichloroethane inhibited spore germination of Diplodia natalensis Evans, Phoma citri, Penicillium digitatum Sacc. and substantially reduced surface lesions caused by Gladosporium Sp. and Alternaria Sp. on figs (Harvey, 1956b).
Dibromotetrachloroethane, a fungicide proposed for the control of dry rot of potatoes (Smith & Wynne, 1958), has also been found to control post-harvest decay of citrus fruits and grapes (Eckert et al., 1962; Nelson et al., 1963).

Tetraidoethylene is used commercially as a 0.3-0.5% suspension in water or 0.2-0.3% suspension in wax emulsion for controlling Alternaria Sp. and Cladosporium Sp. on melons and Botrytis Sp. on grapes (Chiarappa et al., 1962; Christ, 1964).

Hydrobenzoic acid derivatives:

Sodium salicylanilide was used for the control of Penicillium decay (Bates, 1933), Phomopsis stem rot of oranges (Hall & Long, 1950; Leggo et al., 1964), Diplodia and Phomopsis rots, Higrospora Sp. and Cladosporium Sp. on banana (Jardlaw, 1961).

Aldehydes:

Formaldehyde and acetaldehyde have also been used as a dip to kill zoospores of Phytophthora causing rot of lemons (Smith, 1907). Recently, 3% acetaldehyde has been used as a fumigant for the control of Penicillium expansum (Link) Thom. on apples (Stadelbacher & Prasad, 1974) and pathogenic fungi of citrus (Prasad, 1975).

Captan:

Captan (N-trichloromethyl mercapto-4-cyclohexene-1,2-dicar-
boximide) in recent years has been widely employed both as pre- and post-harvest treatment against fungi on strawberries (Dimarco & Davis, 1957b; Thompson, 1958; Salunkhe et al., 1962), peaches (van Blaricom, 1959), cherries (Pierson, 1958), pears (Pierson, 1960), figs (Harvey, 1956b) and potatoes (Cates & van Blaricom, 1961). 

Gleosporium Sp. on apples, Penicillum Sp. on citrus (Spencer & Williamson, 1960); M. fructicola on peaches (Ogawa et al., 1963); Colletotrichum gloeosporioides Penz. on guava, Schizopus Sp. on papaya and grapes (Tandon & Singh, 1969); Penicillum Sp. on citrus (Tandon, 1975) were greatly reduced by 2000 ppm of captan.

Diflutan, an analog of captan, is effective against Monilinia Sp. (Ogawa et al., 1964); Thomopus caricae papayae Petrak & Cif. on papaya (Share & Dhingra, 1975); and Phytophthora nicotianae on tomato (Sokhi & Sohi, 1975).

DCNA:

Clark et al. (1960) first of all described the antifungal properties of DCNA (2,6-dichloro-4-nitroaniline) as an inhibitory agent to the mycelial growth of Botrytis Sp. Preliminary tests carried out in the U.S.A. demonstrated that Schizopus Sp. on cherries (Ogawa et al., 1961), peaches (Cappellini & Strech, 1962) and sweet potatoes (Martin, 1964) could be controlled by dipping fruits in aqueous suspension of 1000-2000 ppm DCNA (Daines, 1965). However, Wells & Cooley (1973) observed that even 950 ppm DCNA
could control Sclerotinia sclerotiorum (Lib) de Bary and Pythium butleri Subram.

2,4-D:

2,4-D (2,4 dichloro phenoxy acetic acid) and other related compounds have been successfully employed in preventing Alternaria rot or citrus fruits (Erikson et al., 1956); Diplodia and Phomopsis stem end rots of oranges (Leggo et al., 1964); Penicillium Sp. and Alternaria Sp. on mandarin (Lodh et al., 1963); Rhizopus arrhizus Fischer on potatoes (Thakur & Chenulu, 1975) and in extending the storage life of lemons (Halton, 1959).

Biphenyl

It inhibited the growth of Botrytis cinerea Pers. on grapes (Tomkins, 1936); Penicillium digitatum Sacc., P. italicum Wehmer, Diplodia natalensis Evans, Phomopsis Sp., Botrytis Sp., Aspergillus Sp., Monilinia Sp., Rhizopus Sp. infecting different fruits and vegetables (Aman, 1940; Ramsey et al., 1944; Heibert & Ramsey, 1946; Godfrey & Ryall, 1948; Winsto, 1948; Littauer, 1956; Dygg et al., 1961; Eckert & Kolbezen, 1963; Leggo et al., 1964); Rhizopus Sp. on peaches (Haller, 1952) and Fusarium Sp. on potatoes (McKee & Boyd, 1962). Biphenyl at 40°F was more effective on valencia oranges against Penicillium Sp. (Rissk et al., 1974) and Rhizoctonia solani Kuhn. (Kataria & Grover, 1975)
Antibiotics:

Aureofungin, Grisoculvin, Pimaricin, Streptomycin, Streptocycline and Tetracycline are the common antibiotics used in control of post-harvest decay of vegetables and fruits.

Nystatine, aureofungin, pimaricin have shown promise for the control of *Monilinia* Sp. and *Rhizopus* Sp. on peaches (Dilworth & Davis, 1957; van Blaricom, 1959); strawberry rots (Ayres & Densien, 1955; Salunkhe et al., 1962); *Gloeosporium* Sp. on banana (Meridith, 1960a,b,c) and *Botrytis cinerea* Pers. on grapes (Stessel, 1956). Nystatin was used to control *Botryodiplodia theobromae* Pat. on guava (Srivastava & Tandon, 1969) and is inhibitory against peach brown rot and anthracnose of banana (Ananthanaryanan & Sheehlah, 1965). Cyclohexamide is effective against post-harvest rots of strawberries caused by *Botrytis* Sp. et al. (Becker, 1958). Aureofungin was successfully effective against *Alternaria* rot of tomato, *Diplodia* rot of mango (Dharamvir et al., 1966-67); *Sclerotinia* rot of peach (Whitehead & Chandler, 1969-70); *Pythium* rot of cucurbits (Sharma & Wahab, 1970-71); *Penicillium* rot of apples (Naik & Joshi, 1973-74). Aureofungin (1000 ppm) gave best results by controlling *Rhizopus* Sp. on grapes and papaya (Sridhar, 1974); *Glomereella* Sp. and *Aspergillus niger* van Tiegh infecting different fruits (Laxminaryana & Reddy, 1974). Tetracycline was found to be effective for the control of *Celinotrichum glaesporioides* Penz. on guava (Gupta et al., 1970).
FULL PLAN OF WORK
FUTURE PLAN OF WORK

Despite extensive literature on the fungal pathogens causing post-harvest diseases of fruits and vegetables, the effect of environmental factors on disease development, changes in biochemical constituents of the infected tissues, success achieved for their control by chemical and other methods, there are still many aspects which need to be investigated:

1. To isolate fungal pathogens from diseased fruits and vegetables viz., papaya, litchi, tomato, mango, banana, guava, pomegranate, apple, brinjal, capsicum and citrus and to diagnose them up to specific level.

2. To test their pathogenicity.

3. To study the effect of different media, different carbon and nitrogen sources and vitamins on the pathogens isolated as in No. 1.

4. To study the effect of temperature, relative humidity and pH on the development of pathogens isolated as in No. 1.

5. To study the post-infection changes in the phenolic, amino acid, carbohydrate, organic acid and ascorbic acid contents in diseased as well as healthy fruits and vegetables.

6. To device control measures, physical as well as chemical.
MATERIALS AND METHODS
MATERIALS AND METHODS

Survey and collection of diseased material:

Diseased fruits and vegetables will be brought to the laboratory at fortnightly intervals, from the local market for identifying the causal organism. Care shall be taken to collect the specimens showing a wide range of symptoms.

Isolation, purification and maintenance of culture:

The diseased specimens collected from the market will be kept in a moist chamber at 20-26°C, to facilitate the growth and sporulation of fungi present in the diseased tissue.

The disease lesions harbouring fungi will be cut into small pieces and then disinfected with mercuric chloride (Riker & Riker, 1934). The isolation of the pathogen will be done by direct tissue transplantation, which involves placement of diseased tissue pieces to the culture medium, contained in petriplates in a sterile chamber. Prior to any transference of the infected parts, all the instruments to be used, will be sterilized by absolute alcohol. Then a portion will be scooped from the disinfecte specimen and it will be introduced into the petriplates containing either of potato-dextrose-agar medium, Czapek's medium and Martin's medium. These plates will be incubated at room temperature.
Pure cultures of the pathogen will be made either by single spore technique (Dickson, 1933) or hyphal tip practices (Brown, 1924). These purified cultures will be stored in the refrigerator to ensure the longer periods of viability.

Pathogenicity test:

It is indispensable to prove the validity of the establishment of the incitant of diseases on fruits and vegetables. So as to confirm Koch's postulates, healthy specimens of nearly same age and size, after having being surface sterilized with 90% ethanol, will be inoculated by making an incision on the intact surface or pin pricking it with the help of sterilized scalpel and needle respectively, or by making a hole by means of sterilized cork borer (Granger & Horne, 1924). In either case the inoculum will be introduced by directly placing the pathogen or by the spore suspension in the incision made earlier. After inoculation the incision will be sealed with wax. The inoculated specimens shall be kept in sterilized desiccators, having saturated atmosphere. These desiccators shall be transferred to an incubator running at room temperature.

After the required incubation period, the diseased areas will be observed under microscope, so as to reveal the presence or absence of the inoculated organism. The identity of the fungus will be confirmed by the use of relevant literature. Wherever the identification will not be possible, the cultures shall be sent to the Division of Mycology & Plant Pathology, I.A.R.I.,
New Delhi and to the Commonwealth Mycological Institute, Kew, England.

Cultural Studies

Effect of different media:

After ascertaining the identity of the fungi, the pathogens shall be cultured on variety of media, the composition of which are given below:

a) Potato-dextrose-agar medium:

Potatoes .. .. .. .. .. .. .. 200 g
Agar .. .. .. .. .. .. .. 20 g
Dextrose .. .. .. .. .. .. .. 20 g
Distilled water .. .. .. .. .. 1000 ml

b) Czapek's medium:

Agar .. .. .. .. .. .. .. 20 g
Sucrose .. .. .. .. .. .. .. 30 g
Sodium nitrate .. .. .. .. .. 2 g
Potassium dihydrogen phosphate .. 1 g
Magnesium sulphate 7H₂O .. .. .. 0.5 g
Potassium chloride .. .. .. .. 0.5 g
Ferrous sulphate .. .. .. .. 0.01 g
Distilled water .. .. .. .. .. 1000 ml

c) Martin's medium:

Agar .. .. .. .. .. .. .. 20 g
Potassium dihydrogen phosphate .. 1 g
Magnesium sulphate 7H₂O .. .. .. 0.5 g
Peptone .. .. .. .. .. .. .. 5 g
Dextrose .. .. .. .. .. .. 10 g
Horse-bengal ... ... ... ... ... ... 100 mg  
Streptomycin ... ... ... ... ... ... 30 mg  
Distilled water ... ... ... ... ... ... 1000 ml 

d) Malt-corn-agar medium: 
Maize ... ... ... ... ... ... ... ... 50 g  
Malt extract ... ... ... ... ... ... 1 table spoon  
Agar ... ... ... ... ... ... ... ... 20 g  
Distilled water ... ... ... ... ... ... 1000 ml 

e) Brown synthetic medium: 
Agar ... ... ... ... ... ... ... ... 75 g  
Glucose ... ... ... ... ... ... ... ... 1 g  
Asparagine ... ... ... ... ... ... ... ... 1 g  
Magnesium sulphate ... ... ... ... 0.375 g  
Distilled water ... ... ... ... ... ... 500 ml 

f) Das Gupta's medium: 
Magnesium sulphate ... ... ... ... 0.75 g  
Potassium phosphate ... ... ... ... 1.25 g  
Asparagine ... ... ... ... ... ... ... ... 2 g  
Glucose ... ... ... ... ... ... ... ... 2 g  
Potato starch ... ... ... ... ... ... ... ... 10 g  
Agar ... ... ... ... ... ... ... ... 15 g  
Distilled water ... ... ... ... ... ... 1000 ml  

Each medium will be prepared by mixing requisite amounts of the ingredients. It will be sterilized in autoclave at 15 lbs pressure for 20-35 mins. The sterilized medium after being poured in the sterilized petriplates will be inoculated with equal quantity of fresh inoculum and will be incubated. 

Radial growth of the test organism on the semisolid medium will be measured in centimeters. On the other hand, in liquid
medium the growth will be measured in grams by filtering the contents through Whatman filter paper No. 1 and drying the fungal mass at 60°C for 48 hours in crucibles. There will be three replicates for each treatment.

Effect of physical and chemical factors:

Temperature: To study the effect of different temperatures, 150 ml Erlenmeyer flasks, filled with 50 ml of sterilized liquid basal medium, will be inoculated with an equal amount of fresh inoculum out by sterilized cork borer. The flasks will be incubated in different cabinets running at 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C and 40°C respectively.

Hydrogen ion concentration (pH): While determining the effect of pH values, the basal nutrient medium will be adjusted to pH 3-10 by employing H/5 HCl for acidic range and N NaOH for alkaline range. After adjusting the desired pH, buffers shall be added to check the increase or decrease of pH.

Erlenmeyer flasks of 150 ml capacity, containing liquid Czapek's medium having different pH, will be inoculated with uniform discs (6 mm diameter) of mycelium punched out from the margins of actively growing fungal colonies. These flasks will be incubated for 15 days at room temperature.

Carbon sources: To study the effect of different carbon sources, various carbon compounds viz., arabinose, ribose, xylose, glucose, fructose, galactose, mannose, lactose, sucrose, maltose,
raffinose, starch, mannitol, sorbitol, fumaric acid and malic acid etc. will be substituted for carbon in basal medium. The concentration of different carbon compounds shall be so adjusted as to contain an amount of carbon equivalent to that present in 50 g sucrose, except for starch which will be added in the same quantity as sucrose.

**Nitrogen sources**: For determining the effect of different nitrogen sources, various compounds containing nitrogen equivalent to nitrogen present in 10 g KNO$_3$ shall be employed, except for peptone, which will be added in same quantity as that of KNO$_3$.

**Flavonoids**: Fungitoxic properties of four main flavonoids viz., hesperidin, tangeritin, narirutin and nobolitin shall be studied in vitro. For the experiment, PDA medium will be used as basal medium. The medium will be divided in three parts and to each part will be added so much amount of flavonoid that the final concentrations of the flavonoid in the medium shall be 50 ppm, 100 ppm and 150 ppm. The medium will be sterilized at 15 lbs pressure for 20 hrs and will be poured into sterilized petridishes. Then these petridishes shall be inoculated with six-day-old cultures, maintained on PDA medium. Basal medium without flavonoids will be taken as control in each case. The dishes shall be incubated at room temperature. The radial growth of fungi in different concentrations of flavonoids will be expressed as per cent of control.
Biochemical Studies

For these studies, inoculations will be made on sterilised fruits and vegetables as indicated in the beginning of this chapter. Later, inoculated as well as uninoculated fruits and vegetables shall be analysed for sugars, amino acids, ascorbic acid, organic acids, phenolics, pectic substances and enzymes.

For the estimation of biochemical compounds, 5 g pulp will be taken from healthy as well as inoculated fruits and vegetables, every third day up to a period of twelve days. It will be macerated in ground-glass homogeniser containing acid-washed sand and 20 ml of 80% ethanol. The pulp after homogenising will be boiled in 20 ml of 80% ethanol on water bath. The boiling will be done at least thrice in order to obtain last traces of organic compounds present. Then the solution will be kept overnight at 10°C. The supernatent, thus obtained, will be centrifuged at 2,000 rpm for 30 mts. However, for the estimation of amino acids, 50 g pulp instead of 5 g will be taken.

Sugars:

Unidirectional ascending paper chromatographic technique will be employed for sugars. The diluted extract (20 ml) will be spotted in triplicate on Whatman paper No. 1. Solvent system employed will consist of n-butanol:pyridine:water (6:4:3). The chromatograms shall be run for 18 hrs at room temperature and shall be air dried overnight. One set of chromatograms will be
sprayed with developing reagent (5 vol. of 4\% aniline + 5 vol. of diphenylamine + 1 vol. of orthophosphoric acid). The sprayed chromatograms shall be dried at room temperature and then heated at 110°C for 15 mts.

For quantitative estimation, the spots from unsprayed chromatograms will be eluted by comparing with sprayed chromatogram with hot distilled water. Sugars in elute shall be estimated with phenol-sulphuric acid method. The optical density will be measured against the blank at 440 n.m. using Bausch & Lomb Spectronic-20 spectrophotometer. The quantities shall be determined by reference to a standard curve prepared with authentic samples of sugars.

Free amino acids and organic acids:

In this case the supernatent will be filtered through glass wool and residue will be extracted thrice with 95\% hot ethanol, and refiltered. The different fractions of ethanolic extract will be mixed and concentrated under vacuum at 40°C and centrifuged at 2,000 rpm for 30 mts. The supernatent will be desalted with a cation exchange column (50 cm X 1 cm) of Amberlite IR-120 resin (H+ form). The effluents obtained by rewashing the column with distilled water will be collected for organic acid analysis.

Amino acids adsorbed on the cation exchange column will be eluted with 200 ml of 1N ammonia at the flow rate of 5 ml/mts. It will then be washed with distilled water several times, and
later dried. The residue thus recovered, shall be mixed with 10 ml of 10\% isopropanol and stored at 10\°C.

For the estimation of amino acids, the two dimensional ascending technique shall be used. Forty ml of samples will be spotted on Whatman paper No. 1. Solvents employed shall be n-butanol:acetone:water (120:30:5:) followed with distilled liquid phenol:water:ammonia (180:20:1). The chromatograms shall be run for 15 hours in each direction and dried at room temperature after each run. Freshly prepared 0.5\% solution of ninhydrin in acetone will be used as developer. The sprayed chromatograms shall be left at room temperature for an hour and later heated at 45\°C for 10 mts to reveal the spots. All the spots shall be marked and cut. All the eluted colours (in 70\% ethanol) shall be read in Bausch & Lomb Spectronic-20 spectrophotometer at 540 n.m., however, in case of proline and asparagine they will be read at 440 n.m. and 530 n.m. respectively. Standard curves shall be prepared under similar conditions for each amino acid with authentic samples. The sample readings shall be converted into absolute amount of ug and calculated to mg/100 g of fresh weight.

For the estimation of organic acids the elute of cation exchange column will be passed through anion exchange column (50 cm X 2 cm) of Amberlite IRA-410 (COOH\textsuperscript{-} form) and the column will be washed with distilled water to remove other components. Organic acids will be washed with distilled water and all the fractions will be mixed and reduced to about dryness under a stream of hot air then redissolved in distilled water to make
the final volume 10 ml and then stored at 10°C.

The organic acids will be analysed by uni-directional ascending paper chromatography on Whatman paper No. 1. Solvent employed will be organic phase of n-butanol:formic acid:water (4:1:5). The chromatograms shall be run for 12 hrs and sprayed with aniline-xylose reagent, then heated at 130°C for 20 mins. For quantitative estimation the spots will be cut and eluted with 70% ethanol. Optical density will be measured at 365 n.m. Authentic samples of organic acids shall help in quantitative analysis.

Phenolics:

The analysis of phenolic compounds will be done daily for five days, then on tenth and fifteenth day. Ten g of pulp tissue and 5 g of peel tissue (in some fruits) from healthy as well as diseased fruits will be scooped and homogenised with 50 ml of 90% hot ethanol. This will be refluxed for 2 hrs and filtered through glass-wool. Residue will be washed twice with 25 ml of hot 90% ethanol and filtrates combined. The quantitative analysis of total phenolics will be done by using n-butanol:acetic acid:water (4:1:3 v/v) and alcoholic FeCl₃ as developer.

Flavanoids:

Unidimensional ascending paper chromatography by using Whatman paper No.1 will be employed for qualitative and quantitative estimation of flavanoids. Chromatograms in triplicate shall be
spotted with 40 ml of ethanolic extract of pulp tissue. The solvent system will be 2.0 sodium chloride solution in distilled water. The chromatograms shall be run for about 12 hrs at room temperature and then air dried. One set of chromatograms will be sprayed with 5% sodium carbonate solution in distilled water and dried at room temperature.

For quantitative estimation of flavanoids, the spots shall be traced on unsprayed chromatograms. The marked area will be taken out from the chromatograms and eluted with 7 ml of distilled water at 90°C for 5 mts. After removal of the paper, the solution will be restored to its original volume. Then 1 ml of Folin-Ciocalteau reagent and 2 ml of 20% sodium carbonate solution in distilled water shall be added and the solution treated for exactly 3 mts at 90°C, cooled for 30 mts and optical density measured at 660 n.m. by using Bausch & Lomb Spectronic-20 spectrophotometer. Concentration of flavanoids will be ascertained by the curves prepared with authentic samples of the flavanoids, where ferulic acid will be used as standard for unidentified flavanoids.

Pectic substances:

The method adopted by Edington et al. (1961) will be used with slight modification for the extraction of pectic substances. In both salthy and diseased specimens analysis of pectic substances will be done after 15 days. Crushed tissue (1.5 g) will be boiled in 95% alcohol for one minute and then left at room
temperature for one day. The supernatent will be replaced with 65% alcohol and again left at room temperature for two days. The process will be repeated thrice after which the liquid will be decanted. The insoluble tissue will be dried and powdered. Two hundred mg sample of the powder will be extracted first with water then with Calgon and finally with 0.013 N HCl. The pectic substances in the three solutions will be estimated by colorimetric method. Two ml of the sample, to be analysed will be taken in test tube kept partly immersed in ice water. Three ml of conc. \( \text{H}_2\text{SO}_4 \) will be added with constant shaking, 9 ml of \( \text{H}_2\text{SO}_4 \) will also be added gradually. Thoroughly mixed contents will be heated on water bath for 20 mts. One ml of 0.15% alcoholic carbazole solution will be added after cooling the contents to room temperature. The intensity of colour will be measured by colorimeter. The colorimetric readings shall be compared with standard curve in order to obtain the amount of pectic substances in the samples.

**Enzymes:**

Peel and pulp (10 g) from healthy as well as diseased specimens will be macerated in ground glass homogeniser with 20 ml of 0.2 M NaCl solution in distilled water (pH 7.6). The homogenates will be filtered through several layers of muslin cloth and squeezed. The extracts thus obtained will be centrifuged for 20 mts at 400 rpm and will be raised upto 25 ml with 0.2 M NaCl solution. This cell-free supernatent solution (test solution)
will be used for enzymatic assay:

a) *Pectin methyltransferase*: It will be measured by continuous titration technique. Ten ml of 1.2% pectin solution adjusted at 7.5 pH will be taken as substrate. Two ml of test solution will be added and the pH adjusted to 7.6 by titrating with 0.1 N NaOH, and incubating for one hour. With the interval of 30 min the pH will be adjusted to 7.6 by adding alkali, and the amount noted. Difference between the amount of 0.1 N NaOH required for the samples as well as control (heat inactivated enzyme preparation) will give the amount of alkali consumed in neutralising the acid. This amount multiplied with 3.1 (constant factor) will give in mg methoxyl gp. removed by 2 ml enzyme sample as PHE activity expressed as the amount of total methoxyl gp. released due to hydrolysis.

b) *Polymethyl-galacturonase (PMG)*: The enzyme will be assayed viscometrically by using Ostwald-Fenske viscometer. The composition of reaction mixture shall be:

\[
\begin{align*}
1.2\% & \text{ pectin pH 4.0 } & \ldots & \ldots & \ldots & 3.5 \text{ ml} \\
\text{McIlvaine's citrate buffer pH 4.0} & \ldots & 1.5 \text{ ml} \\
\text{Distilled water} & \ldots & \ldots & \ldots & 1.5 \text{ ml} \\
\text{Test solution} & \ldots & \ldots & \ldots & 1.5 \text{ ml}
\end{align*}
\]

Thoroughly mixed reaction mixture will be transferred to viscometer. Subsequently, the flow times will be taken at zero hour and after incubation period of 30, 60, 90 and 120 minutes at 25±1°C. Water serves as control. The determination of the percentage loss in viscosity will be calculated according to the
formula: \[
\frac{E_{To} - E_{Tr}}{E_{To} - E_{Tw}} \times 100
\]

Where: 
- \(E_{To}\) = Flow time at zero hours,
- \(E_{Tr}\) = Flow time at the end of incubation period,
- \(E_{Tw}\) = Flow time of water.

c) **Polygalacturonase (PGL):** Polygalacturonase will be assayed viscometrically by employing 1.2% sodium pectate solution in distilled water as substrate, while the quantity and the chemicals of the reaction will be same as mentioned above, but for the pH which will be adjusted to 5.0. The viscosity measurements will be taken by the formula.

d) **Pectin methyl transeliminase (PMT):** Substrate employed will be 1.2% solution of pectin in distilled water, while the reaction mixture consisted of following:

- 1.2% pectin sol. pH 4.0 .... 3.0 ml
- Mollvaine's citrate buffer pH 4.0 .... 1.5 ml
- Distilled water .... .... 1.5 ml
- Test solution .... .... 2.0 ml

After combining thoroughly, the reaction mixture will be incubated for 24 hrs at 25±1°C, later heated on water bath for 30 mts, cooled and centrifuged at 500 rpm for 10 mts. Transeliminase activity of this solution will be detected by increased absorbance at 220-240 n.m. wave length with Unican SP 500 spectrophotometer. Identical reaction mixture containing heat inactivated enzyme will be used as blank.

Thiobarbituric acid test (TBA test) will also be done for confirming the activity detected by the above method. Ten ml of
0.1 N BPA solution in water and 5 ml of H HCl will be added to 3 ml of reaction mixture which will be kept for one hour on a water bath at 100°C. After restoring to original volume absorption will be measured between 500-600 n.m.

e) Polysaccharidase-transcellulase (PGT): The reaction mixture for assaying PGT activity will contain 1.2% sodium polypeptate solution in distilled water, other contents will be same as described above. After detecting PGT activity by spectrometer at 220-240 n.m. wave length, the activity will be continued by BPA test.

f) Pectinase (PP): Discs (0.2 mm thick) will be cut from turbid plugs of 1 cm diameter, taken from medulla of a large potato tuber with the help of hand microtome. Five discs washed with distilled water and drained on blotting paper will be placed in 10 ml of McIlvaine's citrate buffer pH 5.0. The time in mts taken by the disc to lose coherence when gently pulled between blunt forceps will be observed. The activity is expressed as reciprocal of the time taken by five discs. The percentage of PP activity will be calculated by the formula: \[ \frac{100}{MT} \]
(Where: MT = Maceration time in mts of standard potato disc).

g) Cellulase (Gz): Cellulase will be assayed viscometrically by employing 1.2% solution of carboxymethylcellulose (CMC). The reaction mixture will be same but adjusted at pH 4.0. Flow times will be recorded at zero hour and after incubation periods of 30, 60, 90 and 120 mts at 25±1°C. Gz activity expressed in per-
Percentage will be calculated by the formula:

\[
\frac{E_{To} - E_{Tr}}{E_{To} - E_{Tw}} \times 100
\]
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