

INTRODUCTION

The mango (*Mangifera indica* L.), a member of family Anacardiaceae, is the most important fruit crop of India. It has been under cultivation for more than 4000 years and covers the largest area compared to any other fruit crop in the country. In area, production, nutritive value and popularity of appeal no other fruit can ^{be} compare^d with mango fruit. Mango occupies the same position in India as is occupied by the apple in temperate climate and grapes in sub-tropical areas. It is a favourite fruit in all parts of India and has been repeatedly acclaimed as the "king of fruits".

(i) *M. indica* is a 15-30 m tall evergreen tree with light black or blackish brown rough longitudinally fissured bark; leaves alternate, petiolate, entire; flowers small, numerous, polygamous in terminal panicles; fruit a large fleshy drupe with a compressed fibrous stone. Drupes variable in size and shape, yellow to reddish yellow when ripe.

There are over one thousand varieties of mango grown in India. Mango is a commodity of major economic and cultural importance of various states of India. Uttar Pradesh has the largest area accounting nearly 33.2 percent of the total, followed by Bihar (13.5%), Andhra Pradesh (13.4%), Gujarat and Maharashtra respectively (Arya, 1993).

(i) ^{Species name} The mango fruit has many uses and has a high food value, and it is rich in proteins, starch, sugars and vitamins. The wood is used in the manufacture of plywood and the bark for tanning. Many medicinal properties are also ascribed to *M. indica*. Almost every part of the plant is used medicinally. These are used in the treatment of diarrhoea, asthma, cough, chronic dysentery.

catarrh of bladder and gleet, cholera, plague, cutaneous infection, scabies and diphtheria

Various kinds of chemical compounds have been reported from vegetative and floral parts of *M. indica*. Quercetin, Kaempferol, Mangiferin (1, 3, 6, 7 - tetrahydroxy xanthone), gallic acid and m-digallic acid were reported to be present in the leaves of *M. indica* (Elsessi and Satch, 1965). Butin (7, 3', 4' - tri OH flavonone) have been reported from the stem bark (Ansari *et al.*, 1967). Isoquercetin, Kaempferol and laucoanthocyanins were reported from alcoholic extracts of mango panicles (Bose and Siddiqui, 1948; Singh and Bose, 1961). 1, 3, 6, 7- tetra-oxygenated and 1, 3, 5, 6, 7-penta-oxygenated xanthenes and polyphenoles were reported from various parts of *M. indica* (Ghosal *et al.*, 1978). Guha and Chakrabarti (1933) reported vitamins B, B₂ and vitamin C in Indian mango. Free sterols consisting of sitosterol, stigmasterol, campesterol, stigmasterol 7-en-3 β -ol and α - spinasterol and their corresponding ester (steryl esters) of palmitic and stearic acids found in healthy floret ; malformed and artificially infected florets contain a mixture of zoosterol viz. pregnenolone (5-pregnene-3- β -ol-20-one), progesterone and a new lonostane derivative (Ghosal and Chakrabarti, 1988). The seed fat of *M. indica* contain 53 percent molecules of saturated acid and 25.7, 67.5 and 6.5 percent molecules of mono, di and tri saturated glycerides respectively (Narayanan and Kartha, 1962).

The wide range of climatic conditions and environmental situations in which mango grows indicates the nature and the diversity of the associated disease problems. Over 140 fungi (70 diseases) and about 12 nematodes and a dozen of phanerogamic parasites and epiphytes are associated with *M. indica*.

Yet, there are a few diseases which are of great economic importance (Reddy, 1975). Different parts of *M. indica* are known to suffer from a number of diseases caused by fungi, bacteria and insects. Mango is affected by a number of diseases at all stages of its development, right from plant in nursery to the fruits in storage or ^{growing} transit. Hardly any plant organ is immune, and almost every part viz. stem, branch, twig, leaf, root, petiole, flower and fruit are affected by various kinds of parasites. Among the diseases, those affecting flowers and fruits are most destructive ones (Laxminarayan and Reddy, 1977. Singh, 1990). The diseases manifest themselves as several kinds of rots, die back, mildew, necrosis, scab, blotch, stem bleeding, wilt, spot, canker, sooty mould and malformation. Some of these diseases take heavy toll of tree and produce alike, and have become a limiting factor in mango cultivation in some regions. Bloom blight or blossom blight had caused a complete failure of the fruit crop in Florida and Brazil (Singh^h 1960). Black spot on mango fruit in south Africa and black tip, powdery mildew, sooty mould and malformation in India are similar sources of great loss to orchardists.

Mango malformation, also known as bunchy top, is a very serious disease of national importance. This disease poses a very serious problem to mango industry causing considerable loss to growers. The malady has been reported from several mango growing countries such as India, Pakistan, Egypt, Israel, South Africa, Central America, Mexico and U.S.A. (Chadha *et al.*, 1979). In India it was first noticed by Watt (1891) in Darbhanga district of Bihar and then by Burns (1910) in Maharashtra. Mango malformation is of two types - vegetative and floral malformation. Vegetative malformation was first described in young seedlings (Nirvan, 1953), but also appear on mature trees.

Floral malformation as the name implies is a disease of the inflorescence. Flowers born on malformed inflorescence are slightly bigger than those flowers born on healthy normal inflorescence and bear scanty pollen. These flowers fail to produce fruits, resulting in tremendous reduction in the yield of fruits

Besides mango malformation, leaves of the plant are also affected by a number of fungal diseases like anthracnose, die back, leaf blight, twig blight *Alternaria* spot, *Pestalotia* leaf spot, *Phoma* leaf blight, *Curvularia* leaf spot (Hingorani and Sharma, 1956, Srivastava *et al.*, 1965; Yadav and Udainarain, 1970; Singh, 1971; Prakash and Singh, 1977; Prakash and Raoof, 1985; Prakash and Srivastava, 1987).

Mango fruits both ripe and unripe are also vulnerable to variety of diseases. Diseases of fruits occurring in transit and storage results in to great spoilage because of the lack of proper storage facilities. Among the several spoilage organisms, fungi are the most destructive, causing extensive damage during storage and transport of mango fruits. Most of the spoilage caused by various fungal infections amongst which *Colletotrichum gloeosporioides* Penz., *Gloeosporium mangiferae* P.Henn., *Phomopsis* sp., *Diplodia natalensis*, *Dothiorella ribis* (Del) Sacc., *Fusarium* sp, *Aspergillus niger*, *Rhizopus* sp, *Cladosporium herbarum* and *Botryodiplodia theobromae* Pat are quite important (Mathur ^{et al.} 1953; Singh, 1960; Srivastava *et al.*, 1965; Thakur and Chenulu, 1966; Tondon, 1967; Laxminarayana and Reddy, 1975; Arya, 1993) Beside these, there are several other fungi which damage the fruits either on the tree or in storage such as soft rot (*Phoma* sp.), *Pestalotia* rot, *Alternaria* rot (*Curvularia*, rot (Srivastava *et al.*, 1965; Prakash and Srivastava, 1987).

Disease is an interaction among the host, parasite and environment. These are widely distributed in all existing things. Human beings, plants and animals are affected by a large number of microorganisms which leads into sickness, malformation or death. Disease resistance is the capacity of an organism to prevent, restrict or retard disease development and occurs at high, moderate or low level. All plants have resistance to some pathogen under certain conditions (Stakman and Harrar, 1957). Host-parasite relationship may be inhibited or completely hindered if the host is able to ward-off the pathogen by passive or active process.

Protection and defence are two primary components of disease resistance. Protection is a static phenomenon which involves either fungitoxic or fungistatic compounds in or on the host tissues. It also includes presence of pre-formed structures within the tissues of host plant. Defence is a dynamic phenomenon and does not occur until the host and pathogen have made physiological contact. Like protection, the defence reactions may involve either structural or chemical barriers. Structural, morphological and chemical defence mechanisms are the categories of defence process.

Certain structural features act as natural barriers for the entry of pathogen. These morphological structures may be present before infection or may develop afterward as a result of infection. Thick walled epidermis, cutin, wax-deposition, natural opening, formation of cork layer around the infection zone, abscission layer, tyloses, gum deposition, lignin deposition, resins, tannins and swelling of cell walls and sheathing of hyphae impart the role in disease resistance against the pathogen (Akai, 1959).

The biochemical defence mechanism may consist of the presence or absence of a particular chemical substance or a group of substances in the host plant which interferes with the growth and multiplication of the pathogen. The biochemicals may be present before infection or may be produced by the interaction of the host and pathogen (post-infectious). Biochemical defence can be categorized into passive (pre-formed) and active (post-infectious) substances. Pre-formed substances are those substances present in the plant before to their contact with pathogen. A large number of substances with their antimicrobial properties have been involved in resistance. These compounds are present in high concentration in healthy plant or may change into effective toxin as a result of host-parasite interaction. Many workers assessed the role of preformed substances in disease resistance (Schonbeck and Schlosser, 1976; Weinhold and Hancock, 1980) and many secondary metabolites such as phenols, flavonoids, steroids, terpenes, tannins and alkaloids also play an important role in the mechanism of defence (King, 1953; Skinner, 1955; Hiller, 1964; Birch, 1966; Tokin, 1967; Wain, 1969).

Infection persuades formation of antifungal and postinfectious compounds. These compounds also take part in defence mechanism. These compounds are either absent or present at low levels and are produced or activated upon infection. They are formed as a result of change in metabolic pathway due to host-parasite interaction. The formation or release of antifungal substances takes place after the inoculation of fungi in different host-parasite combination in plants such as sweet potato, pea, soybean and rice (Hiura, 1943; Gunmann *et al*, 1950; Mizukani, 1953; Kuc, 1955; Uehara, 1958; Muller, 1958).

Never possible

In human beings and animals, the formation of antibodies in blood to impart resistance against infection by microorganism is well understood. A functionally similar defence mechanism was found by Muller and Borger (1940) in plants. They found that when potato tubers were exposed to virulent race of the fungus to which they were resistance, potato tubers developed localized resistance. This phenomenon lead to the theory of phytoalexins.

Phytoalexins are defined as low molecular weight antimicrobial compounds that are synthesized by and get accumulated in plants as a results of microbial attack. These compounds are strictly formed after infection. They get accumulated at infection site in concentration enough to restrain the development of microorganism. In the last few years, many workers have studied the role of phytoalexins in disease resistance (Deverall 1976; ⁷Stoessl *et al.* ^{Ref year} 1977; Albersheim, 1977; Keen, 1981; Kuc and Rush, 1985). Accumulation of phytoalexins at the site of infection clearly points out their defence function. Phytoalexins are not only induced by fungi, but also by bacteria, viruses, nematodes, toxic chemicals, physical treatment and stress conditions (Bell, 1967, Hadwiger and Martin, 1971; Stoessl *et al.*, 1976; Rich *et al.*, 1977; Misaghi, 1982; Kulshreshtha, ^{et al.} 1985). 8

The phenomenon of phytoalexin production is wide spread among the higher plants. Their accumulation shows self defending ability of the higher plants. Phytoalexins are not only antimicrobial, some phytoalexins are also toxic to nematodes (Kalpan *et al.*, 1980), Plants (Shiraishi *et al.*, 1975; Skipp *et al.*, 1978; Glazner and Van Etten, 1978) and animals (Van Etten and Bateman, 1971; Oku *et al.*, 1976). However these are more effective against the fungi and some are considered to be capable of limiting fungal colonization

in plant tissues (Johnson *et al.*, 1976; Skipp and Bailey, 1976, Deverax⁶, 1977; Smith, 1978; Yoshikawa⁶ *et al.*, 1978) The variety of chemical classes such as terpenoids, flavonoids, isoflavonoids, stilbenes, polyacetylenic alcohol and phenols have been identified in beans, potatoes, orchids, beet roots, cotton and other crop plants (Cruickshank and Perrin, 1971⁶⁴; Frail *et al.*, 1980; Kuc and Rush, 1985)

With the development of disease, a complex series of biochemical reactions proceed in an orderly and highly interacted manner. In an infected plant the normal sequence of metabolic reaction is replaced by other biochemical sequence which has a detrimental role on the host. The changes may result from the interaction of fungal products with the compounds already present in the host or with host genome (Drysdales^{and Langcake}, 1973). The successful infection of a host by the pathogen may result in balanced parasitism on the one end and restricted infection or death of the host tissue on the other end. Pathological studies concerned with the changes in ascorbic acid content, peroxidase activity, total soluble sugars, total phenols and mangiferin content have been carried out in order to understand the pathogenic nature of fungi and also helps in understanding some aspects of biochemical defence mechanism.

In Gujarat state mango cultivation covers about 32,000 hectares of land. Mango varieties such as *rajapuri*, *kesar*, *alphonso*, *totapuri*, *dadmo*, *sardar*, *langra*, *dasheri*, *ladva*, *neelam*, *karangio*, *jamadar* and *vashibadam* are considered to be the popular varieties of the state. The commercial variety of Gujarat state is *rajapuri*. Mango malformation disease is found to be more pronounced in all these varieties, but the severity of the disease is found to be more in *rajapuri* (50 - 60 %). Beside mango malformation, leaves woods and

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fruits are also susceptible to a number of diseases. Though *rajapuri* is considered as popular variety of the state, detailed account on the disease causing organisms and the interaction on this variety is not available

Therefore in the present work an attempt has been made to investigate the post infectional phytochemical, biochemical and anatomical changes of different organs of *M. indica* var. *rajapuri*. Along with the var. *rajapuri* some other popular varieties like *kesar*, *alphanso*, *totapuri*, *ladva*, *langra* and *dasher* have also been studied for their phytochemical and biochemical changes

The principal objectives of the thesis are as follows:

1. Isolation, culture and identification of pathogenic fungi from various diseased organs.
2. Comparison of healthy and infected inflorescences and leaves of different varieties in relation to their post infectional chemical changes.
3. Determination of antifungal activity of phytoalexins by different bioassay systems.
4. Study of biochemical changes in healthy and diseased inflorescences, leaves and fruits of different varieties.
5. Study of anatomical and histochemical changes associated with healthy and diseased organs