DISCUSSION

Fungal infection brings about a number of changes in chemical and metabolic constituents and structure of plant Plant respond in such a way to protect themselves from the causal organisms. Several metabolic activities get activated when a plant is attacked by a pathogen (Paxton, 1995). Post infectional chemical, biochemical and structural changes are the defence actions, exhibited by most of the plants to protect themselves against the pathogen. Studies of post infectional chemical, biochemical and histological changes in leaves, inflorescences, fruits and wood of *M. indica* L. have shown interesting results

India, being geographically subtropical country with warm and humid climate, provides suitable conditions for development and spread of a number of plant diseases. Many of these diseases are caused by different groups of fungi Fungi imperfecti, consist of large number of parasitic and saprophytic organism, is the major disease causing group Fungi imperfecti were well equipped to complete their life cycle within a very short span and highly evolved to adapt themselves into changing environment and can survive well even in extreme adverse conditions They not only inhabit almost all parts of the plant before harvest but also deteriorate fruits and seeds during post harvest phase In the present study thirty two organisms of fungi imperfecti and eight organisms of Ascomycetes have been isolated from different parts of mango (*Mangifera indica* L.).

Post infectional phytochemical changes

In response to fungal attack, plants are known to accumulate a variety of chemical compounds in large quantities or produce new compounds (Theodora *et al.*, 1982 ; Purkayastha *et al* , 1983 ; Sadiq and Vyas, 1991 ; Daniel , 1994).

In the present study six popular mango varieties of Gujarat state have also been selected along with variety *rajapuri* to study the post infectional chemical changes.

Malformed inflorescences and diseased leaves of all the varieties have shown significant post infectional changes In most of the diseased leaves and inflorescences analysed, an increase in concentration of phenolic compounds was observed. The appearance in high concentration of these compounds is the result of an enhanced synthesis of phenol by the host through sikmic acid pathway. These results support that phenolic compounds accumulate in more concentration in diseased plant tissues as a result of infection (Tomiyama, 1963 ; Kuc, 1964 , Mace, 1964 ; Rohringer and Samborski, 1967 ; Kosuge, 1969 ; Glazner, 1982)

Some notable changes have been observed in the distribution of flavonol, quercetin and its derevatives and phenolic acids like p-OH benzoic acid and ferulic acid. The malformed inflorescences and diseased leaves of *rajapuri, ladva, alphanso, langra* and *dasheri* varieties exhibit higher concentration of flavonol quercetin. The accumulation of quercetin in diseased organs indicates toxic nature of this compound to fungi It seems that the plant resortes to the production of this compound when it is infected It is evident that quercetin is an antifungal compound inhibits the growth of fungi such as *Daelalea quercina* and *Fomes annosus* (Walchii and Scheck, 1976 ; Alcubilla-Martin, 1970 ; Sporoston, 1957 ; Dixit *et al.*, 1978). Therefore, antifungal compound quercetin could be considered as a phytoalexin providing a better mode of resistance. In the present study quercetin has been assayed for its antifungal activity and noticed the inhibition of *F. moniliforme* growth and incombination with p-OH benzoic acid against the fungus *P. mangiferae*.

The increase in concentration of phenolic compounds may be an instant reaction to infection and could possibly be involved with mechanism of disease resistance. An increase in p-OH benzoic acid concentration is observed in malformed inflorescences and diseased leaves of all varieties of *M. indica* L. except in the

inflorescences of *langra* variety Though p-OH benzoic acid occur in plants as component of lignin, its role in imparting disease resistance to plants has already been evidenced by the studies on carrot slices using *Botrytis cinerea* (Harding and Heale, 1980, 1981). The production of more p-OH benzoic acid indicates the active lignification process in diseased organs. Lignification either directly or indirectly would also form a potential barrier to infection (Kolattukudy, 1975 ; Ride, 1975 ; Henderson and Friend,1979) Gallic acid concentration is found to be high in malformed panicles and diseased leaves of all the varieties. The presence of gallic acid in infected fruits and leaves is reported by Ghosal *et al* (1978). Post infectional increase in phenolic contents involves the enhancement of synthesis and translocation of phenolics to the site of infection and hydrolysis of phenolic glycosides by fungal glycosides to produce free phenol (Pridham, 1965 , Rohringer and Samborski, 1967 , Glazner, 1982 ; Sharma *et al* , 1983)

Mangiferin (1, 3, 6, 7 - tetra hydroxy Xanthone- C_2- β -D-glucoside), a natural metabolite has been found in all the parts of *M. indica* L. and it acts as a phytoalexin - like compound The concentration of this compound is found to be very high in malformed panicles It usually accumulates in the cells of the plant organs close to the site of pathogen invasion and thus prevents the ingress of the pathogen into the host tissue (Chakrabarti and Ghosal, 1985, 1989 ; Chakarbarti *et al* , 1990, 1994 ; Ghosal *et al* , 1977, 1979) The accumulation of mangiferin in high concentration in malformed panicles, diseased leaves and treated plant parts clearly indicates its antifungal nature In the present study antifungal nature of this compound has been assayed against the *Fusarium moniliforme* and *Curvularia lunata*.

In addition to quantitative changes in chemical constituents, a number of qualitative changes have also been observed in the present investigation

(i) Demethylation of methoxylated compounds

In this process, the chemical nature of a compound already existing within the plant is altered resulting in the production of more reactive compound. The demethylation of methoxy flavonol was observed in the leaves of *alphanso* and in the inflorescence of *alphanso* and *langra* varieties, where 3' OMe quercetin and 3' 4' di OMe quercetin are replaced by its hydroxyl derevative quercetin. The presence of more hydroxy group give more reactive power to this compound Similar results were observed in *Cassia fistula, Morinda tomentosa, Citrus limon* L. and *Calotropis giantea* (Abraham, 1989 ; Parikh, 1995).

(ii) Formation of new compound

Formation of a new compound is noticed in malformed inflorescences of *rajapuri, ladva, alphanbso, langra* and *kesar* varieties and diseased leaves of *dasheri* and *alphanso.*

Quercetin is the compound formed in malformed panicles of *alphanso* and *langra* varieties and diseased leaves of *alphanso.* The antifungal nature of the compound against *F. moniliforme* is established in the present sutdy. Abraham (1989) and Parikh (1995) also reported inhibitory action of quercetin towards *Aspergillus niger, Colletotrichum gloeosporides. Fusarium semitectum, Colletotrichum capsici* and *Chaetomium globosum.*

Production of p-OH benzoic acid in malformed panicles of *rajapuri* and *ladva* indicates that this compound also involves in the disease resistance process. The antifungal nature of p-OH benzoic acid against *Fusarium moniliforme* and *Drechslera specifer* has been proved in the present study The immediate production of p-OH banzoic acid in response to fungal attack and its antifungal activity leads to the conclusion that p-OH benzoic acid could be a phytoalexin-like compound. The

presence of p-OH benzoic acid in infected tissues and its role in defence mechanism has been reported earlier (Swinburne, 1975 ; Kolattukudi, 1975 , Henderson and Friend, 1979 , Harding and Heale, 1980)

The formation of ferulic acid in malformed inflorescences of *alphanso* and *kesar* varieties and diseased leaves of *dashert* variety indicates that this compound is also involved in the defence reaction The antifungal property of ferulic acid against *Porta weirri* (Li *et al* , 1972) and *Fusicocum amygdali* (Borys and Childers, 1964) is known The inhibitory action of ferulic acid towards *F. moniliforme* and *Chaetomium globosum* growth supports the antifungal role of this compound.

(iii) Degradation of compound

Fungi play an important role in destroying the active principles of the host plants. As alkaloids, phenolics and other secondary metabolites play an important role in the defence of plants, detoxification of these compounds helps the parasite to grow successfully within the host The degradation of quercetin is found in malformed panicles and diseased leaves of *totapuri* and *kesar* varieties Degradation of ferulic acid is also seen in malformed inflorescence of *dasheri* and *langra* varieties. The detoxification of phytoalexins by pathogens is accepted phenomenon in plants (Westlake, 1965 ; Higgins and Millar, 1979 ; Boominathan, 1986)

BIOCHEMICAL CHANGES

During pathogenesis the metabolic constituents of host cells and enzymatic activities undergo drastic changes Changes in ascorbic acid content, peroxidase activity, total sugars, total phenols and mangiferin content have been observed. These changes may have resulted from altered host metabolism due to infection and also due to the metabolism of the pathogen in the host An analysis of such biochemical changes

in the host tissue may lead to the understanding of some aspects of biochemical defence mechanism.

Ascorbic Acid Content: Nutritional value of fruits is largly due to their high vitamin content especially ascorbic acid (Vitamin C). Mango is an important source of ascorbic acid The quantity of ascorbic acid decreases during storage of healthy fruits and during infection (Ghosh *et al.*, 1965, 1966, Srivastava and Tondon, 1966, Rai, 1982).

In *M. indica*, compared to healthy plant parts, infected and fungus treated ones showed considerable reduction in ascorbic acid content Mangiferin treated leaves, inflorescences and fruits show increased quantity of ascorbic acid content. Reduction in ascorbic acid content under the pathogenesis is due to the production of suitable ascorbic acid degenerative enzyme either by the fungus alone or by the activity of host-pathogen complex (Ghosh *et al* , 1966, Tondon, 1970)

Samborski and Shaw (1956), Daly *et al* (1961) and Srivastava and Tondon (1966) have reported that increased respiration under pathogenesis caused a rapid decline in ascorbic acid of the infected tissues It is known that L - ascorbic acid is easily oxidized to dehydro- L - ascorbic acid by the enzyme ascorbic acid oxidase or by certain oxidative enzymes like polyphenyl Oxidase, Cytochrome Oxidase, peroxidase etc , according to the following reaction

L-ascorbic acid $\xrightarrow[-2H]{\text{oxidase}}$ dehydro-L-ascorbic acid $\xrightarrow[+H_{20}]{}$ 2, 3 - di keto gluconic acid

An oxidative enzyme, specific for L-ascorbic acid, has also been demostrated by Mandels (1953) in the spores of *Myrothecium verrucaria.*

Mangiferin treated plant parts show low respiratory activity and high chlorophyll content (Chakrabarti *et al* , 1990) The reduction of ascorbic acid content

in diseased plant parts may be also due to the reduction in chllorophyll content and increased respiration (Siddaramaiah *et al*, 1979, Isherwood and Mapson, 1962) Thus the increase in chlorophyll content and decrease in respiration activity by mangiferin treatment may help in the enhancement of ascorbic acid content

Many investigators (Chahal and Grower, 1972, Patil and Kulkarni, 1977; Agrawal and Ghosh, 1979, Reddy *et al.*, 1980, Chaudhary *et al*, 1980; Prasad, 1980; Parmar *et al.*, 1983, Taneja *et al*, 1983a; Tulsi Raman and Sankaran, 1989) have indicated that the loss in ascorbic acid is more pronounced when leaves and fruits are infected with pathogen Therefore, it is evident from the present study that the ascorbic acid content in plant parts decreases following fungal infection

Peroxidase Activity: The peroxidase enzyme plays a key role in defence reactions. Plant peroxidases consist of a multiple isoenzyme which plays an integral role in the biosynthesis of cell wall components, including lignin, suberin and cross-linked extension (Grisebach, 1981, Greppin *et al*, 1986). Its role in cell wall thicking and lignification as plant's defence mechanism against pathogen is well known (Vance *et al*, 1980; Hammerschmidt and Kuc, 1982) Peroxidases have also been suggested to play a role in the induction of systematic resistance (Iriving and Kuc, 1990) as a defence mechanism of plant against pathogen (Flott *et al.*, 1989; Kebry and Somerville, 1992; Ludwing-Muller *et al*, 1994).

The results show that the peroxidase activity increases significantly in the diseased panicles, leaves and fruits of *M. indica.* Mangiferin treated plant parts show decreased peroxidase activity High peroxidase activity in the diseased tissues is considered to be a resistance mechanism following oxidation of phenolic compounds (Sempio *et al*, 1975) The increase in mangiferin concentration is inversely proportional to the activity of peroxidase (Chakrabarti *et al*, 1990)

Results of the present study evidently support the previous work correlating the increased peroxidase activity in various host-parasite combinations with the disease resistance The enhanced activity of this enzyne might result in augmented rate of oxidation of phenolic substances resulting in the formation of toxic quinones participating in the defence reaction of the host (Tomiyama, 1963; Fehraman and Diamond, 1967; Fric, 1976, Shekhawat and Arya, 1979; Purohit *et al* , 1980; Agrawal *et al* , 1982, Sakare and Thite, 1986, Gupta *et al* , 1992; Gupta *et al*., 1995, Alcazar *et al* , 1995)

Total Soluble Sugars: Carbohydrates are the main source of carbon, which consititute the first choice of phytopathogen Breakdown of these compounds is a regular feature of diseased host tissue The changes brought about by the pathogen significantly reduce the quality of fruits and render them nutritionally unfit for human consumption.

The present study indicates that healthy leaves, inflorescences and fruits of all varieties contained more sugars compared to diseased and mangiferin treated ones. The reduction in sugar content indicates its utilization by the pathogen for its growth and proliferation in the plant organs. (Mehta *et al* , 1975, Mondokhot *et al*., 1979; Kaur and Dhillon, 1989)

According to Goodman *et al*. (1967), Farkas and Kirally (1954) and shaw and Colotelo (1961), the carbohydrates may also be utilized to meet the energy requirement of host plant due to the increased respiratory activity. The reduction in sugar content also could be attributed to the fact that major part of these sugars are utilized by the pathogen for its growth or they are being shifted for the synthesis of polyphenol (Neish, 1964, Kaur and Dhillon, 1989)

Total Phenols: The metabolic changes occurring in disesed plant frequently lead to the accumulation of aromatic and phenolic compounds. Phenolic compounds and related

oxidative enzymes are mostly considered as one of the important biochemical parameter for disease resistance (Kosuge, 1969, Cruickshank and Perrin, 1964; Rohringer and Somborski, 1967). It is evident form the results obtained that the diseased and fungus treated plant parts of all varieties of *M. indica* exhibit higher accumulation of phenolic contents. Similar observations are also made by many workers (Parmar *et al*, 1983, Taneja *et al*, 1983, Thomson and Eribo, 1984; Sinha and Sinha, 1984; Prasada *et al*, 1987)

Accumulation of phenolic compounds following host-parasite reaction is considered as a general phenomenon of disease resistance in plants The rate of accumulation and break down of phenolic compounds determine the degree of resistance (Tomiyama, 1963, Kosuge, 1969) The phenolic concentration might increase by the release of phenols from their glucosides by B-glycosidase activity in either host or pathogen (Pridham, 1965) and / or enhanced synthesis by the host through shikmic acid pathway (Neigh, 1964).

Mangiferin Content: Mangiferin (1, 3, 6, 7 - tetra hydroxyxanthone - C_2 - β -D glucoside) is a natural metabolite of *M. indica* L It being phenolic in nature, acts as a defensive chemical (Ghosal *et al*, 1979, Sen, 1981) It is synthesized in leaves and remain stored in bark. Tender green leaves, diseased leaves and malformed inflorescence contain higher concentration of mangiferin. In healthy inflorescences either it is totally absent or present in a very low concentration (Ghosal *et al*, 1977). As soon as the pathogen invades the host a large amount of mangiferin is accumulated in the cortical cells surrouding the infected region and may prevent the pathogen to go deep in to the host cells due to its antifungal nature (Ghosal *et al*., 1977, 1979; Chakrabarti and Ghosal, 1989, Sen, 1981; Chakrabarti and Kumar, 1997)

In all varieties studied it is clearly evident that diseased leaves, inflorescences and fruits contain more amount of mangiferin as compared to the healthy ones Fungus and mangiferin treatment also enhanced the mangiferin accumulation in the organs which supports the work of Chakarbari *et al* (1990) and Kumar and Chakarbarti (1992) Higher concentration of mangiferin in deseased and fungus treated plant organs of *M. indica* proves the antifungal nature of this compound.

ANATOMICAL STUDIES

The structure of plant organs in relation to their resistance against pathogen has been reviewed by Royle (1976), Campbell *et al* (1980) and Akai and Fukutomi (1980) These studies indicate that structural modifications represent the resistance mechanism developed by the plant to defend itself against infection agent.

The presence of cuticle on epidermal layer of plant organs prevents the entry of parasitic fungi The outer epidermis is strengthened by thick cuticular layer acting as a chemical and physical barrier to the germination and penetration of fungi into the leaves (Bell, 1981). Thus the cuticle thickness has been correlated with the level of resistance to fungi (Wang and Pinckard 1973) The presence of relatively thick cuticle on the diseased leaves of *M. indica* supports the view of previous workers that cuticle acts as a barrier and helps in disease resistance.

The plant cell wall is undoubtedly a most important line of defence against invasive parasite (Albersheim, 1975; Pridham, 1974) The polysaccharides of most cell wall are acylated with hydroxy aromatic acids, especially ferulic acid. Acylation represents a primitive defence mechanism against pathogen and this phenomenon is also a forrunner of lignification (Hartley, 1973; Swain, 1977)

Cell with lignified or otherwise altered walls are the other phenomenon of resistance and in some cases the presence of such cells limits the area of spread of the

parasite (Brown, 1936) The thickness of wall of invaded cells or modification as a result of infection helps in disease resistance (Ride, 1978, Whitmore,1978, Asada *et al*, 1979) Lignification and other cell wall modification may decelerate fungal development and allowing phytoalexin to accumulate to effective levels Phytoalexins on the other hand, may retard fungal growth providing enough time for cell wall modification to become effective (Misaghi, 1982) Lignin-like polymers are synthesised in cell wall of young tomato fruits infected with *Botrytis cinerea* may be responsible for the restriction of fungal development to few epidermal cells after penetration (Glazner 1982). Relatively thick walled and lignified vessel elements and fibres are commonly found in diseased leaves and wood of *M. indica* L. This supports the view of previous workers that lignification is a important way of disease resistance (Grisebach, 1977, Ride, 1978, Asada *et al*., 1979, Vance *et al*., 1980)

In the present study occurrence of tyloses has been noticed in the vessels of diseased wood Tyloses are formed by the protrusion of cell wall of parenchyma cells through pits into the lumen of xylem vessels Once a tylosis is formed it may undergo secondary wall thickening Resistance of cotton to *Verticellium albo-atrum* is attributed to the reduced systematic spread of fungus in plants due to a rapid occlusion of xylem vessels by tyloses followed by the synthesis of fungitoxic terpenoid aldehyde. Phytoalexins are formed immediately after the tyloses formation and coat them, possibly offering protection from microbial attack (Mace, 1978) Without tyloses, the phytoalexins would be diluted in the xylem stream, probably making them ineffective. Thus the two components working together are needed for effective resistance (Bell, 1981). It is evident that the presence of tyloses in wood of mango may also helpful in providing resistance against *Botryodiplodia theobromae*

Infected leaves of *M.indica* showed narrow gum- resin ducts filled with darkly stained substances Earlier it was reported that resin ducts of *M.indica* contained mainly terpens, phenols, proteins and carbohydrates and the duct system may be an effective factor in defending certain mango fruits against the meditterrarean fruit fly (Bhatia *et al* , 1967; Ansari *et al* , 1967; Joel, 1980; Joel and Fahn, 1980). Therefore, it is evident from the present study that gum-resin ducts of infected leaves may also impart resistance against the fungal pathogen

HISTOCHEMICAL STUDIES

Protein content : Infection has a marked influence on the total protein accumulation in diseased leaves. Protein changes after infection are related to defence action of plants Infection induces production of abnormal metabolites in adjacent non-infected tissues followed by their accumulation in the infected region (Uritani, 1971) Diseased leaves of *M. indica* show more protein content as compared to the healthy ones Changes in protein metabolism in plant tissues during the initiation of pathogenic infection and course of disease development have been reviewed by Uritani (1976). Vidhyasekharan *et al* , (1973) and Parmar *et al* , (1983) reported an incerease in protein content of diseased rice and *Cichorium intybus* leaves infected with *Helminthosporium* and *Alternaria cichorii* respectively Similar observations are also made by many workers (Kaur and Dhillon, 1979, Patel and Vaishnav, 1986) Findings of Grzelinska (1969), Chattopadhyay and Bera (1978) and Upadhyay and Dwivedi (1979) also indicate that more postinfectional increase in protein level of succeptible varieties in comparison to resistance varieties

Accordings to Bhattacharya *et al* (1965), *Puccinia graminis tritici* increases total nucleoprotein after inoculation in wheat seedlings. More intensity of protein in hypertrophied inflorescence axis of *Brassica juncea* due to *Albugo candida* was observed by Maheshwari *et al* (1985)

An increase in protein content following infection may also be due to super imposing of pathogen protein fraction on host protein or due to their role in cell protection mechanism (Von-Broembsen and Hadwiger, 1972) Increase in protein content may also be due to the synthesis of new protein or modification of the existing protein by the pathogen according to its need (Taneja *et al*, 1980 , Parmar *et al.*, 1980; Parmar *et al* ,1983) Thus the increase in protein content in diseased leaves of *M. indica* indicates its role in disease resistance mechanism

Starch content: Diseased leaves and wood of *M indica* showed less amount of starch as compared to the healthy ones Changes in starch content following infection have been observed in many plants The general pattern is an initial decrease of starch content followed by a marked increase with heavy accumulation around the margin of lesions There after the polysaccharide content again declines

It appears that polysaccharide accumulation was associated with disease development and it acts as a carbon source of fungi Wang (1961) has shown in the case of bean rust, that the regions termed green islands at the edge of the infection, are photosynthetically active and starch tends to accumulate in them to a greater extent than in the surrounding areas infected by *Uromyces phaseoli*, while Schipper and Mirocha (1968a) showed the depletion of starch in the same infected tissues

According to Vidyasekaran and Kandaswamy (1972), the powdery mildew (*Oidium* sp) infected tissues of *Phaseolus aureus* showed a severe reduction in both starch and sugar contents Schipper and mirocha (1969b) explained that this depletion may be due to the activation of starch hydrolysing amylase in infected tissues Absence or less amount of starch grains in deseased plant suggest the inhibition of its synthesis as well as degradation of starch and consumption by the fungus Starch is consumed due to increased host metabolism to cater the need for growth and proliferation of the pathogen (Howel and Krusberge, 1957) Findings of Aulakh and Sandhu (1970), Padmanabhan (1973) and Kaur and Dhillon (1989) also reveal the same view.

The carbohydrate synthesis has also been correlated with the quantity of chlorophyll (Hopkins and Hampton, 1969) The decrease in chlorophyll content in infected leaves may be due to toxic metabolites relased by the pathogen which may inhibit chlorophyll synthesis (Pero and Main, 1970) or by the action of chlorophyllase which caused breakdown of chlorophyll molecules (Goodman *et al*, 1967) The increased respiration together with decreased photosynthesis eventualy lead to the depletion of host sugar (Patil and Kulkarni, 1977, Agrawal *et al.*, 1982, Parmar *et al.*, 1983; Kaur and Dhillon, 1989). Therefore a decline in intensity of polysaccharides in tissues of diseased leaves and wood of *M. indica* probably due to the factors mentioned above.

Phenolic contents: High accumulation of phenolic contents in infected leaves and wood has been observed Accumulation of phenolic compound in host-parasite reaction is a general phenomenon of disease resistance (Farkas and Kiraly, 1962, Tomiyama, 1963, Kosuge, 1969)

The post infectional increase in phenolic content could be due to enhancement of synthesis, translocation of phenolics to the site of infection and hydrolysis of phenolic glycosides by fungal glycosides to yield free phenols (Pridham, 1965) or enhanced synthesis of phenolics by the host-through sikmic acid pathway (Neish, 1964) Similar views are expressed by many earlier workers (Vir and Grewal, 1974; Agrawal *et al.*, 1982; Parmar *et al.*, 1983; Sharma *et al* , 1983, Thomboson and Eribo, 1984). The consistant accumulation of phenolic contents at the site of infection in diseased organs of *M. indica* also supports the views of earlier workers