VI. DISCUSSION

The fundamental basis of pathogenicity in microorganisms and resistance in plants is awaiting solution and gradually becoming more complex day by day (Kuc and Preisig, 1984). However one of the important areas of research on host-parasite interactions in plant pathology is contemplated to the pathophysiological studies involving changes in host's metabolism brought about by the pathogen. The leaf-spot symptoms in five cultivars of *P. betle* viz. Kali, Dhal, Mitha-Bangla, Sanchi and Mitha are well characterised with the development of lesion and its gradual spreading caused by *Fusarium scirpi* except in cv. Mitha-Bangla where it is found to be almost confined to in and around the infection site. An attempt has been made to understand the underlying mechanisms of this two type of reactions and also an attempt has been made to find out any possible biological control method of the disease as the people are used to chew the betle leaves in fresh green condition and some salient features out of the experimental findings are discussed here.

The experimental data on the electrolytic leakage clearly show that it starts after 6 hrs of inoculation and increases upto 24 hrs after inoculation. The maximum electrolytic leakage is observed in cv. Dhal and the minimum in cv. Mitha-Bangla of *P. betle*. This is possibly due to damage of cell membrane and/or increase in permeability of plasma membrane due to some changes brought about in structural constituents by the pathogen.
Several experimental findings have so far revealed some positive role of pectin-degrading enzymes of pathogenic origin in disease development caused by different species of *Fusarium* in host plants (Gothoskar *et al.*, 1955; Winstead and Walker, 1954). The production of PME and Endo-PG in culture filtrate and increased production of these two enzymes in the infected host leaf tissues in all the more susceptible cultivars (viz. Mitha, Sanchi, Kali and Dhal) and less increased production in the cv. Mitha-Bangla (less susceptible cultivar) (Tables 5-14) and subsequent changes in the concentrations of pectic substances and calcium in the respective leaf tissues of different cultivars (Tables 3, 4) indicate the involvement of these two enzymes in the present disease symptom development. These findings support the earlier similar observations made by Deese and Stahman (1962 a, b), Patil and Dimond (1968), Jones *et al.* (1972), and others.

The experimental findings on the phenol metabolism reveal that the amount of free phenols, free phenolic acids and bound phenolic substances are high in the infected leaf tissues of all the cultivars of *P. betle* than the healthy ones (Tables 16-18). Cardoso and Garraway (1971), Dimond (1970), Matta *et al.* (1969, a & b), Kuc *et al.* (1956), Muller and Behr (1949), Schaal and Johnson (1955) and Uritani and Akazawa (1959) have also reported similar accumulation of phenolic substances and their oxidised products and the characteristic symptoms (browning) in *Fusarium*.
infected plant tissues and in other plant diseases. A possible correlation between the accumulation of phenolic substances and resistance reaction in host plants has been reported by Matta and Dimond (1963). It has been stated that both the enzymes peroxidases and phenoloxidases are capable of oxidising phenolic substances within plant tissues. Bhaskaran and Prasad (1971), Kedar (1959), Hislop and Stahman (1971), Simons and Ross (1971), Stahmann et al. (1968), Grzelinska (1969), Matta and Dimond (1963) and Solomosy et al. (1959), Jennis and Kuc (1979), Stoessl (1982), Hammerschmidt and Kuc (1980), Hammerschmidt et al. (1982) have demonstrated a correlation between the PO and PPO activities and resistance mechanism of host plants in several plant diseases including *Fusarium*-infected plants. In the present investigation it has been found that PPO and PO activities increase appreciably in the infected leaf tissues of all the cultivars of *P. betle* infected with *F. scirpi* and the increase is more in cv. *Mitha-Bangla* in comparison to other four cultivars. Moreover *F. scirpi* is possibly able to produce β-glucosidase enzyme which releases the bound phenolic compounds of the host and thereby increasing the phenol fraction after infection. Retig (1974), Hammerschmidt and Kuc (1980) and Hammerschmidt et al. (1982) have stated that PO and PPO increase in induced resistant mechanisms of plant diseases. All these observations indicate the possible involvement of PPO and PO enzymes in this disease development.

The increase in phenol contents in the induced resistant *P. betle* cv. *Mitha-Bangla* by inoculating with nonpathogenic
species of Fusarium e.g. F. *udum* and F. *oxysporum* supports the findings of Matta *et al.* (1969). It has also been found that *F. betle* cv. Mitha-Bangla plants fail to respond to the non-pathogenic species in presence of the pathogenic one i.e. *F. scirpi* during mixed inoculation study. During mixed inoculation study, the phenol content of the infected leaf tissue is found to be lower than the individual inoculation cases. This finding supports the view of Davis (1967), Johnson and Schaal (1957), Paxton and Chamberlain (1967) and Phillips *et al.* (1967).

It has been also observed that *F. betle* cv. Mitha-Bangla cell wall protein and phenols, of the preinoculated plants with nonpathogenic species, *F. udum* and *F. oxysporum*, have more pronounced inhibitory effect on the PME and Endo-PG activities of the pathogenic species *F. scirpi*. This inhibitory role has helped the *F. betle* cv. Mitha-Bangla plant to acquire more resistance against the pathogenic species, *F. scirpi* in comparison to other four cultivars. Similar observations have been reported by Byrde *et al.* (1960), Williams (1963), Hunter (1974), Keen and Long (1972), Keen *et al.* (1972) and *Jones, Anderson* and Albersheim (1972).

It has been found in this investigation that some of the saprophytic microorganisms isolated from the leaf surfaces of *F. betle* cv. Mitha-Bangla are antagonistic to the pathogen *F. scirpi* in *vitro* as well as in *vivo*. The data suggest that the saprophytes which are antagonistic to pathogen are widespread in occurrence and the growing season tested (Table 34).
In order to understand the mechanism of antagonism, it has been found that culture filtrates of *Cladosporium oxysporum*, *Aspergillus terreus*, *A. flavus*, *A. oryzae*, *Pseudomonas* sp. and *Streptomyces* sp. could inhibit the growth of the pathogen (Table 35). Of these, the culture filtrate of *Streptomyces* sp. fail to inhibit the growth of *F. scirpi* in cup assay test (Table 36). This suggests that all the above mentioned microorganisms except *Streptomyces* sp. produced some metabolites in the culture filtrates which is inhibitory for growth of *F. scirpi*. The results are in confirmation of previous work (Hsu and Lockwood, 1969). In case of *Streptomyces* sp. it is possible that nutrient competition on the surface is responsible for the inhibition of *F. scirpi* in PDA agar plate culture test. Similar results here have been obtained by Hsu and Lockwood (1969).

The experimental findings of in vivo experiments indicate the possibility of biocontrol of *F. scirpi* by saprophytes like *Cladosporium oxysporum*, *A. terreus* and *Pseudomonas* sp. In each case the simultaneous application of saprophytes and the pathogen has yielded significant decrease in disease severity in *P. betle* cv. Mitha-Bangla as compared to plants inoculated with the pathogen alone (Tables 38-40). It has also been found that the cell free culture filtrate of all the above mentioned saprophytes when applied simultaneously with the spore of the pathogen have significantly reduced the disease severity in *P. betle* cv. Mitha-Bangla plants caused by *F. scirpi* (Tables 38-40). Moreover any
phytotoxic effects of the culture filtrates of the above-mentioned saprophytic microorganism have not been found in betel plants. Similar results have also been reported by Leben (1965), Barnes (1971), Blakeman and Brodie (1976), Fokkema (1976) and Skidmore and Dickinson (1976). The data of the in vivo experiments are based on greenhouse grown plants, as such it needs field verification by conducting experiments under field conditions in different agro-climatic zones of West Bengal for suggesting this method of biocontrol of F. scirpi under field conditions.

It has also been found that nonpathogenic species of Fusarium, i.e., F. udum and F. oxysporum are able to protect the P. betle cv. Mitha-Bangla plants from the infection by F. scirpi, the pathogenic one (Tables 31-33) by bringing changes in the protein and phenolic compounds of the host leaf cell walls which have been found to be inhibitory to the PME and Endo-PG activities of the pathogen i.e., F. scirpi. Similar findings on other diseases by immunizing the host plants by infection with viruses, bacteria and fungi for controlling the diseases caused by viral, bacterial and fungal pathogens have already been reported by several authors (Caruso and Kuc, 1977a; 1977b; Gessler and Kuc, 1982; Hammerschmidt et al. 1976; Jenrs et al. 1979; Jenrs and Kuc, 1977, 1979; 1980; Kuc, 1981, 1982a, 1982b, 1982c; Kuc and Caruso, 1977; Kuc et al. 1975; Staub and Kuc, 1980; Cohen and Kuc, 1981; Kuc and Tuzun, 1983; Tuzun and Kuc, 1983; Kuc and Preisig, 1984). However, the mechanism of immunization in this case needs further elaborate study.
However from the evidence of the present investigation, it can be assumed that PME and Endo-PG enzymes of *F. scirpi* are involved in the leaf spot disease development of five cultivars of *P. betle*. The less susceptibility of *P. betle* cv. *Mitha-Bangla* to *F. scirpi* is possibly due to oxidation of phenols by PPO and PO of *F. scirpi* and might also be due to release of bound phenolic substances of host by β-glucosidase activity of the pathogen. These phenolic substances have been found to be inhibitory for PME and Endo-PG activities of *F. scirpi*. It might be also due to protein of cell wall of leaf tissues of *P. betle* cv. *Mitha-Bangla* which has also been found to be inhibitory for PME and Endo-PG activities of *F. scirpi*. It has been further found that saprophytic microorganisms of leaf surface eg. *Cladosporium oxysporum*, *Aspergillus terreus* and *Pseudomonas* sp. are able to check the growth and germination of species of *F. scirpi*. So there is a possibility of biocontrol of the disease either by saprophytic microorganisms or by immunization by non-pathogenic forms but mechanisms of the steps are yet to be revealed. Further research works are in progress in these lines of the disease aspect.