DISCUSSION
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In the foregoing chapter of experimental findings, observations on morphological, cultural, physical, nutritional and biochemical characteristics of Xanthomonas campestris pv citri are presented. Also, the interaction of Xanthomonas campestris pv citri with citrus plants by taking different parameters were studied. The post infectional changes in the major biochemical components of leaves and fruits and histological changes in the plant parts due to interaction with bacteria were studied. Influence of surface microflora on growth of causal bacteria and their interaction were also studied.

It was evident from the survey conducted in the vicinity of Raipur city on available citrus species for canker disease, that the canker was more severe on acid lime (Citrus aurantifolia) plants or in other words, acid lime plants were found to be most susceptible for infection of bacteria and subsequent canker disease development. This result is in confirmity of the findings of Govinda Rao, (1954) and Aiyappa, (1958). Also, according to reports of All India Coordinated Project on Citrus Improvement (Anon., 1993) and Kumar, (1992) the canker is a serious problem wherever acid lime is grown on commercial scale or even a single plant.

The cells of isolates C₁, C₂, C₃ and C₄ (isolated from Citrus aurantifolia, Citrus limon, Citrus reticulata and
Citrus paradisi, respectively) were Gram negative, rod shaped with monotrichus polar flagellum. These characters were defined by the Society of American Bacteriologist (1957) in their manual and Bergey's Manual of Systematic Bacteriology (1984) for typical Xanthomonas campestris. Chohan and Knorr (1970) also reported that the bacterium Xanthomonas citri is rod shaped, motile and Gram negative. Kumar (1992) further confirmed that the bacterium is Gram negative having yellow pigmented rods and is motile by means of single polar flagellum.

In the Manual of Society of American Bacteriologist (1957) and Bergey's Manual of Systematic Bacteriology (1984), the colony characters of genus Xanthomonas described as circular form, convex elevation entire margin, viscid consistency, yellow pigment, smooth surface and glistening optical characters on agar plate. Similar characters were recorded in all four isolates during present investigation. The growth character on agar slant and Gelatin (stab) of all the isolates were tallied with the characters described by Society of American Bacteriologist (1957) and Bergey's Manual of Systematic Bacteriology (1984) for genus Xanthomonas.

According to Dye (1962) the genus Xanthomonas has got a wide range of temperature for its growth. Temperature below 5°C and above 40°C, do not allow the growth of bacteria. In the present study, at the temperatures of 5 and 40°C, no growth was recorded in any isolate. All the isolates could grow between 10-35°C. Maximum growth was observed at 30°C.
followed by 25°C. The influence of temperature on the growth of isolates revealed that the isolate C3 could not show any growth at 10°C while isolate C1 was appeared as faster growing at 30°C as compared to other isolates. Similar trend was also observed at 25°C. Several workers also reported the optimum temperature for the growth of *Xanthomonas oryzae* as 25-30°C (Sulaiman and Ahmed, 1965; Sulaiman et al., 1965; Chakravarti and Rangarajan, 1967; Shekhawat and Srivastava, 1968 and Dileep Kumar, 1971). Kore and Phad (1988) reported that *Xanthomonas campestris pv oryzae* had maximum growth at 29°C.

All the isolates could grow between pH 5.5 to 8.5. The maximum growth was recorded at pH 7.0 and no growth was recorded at pH 5.0 and 9.0 in all isolates. The influence of different pH levels on growth of isolate C1 is comparatively less than the other isolates while isolate C4 showed comparatively higher reduction in the growth at pH 7.5 and above as compared to other isolates. Mizuta (1953) reported that the growth of *Xanthomonas oryzae* was best at pH range of 4.0-8.0. Mizukami and Murayama (1959) and Mizukami (1961) found pH 6.8 was optimum whereas Devadath (1969) reported the best growth between pH 6.5-7.5. The growth of *Xanthomonas campestris pv oryzae* was best at pH 6.5 (Kore and Phad, 1988).

In antibiotic sensitivity test of different isolates, it was observed that at 100 ppm, no antibiotic treatment
showed inhibition zone. Sinha (1989) found that Plantomycin at 100 ppm was effective against Xanthomonas campestris pv cucurbitae followed by Paushamycin and Streptocycline. Streptocycline at 500 ppm appeared as best retardent of growth of all isolates as compared to other antibiotics tested but the inhibition of growth vary among isolates. Several workers also reported the effectiveness of Streptocycline against various species and pathovars of genus Xanthomonas (Rangaswami, 1959; Sangam Lal et al., 1970; Kore and Dahiwal, 1980; Chakravarti et al., 1980; Kishun et al., 1981; Jindal and Madhumeeta, 1988; Kore and More, 1989; Parashar et al., 1992; Mishra and Om Prakash, 1992; Kore and Dhutraj, 1992; Chourasia, 1994).


In the present study, none of the bacterial isolates was found sensitive to Penicillin as no inhibition zone was
produced in any isolate. Since, only Gram positive bacteria were reported sensitive to Penicillin (Misra, 1980).

Studies on effect of different growth regulators on growth of isolates, clearly showed that all the growth regulators tested were found to be inhibitory of growth of all isolates in comparison to control. Among them Maleic Hydrazide appeared as best retardent followed by Indole Acetic Acid, Indole Butyric Acid, 2, 4-Dichlorophenoxy acetic acid, Gibberelic Acid, and Naphthalene Acetic Acid. The inhibitory property of Indole Butyric Acid and 2, 4-Dichlorophenoxy acetic acid was reported by Hiremath et al., (1973) and that of Indole Acetic Acid and Indole Butyric Acid was also reported by Pandurangarao and Hiremath (1986) against the growth of Xanthomonas campestris pv vignicola but Cycocel, Gibberelic Acid and Indole Acetic Acid (Hiremath et al., 1973) and Gibberelic Acid and Naphthalene Acetic Acid, Cycocel and 2, 4-Dichlorophenoxy acetic acid were found to favour the growth of bacteria.

All the isolates differ in their growth depending upon the kind of carbon sources used in the medium. Sucrose as carbon source appeared best, among the eight sources tested. Sucrose is the major component in the selective medium of Xanthomonas campestris pv citri given by Canteros De Echenique et al., (1985). Therefore, it is confirmed from the present investigation, that Sucrose is the major and essential component for the growth of Xanthomonas campestris.
pv citri. The isolates differed in the utilization of carbon sources showing variation in the amount of growth produced on particular carbon sources. This might be due to differences in the physiology of isolates. Similarly, isolates of Xanthomonas oryzae also showed differences in the utilization of different carbon sources as reported by several workers (Watanabe, 1963; Goto, 1964; Muko and Ishaka, 1964; Sulaiman and Ahmed, 1965; Sulaiman et al., 1965; Chattopadhyay and Mukherjee, 1968; Mahmood and Singh, 1970; Dileep Kumar, 1971 and Misra, 1993).

The results of utilization of nitrogen sources clearly indicate that not only the differences in the amount of growth among nitrogen sources, but also among different isolates. None of the nitrogen sources tested could produce significantly higher growth of any isolates over control. Least amount of growth was produced by Thymine nitrogen source which was significantly inferior to all other nitrogen sources. The differences in the amount of growth of different isolates might be due to differences in the physiology of the isolates. The average growth of isolates in Asparagine nitrogen sources was more than other sources, but Deepa Ganesh et al., (1994) reported that isolates of Xanthomonas campestris pv citri failed to grow on Dye's medium C with Asparagine as sole nitrogen source. The result in the table reveal clearly that the isolates differed in the utilization. Similar results have been reported by several workers with the isolates of Xanthomonas oryzae (Tanaka, 1963; Watanabe,
Among the trace elements, Magnesium was found to be the best trace element, as it supported the growth of all isolates significantly over other treatments including control. In the composition of selective medium for cultivation of *Xanthomonas campestris pv citri* given by Canteros De Echenique *et al.* (1985), Magnesium is one of the important component. This confirm that Magnesium is essential for the growth and multiplication of causal bacteria. All the other trace elements showed inhibitory effect and the inhibition was varied among them. Moreover, all the treatments except Magnesium showed lower growth than control. Copper appeared as most inhibitory for the growth of all isolates. It is probably one of the reason for use of Copper oxychloride fungicides in combination with Streptocycline to control the canker as well as other disease caused by genus *Xanthomonas* as reported by several workers (Jindal and Modhumeeta, 1988; Patil and Mehta, 1989; Thombre *et al.*, 1989; Jindal, 1990; Khetmals *et al.*, 1992; Kore *et al.*, 1992; Holev *et al.*, 1993; Andhale and Raijadhav, 1994; Kale *et al.*, 1994 and Chakravarti *et al.*, 1995). Some other workers also reported the another copper based primitive fungicide, Bordeaux Mixture was effective against canker pathogen (Fawcett, 1936; Naik, 1949; Cheema *et al.*, 1954; Ramakrishnan, 1954; Govinda Rao, 1954; Prasad, 1959 and Paracer, 1961). The growth inhibitory property of copper was also reported by
Watanabe (1963) and Misra (1993) working with Xanthomonas oryzae pv oryzae. Different trace elements were found to be essential for the growth of other species of Xanthomonas and also for different isolates of a particular pathogen. Watanabe (1961) obtained maximum growth with Ferrous (Iron) as the trace element. Watanabe (1963) found that addition of Ferrous (Iron), Magnesium and Manganese markedly increase the growth of Xanthomonas oryzae. Misra (1993) reported that Boron was found to be best trace element for the growth of Xanthomonas oryzae followed by Ferrous (Iron) and Zinc.

All the isolates differed among each other for gelatin liquefaction, hydrogen sulphide production, cellulase production, nitrate reduction and starch hydrolysis, however, by showed similar reaction in litmus milk and fermentation carbohydrates. Therefore, the present investigation licated the existence of variation in the biochemical characters of the tested isolates. Rangaswami and Soumini Lagopalan (1957) observed certain differences in the chemical properties among 10 isolates of Xanthomonas citri and considered the possibility of existence of biochemical varieties in the pathogen. Masao Goto (1969) also differentiated 300 isolates of Xanthomonas citri into 5 groups by their ability to oxidise Mannitol and Lactose, and rapidity of breakdown of Mannose. Khan and Hingorani (1970) collected 15 isolates and classified these isolates into six groups on the basis of their capacity to liquefy starch and into these groups depending upon the starch
hydrolysis. Buragohain et al., (1991) distinguished isolated of *Xanthomonas campestris* pv *citri* into four groups on the basis of biochemical characters. On the contrary, Vasudeva (1958) did not notice any variation in the biochemical properties of 13 isolates of *Xanthomonas citri*. Similarly, Rao and Hingorani (1963) reported that isolates of *Xanthomonas citri* can not be delimited into different strains on the basis of their biochemical characters.

All the isolates were able to produce acid from fermentation of carbohydrates in the present study. This result is corroborating with findings of Deepa Ganesh et al., (1994) that isolates of *Xanthomonas campestris* pv *citri* produced acid by fermentation of sugars in Dye's medium C.

Since, 'biochemical tests are important parameters in distinguishing the isolates from each other of a particular pathogen, several workers had reported differences in isolates for the biochemical properties of *Xanthomonas oryzae* (Schure, 1953; Dye, 1962; Goto, 1964; Muko and Isaka, 1964; Sulaiman and Ahmed, 1965; Sulaiman et al., 1965; Reddy, 1966; Goto and Okabe, 1967; Chakravarti and Rangarajan, 1967; Shekhawat and Srivastava, 1968; Devadath, 1969 and Dileep Kumar, 1971).

It is evident from the study conducted to know the pathogenic behaviour of all four isolates by inoculating them on their parent species as well as on other species also, that isolate C1 appears as most virulent and isolate C4 as
least virulent while isolate C_2 and C_3 appeared as moderately virulent. This study establishes the variation in all four isolates in confirmation of variations in the physical, nutritional and biochemical characteristics. Rangaswami and Rajagopalan (1957) reported that all the ten isolates of *Xanthomonas citri* infected the fruits and leaves of acid lime, sathgudi, nepali lemon and jambhiri. On sathgudi and jambhiri, the isolates were comparatively less virulent than other citrus species. Khan and Hingorani (1970) collected 15 isolates and separated them into three groups of strains like severe, mild and avirulent by testing them on *Murraya exotica*. Similarly, Buragohain et al., (1991) classified the isolates into four distinct groups based on virulence reaction on seven citrus cultivars and all the four groups were designated as four biological forms of *Xanthomonas campestris pv citri*.

The study on comparative efficacy of different methods of inoculation on disease development indicates that insect injury was found to be best as it produces highest disease severity followed by pin prick, rub and spray methods. The disease severity in insect injury and pin prick method did not differ significantly. Similarly, several workers also reported that citrus leaf minor (*Phyllocnistis citrella* and *Throscoryssa citri*) helps in dissemination and infection of citrus canker because injury to the leaf epidermis made by the burrowing of leaf minor serves as an easy opening for entrance to the bacteria (Hayes, 1957; Mundkar, 1961; Chohan
and Knorr, 1970; Nirvan, 1961; Gottwald and Garsney 1990 and Venkateswarlu and Ramapandu, 1992). The efficacy of pin prick method was also established by some other workers during the investigation on host pathogen interaction (Matsumoto and Okudai, 1988; Ramesh Chandra and Ram Kishun, 1991; Kore and Komble, 1992; Reddy and Murti, 1990 and Shukla et al., 1995). By this study it is confirmed that entry of bacteria in the host is passive as they required insect or mechanical injuries for the entrance. The surface injuries caused by winds and spines of host plant could be minimized by planting the suitable wind breaks around the citrus orchard across the direction of winds.

The results on influence of age of the inoculum on disease development indicate that the young age inoculum (bacterial culture) viz, 1, 2 and 3 days old, produced higher disease severity than old age culture. With the increase in the age of inoculum a decreasing trend in the disease severity was observed. Similar results were also reported by Verma and Singh (1987) while studying the interaction between *Xanthomonas campestris pv malvacearum* and cotton plants. The reduction in the disease severity with the increase in age of the inoculum might be due to reduction in active and viable bacterial cells. The reduction in active and viable cell in the old age cultures is a common phenomenon in case of bacterial plant pathogens because of the secretions from the bacterial cells in the form of extracellular polysaccharides which oftenly get deposited around the bacterial cell and
finally ceases the activity of that particular cell. Some times harmful by products are produced in the process of metabolism and catabolism which gradually increase in quantity in old cultures and probably became a major cause in reducing the number of viable cells.

The inoculum concentration of 1.0 and 0.5 optical densities produced higher disease severity than lower inoculum concentration. A decreasing trend in the disease severity was observed as the inoculum was diluted further and at 0.01, 0.05, 0.005 and 0.001 optical density, no disease symptoms were produced as in case of sterile water (control). Verma and Singh (1987) also found that when the inoculum of Xanthomonas campestris pv malvacearum was diluted, the symptom did not appear at $10^2$ c.f.u./ml onwards. The reduction in the disease severity with the increase in dilution of inoculum concentration might be due to non-availability of required number of cells for successful infection. The population of infection propagules in a particular number is essential for successful host-pathogen interaction because the number of cells present in the inoculum must be in a position to overcome the physical as well as biochemical defence mechanism of the host plant. In the process of interaction the loss in the number of bacterial cells is very high, it might be due to unfavourable environmental conditions as well as competition for nutrition which makes strongest to survive and cause infection. Therefore, when a particular population of inoculum is not
available, the number of cells present in the inoculum concentration are not enough to cause infection and subsequent disease symptoms expression.

Among the three bearing seasons of the citrus plants, Mrig bahar appeared to be most favourable season for disease development than Hasta Bahar and Ambe Bahar. This corroborates with the findings of Co-ordinated project workers (Anon., 1993).

The possible reasons for the higher intensity of canker on Mrig bahar flush might be due to favourable environmental conditions and seasonal variation in the nutritional status of flushes.

Ramakrishnan (1954) reported that the disease spread was favoured by mild temperature (20-25°C), high humidity, wet weather and presence of moisture on the host surface. A positive correlation exists between rain and disease incidence. Since, Mrig bahar flush appears in the first fortnight of June month, this period coincides with the onset of mansoon, which provides mild temperature and high humidity which are quite favourable for disease development.

Bose and Mitra (1989) reported that starch content was maximum in bearing shoots of Mrig bahar flush and decreased with the advancing season. The Mrig bahar flush has the maximum reducing sugar content while non-reducing sugar content showed similar trend. They also observed that the total sugars were highest in Mrig bahar flush and least in
Ambe bahar while it was intermediate in Hasta bahar.

On the basis of differences in the nutritional status of plants in different flushes, it appears that the availability of nutrients in Mrig bahar flush is more, favourable for the growth and development of the pathogen resulting in higher disease severity.

The relative susceptibility of first, second, third, fourth, fifth, sixth and seventh leaf of a twig from the top were studied and results indicate that first leaf from the top of twig was found to be most susceptible than other leaves. A decreasing trend in disease severity was observed in the lower leaves of the twigs. Similar observations have been reported by several workers while studying with interaction between various species of Xanthomonas and their hosts (Chand et al., 1970; Kore and Dahiwal, 1980; Jones et al., 1985; Kore and More, 1989; Wasnikar and Nayak, 1989; Graham et al., 1992; Reddy and Murti, 1990; Singh and Jain, 1995).

For the susceptibility to younger tissues, probably different factors are responsible. Analysis of leaves at all the positions on twigs revealed that the moisture content and nitrogen content of first leaf from the top were higher and amount of surface wax was lower than the leaves at other positions. The decreasing trend in the moisture and nitrogen content and increasing trend in amount of surface wax was observed in the lower leaves of the twigs. It is a well
established fact that moisture is essential for the movement of bacteria with the help of flagellum. In the present investigation, it is found that test bacteria having monotrichus polar flagellum which helps them to move from one place to another in the film of water, which is available in more quantity in the first leaf as compared to other leaves. Since, nitrogen is increasing the permeability of host cell walls which facilitates the availability of water and nutrients from the host cells to the bacterial cells. The excess water and nutrients helps in the growth and rapid multiplication of bacteria. This might be one of the reason for greater susceptibility of first leaves than other leaves.

The surface wax is known for providing resistance in many crop plants against pathogenic entities. A thin film of wax acts as a barrier between film of water and host surface. This wax film prevents the bacterial cells to enter in the host tissue, though enough moisture is available for movement. In the present study, analysis of leaves revealed that the amount of surface wax was less on first leaf than other leaves. Therefore, the entry of bacterial cells could not be prevented to that extent as in case of lower leaves and finally more disease severity produced.

Shukla et al., (1975) reported that high stomatal frequency, higher nitrogen content and higher moisture content of young leaves of sesame may be responsible for their greater susceptibility to Xanthomonas sesami. Rani and
Parashar (1981) also found that nitrogen content was higher in leaves of susceptible cultivars of *Vigna radiata* while less nitrogen content in moderately resistant and resistant cultivars to *Xanthomonas campestris* pv *phaseoli*.

The study on influence of different developmental stages of the fruits revealed that the full size and ripe fruit stages were most vulnerable while 1/3 and 3/4 of full size stages showed moderate reaction. The pea sized fruit stage showed no disease reaction. Rossetti et al., (1982) and Danos et al., (1984) reported that developing fruits (3/4 of full size and full size) of citrus were more vulnerable to *Xanthomonas campestris* pv *citri*. The findings of Graham et al., (1992) is in agreement with the present findings, that an aggressive strain of *Xanthomonas campestris* pv *citri* readily infect the developed fruits of citrus whereas fruits of smaller diameter were not readily infected. Generally there is a increase in respiration rate in ripe fruits, which result in breaking of complex compounds in to simpler forms. This breakdown of complex compounds might facilitate the availability of nutrients in simpler forms which could be utilized by bacteria for their growth and multiplication and finally more disease results.

The post infectional changes in the biochemical constituents of infected leaves and fruits are conspicuous due to infection of *Xanthomonas campestris* pv *citri*. Reduction in reducing and non-reducing sugars, Chlorophyll 'a' and 'b', total Chlorophyll, total phenols and protein
contents were recorded in infected leaves as compared to healthy leaves. In the infected fruits reduction in reducing and non-reducing sugars, Ascorbic acid, total soluble solids and juice content and increase in protein content and total acidity were recorded.

The reduction in the reducing and non-reducing sugars, Chlorophyll 'a' and 'b', total chlorophyll and total phenols of leaves were reported by several workers while studying the interaction between *Xanthomonas campestris* pv *citri* and citrus plants as well as with other species of genus *Xanthomonas* and their host (Padmanabhan et al., 1974; Prasad and Lal, 1977; Ram, 1979; Thind et al., 1981; Kumar, 1983; Khatri et al., 1983; Nema, 1989 and 1991 and Khare and Shukla, 1993).

The findings of Vidhyasekaran and Durairaj (1971) is corroborating with the findings of present investigation on post-infectional changes in the fruits infected with *Xanthomonas campestris* pv *citri*. The reduction in total sugars, reducing and non-reducing sugars and vitamin C (Ascorbic acid) content was also reported by Kumar and Prasad (1994).

There are differences in the views of various workers on the post-infectional changes in the host plant parts. Yarwood and Jacobson (1955) attributed that sugars may be transported to the infection sites from non-infected sites due to metabolic sink which may result from increased
metabolic activity at infection site leading to reduction in sugar content. Vidhyasekaran and Durairaj (1971) and Mehta et al., (1975) opined that reduction in the sugar content because of utilization of sugar by the bacteria for their growth and development. Padmanabhan et al., (1974) suggested that the reduction in the total sugar content in the infected leaf tissues may be due to impairment of photosynthetic activity. According to Kosuge (1978) it was also possible that reduction in reducing sugar may be due to increased activity of oxidative enzymes of Pentose-phosphate pathway. Prasad (1986) opined that the fall in sugar content was obviously due to breakdown of sugars by enzymes which increase the rate of respiration or utilization by pathogen. Nema (1989) attributed that reduction in sugars during disease development may be due to utilization of sugars probably for energy and synthetic reactions involved in the multiplication of bacteria.

Therefore, it appear that the reduction in sugar contents in infected plant parts may be due to utilization of sugars by the bacteria for its growth and multiplication. The reduction in Chlorophyll content in the infected leaves might be due to proteolysis which degenerate the chloroplast as suggested by Meyer et al., (1960) and proteolytic enzymes which helps in proteolysis may be produced by bacteria during interaction with the host (Keen et al., 1967). Doby (1965) suggested that a reduction in protein nitrogen content after infection may decrease the synthesis of Chlorophyll.
Similarly, Padmanabhan et al., (1974) also reported the reduction in protein nitrogen content in both canker and hole region and more in the canker lesions. Nayudu and Walkar (1961) suggested that Chlorophyll loss may be due to metabolic disruption rather than direct destruction.

The reduction in the quantity of phenols in diseased plants may be due to the reduced quantity of sugars available for the phenol synthesis (Raghunathan et al., 1966). Mehta et al., (1975) attributed the reduction in phenolic compounds may be due to the assimilation or degradation of this compound during pathogenesis. Nema (1989) suggested that the infected host was unable to manufacture phenolic compounds in the presence of pathogen, thus reduction in phenol content occurs.

The details of the physiological functions of Ascorbic acid are not well understood so far but it was believed that Ascorbic acid can be easily oxidized to Dehydro-L Ascorbic acid by different oxidative enzymes as suggested by Fruton and Simmonds (1958). Singh (1986) attributed that Ascorbic acid degenerating enzymes were produced by either pathogen or host-pathogen interaction. Abery (1958) opined that synthesis of Ascorbic acid depends upon the quantity of sugar precursors and the intensity of respiratory activity. In the present study, the depletions in amount of sugars in the infected fruits might be due to its utilization by bacteria for its growth and multiplication. It may also be due to
increased sugar consumption during increased respiration, in fully developed fruits as well as during pathogenesis, these are the probable reasons for non availability of more quantity of sugars for Ascorbic acid synthesis. Therefore, reduced amount of Ascorbic acid was observed in infected fruits.

The decrease in total soluble solids in infected fruits might be due to rapid fall in Citric acid and Ascorbic acid contents during pathogenesis and utilization of sugars by the pathogen for its growth as suggested by Reddy et al., (1984). But in the present study, citric acid in terms of total titrable acidity was increased in infected fruits still than reduction in total soluble solids in infected fruits might be due to reduction in Ascorbic acid and sugar content.

The increase in Citric acid content in the infected fruits was observed. The inhibition of sugar synthesis or breakdown of sugars would have resulted in the increase in acid content as suggested by Sinclair (1961). In the present study, depletion in the amount of sugars after infection in the fruit might be one of the reason for increase in the amount of citric acid in terms of total titrable acidity during the study.

The histopathological studies of infected plant parts revealed that after entrance into the host, bacteria progressed intercellularly among parenchymatous cells followed by thickening and darkening of cells. The infected
cells were completely destroyed and a cavity within host tissues was formed, filled with bacterial cells. This results in the formation of bacterial pocket. The cavity enlarge gradually, reached to epidermis and finally epidermis was ruptured which facilitate the bacteria to come out and deposited over the host surface known as canker. In case of twigs, infection was confined only in the cortex region and wood portion was not affected.

In the process of infection, entrance of bacteria into the host followed by multiplication and subsequent destruction of parenchymatous cells leading to the formation of cavity filled with bacteria are the common events in many host-pathogenic bacterial interactions as reported by several workers (Nelson and Dickey, 1966; Williams and Keen, 1967; Vohra et al., 1971; Karbhari, 1976; Shekhawat et al., 1977 and 1979; Ikotun, 1978; Shekhawat and Patel, 1979; Singh and Thind, 1987; Srivastava et al., 1990 and Khare et al., 1996). The disruption of host cell walls after infection of bacteria might be due to the pectinolytic and cellulolytic enzymes produced by bacteria during interaction with the host tissues. The involvement of enzymatic activity in the disruption of cell walls was also suggested by Khare et al., (1996).

The effect of surface microflora on growth of causal bacteria in vitro and in vivo conditions was studied and result indicates that out of 11 mycoflora isolated and identified, non could produce inhibitory effect on the growth
of causal bacteria in both the conditions. Masroor and Sudhir Chandra (1987) found inhibitory effect of *Aspergillus niger* and *Aspergillus flavus* against *Xanthomonas campestris pv citri*. On the contrary, both species of *Aspergillus* were isolated in the present study, did not show any inhibitory activity against *Xanthomonas campestris pv citri*. Some other workers also recorded inhibitory effect on *Xanthomonas campestris pv citri* by *Erwinia herbicola*, *Pseudomonas fluorescens*, and *Aspergillus clavatus* (Goto, 1979; Ota, 1983; Unnamalai and Gnanamanickam, 1984 and Masroor and Sudhir Chandra, 1987). In the present study, a fungus *Penicillium chrysogenum* was isolated from plant surface. This fungus is the source of origin of well known antibiotic 'Penicillin'. Antagonistic effect might be produced due to secretion of an antibiotic by mycoflora or change in the pH of medium which could be unfavourable for growth of bacteria. Since, antibiotic 'Penicillin' is effective against Gram-positive bacteria and the test pathogen is Gram-negative one. This might be one of reason for non-antagonistic effect of *Penicillium chrysogenum*. In the antibiotic sensitivity test also, bacterial isolates were not sensitive to Penicillin as it had no inhibitory effect on growth of any isolate. Several workers also reported the antagonistic effect of different micro-organism like *Flavobacterium* spp., *Bacillus subtilis*, *Pseudomonas fluorescens*, *Aeromonas*, *Erwinia herbicola*, *Serratia mercescens*, *Penicillium oxalicum* on important phytopathogenic bacteria like *Xanthomonas campestris pv*
malvacearum, Xanthomonas campestris pv vignicola and Xanthomonas campestris pv vignaeradiatae (Verma et al., 1983; Anuradha and Gnanamanickam, 1987 and Bora et al., 1993). No bacteria except Xanthomonas campestris pv citri could be isolated from plant surfaces.