PART IV MISCELLANEOUS
GENERAL SUMMARY AND CONCLUSIONS

Tomato fruits are very commonly used as one of the most delicious vegetables and in the form of salad, juice, paste, ketchup, sauce, soup etc. in almost all parts of this country. Huge losses are incurred every year due to the spoilage of fruit crop by various infectious fungal diseases. In Sagar a lot of damage is done both in vegetable fields as well as in storage and transit conditions, by the fruit rot diseases caused by *A. solani* and *A. tenuis*. Hence the present work was undertaken which deals with the investigation on *Alternaria* fruit rot of tomato with a back-ground of recent concepts and developments which have taken place in the field of physiological plant pathology. Particular attention has been given to pathogenicity tests, various factors affecting the development of fruit rot, pathogenesis with special reference to the production and activity of pectolytic and cellulolytic enzymes in vivo, factors affecting the production and activity of various pectolytic and cellulolytic enzymes by pathogens in culture, analysis of metabolites of healthy and diseased fruit and some experimental trials for control measures.

Repeated inoculation experiments confirmed the pathogenicity of both *A. solani* and *A. tenuis* on tomato fruits.
Both the pathogens produced the typical fruit rot symptoms as observed in nature. In case of *A. solani*, the rotten area appeared as dark coloured spots with concentric zones while no such zones were found with *A. tenuis*.

A number of factors, including the methods of inoculation, the type of inoculum, temperature, relative humidity, age of the culture and age of the fruit were found to cause considerable effects on the development of rot. It was observed that the rotting was more significant and severe in case of injured fruits than uninjured ones. Thus the behaviour of both *A. solani* and *A. tenuis* appeared to be like that of wound parasites. The inoculum when provided in the form of an agar disc or spore mycelium suspension in host extract, caused a more severe fruit rot than when the inoculum was used in the form of spore mycelium suspension in water. The optimum temperature for the growth of fungi in *vitro* was $26^\circ - 28^\circ$ C and the maximum amount of rot was also observed at $26^\circ - 28^\circ$ C. Both low ($8^\circ - 12^\circ$ C) and high ($35^\circ - 40^\circ$ C) temperatures were unfavourable for fungal growth and the development of rot as well. The maximum disease incidence was found at 100% R.H. and the amount of rot was decreased with decrease in the relative humidity. With regard to the
effect of the age of the culture, it was observed that the
inoculum taken from 4-8 day old cultures was more virulent
in producing the severe fruit rot as compared to very young
(2 days) and old (20 days) cultures. Comparatively semiripe
tomato fruits were more susceptible than the uninjured green
and fully riped fruits to both the pathogens.

To understand, certain aspects of pathogenesis
caused by *A. solani* and *A. tenuis* during the development
of tomato fruit rot, enzymological studies with special
reference to pectolytic and cellulolytic enzymes were made.
Standard viscometric procedure was followed for these studies.

Both *A. solani* and *A. tenuis* produced four pectolytic
enzymes including PG, PMG, PGTE and PMTE in culture and in
diseased fruits, infected with both the test fungi. Though
the extract of healthy tomato fruits also showed some activity
of the same four pectic enzymes, the amount of enzyme activity
in healthy fruits was much less as compared to the diseased
fruits and the culture filtrates of both the pathogens. The
increased enzyme activity in the diseased tissues therefore,
suggested the active participation of these pectolytic enzymes
in pathogenesis.

For the production of all the four pectolytic enzymes
by both the organisms in culture, synthetic medium comparatively
was found to show a better performance. Richard's medium,
however, was most favourable for the mycelial growth of both the fungi. The 12 day incubation time in general, was proved to be the optimum incubation period for the production of enzymes. Therefore, synthetic medium and 12 day incubation period were selected for further studies. In both the organisms, the enzyme activity in case of A. solani with sodium polypectate was comparatively lesser than that of control containing glucose as the sole source of carbon. However, the fungal growth of both the organisms was greater in glucose than in pectin and sodium polypectate. On the basis of overall experimental results it was concluded that both the species of Alternaria were capable of producing various pectic enzymes constitutively and the increased production in presence of certain native pectic substances could be considered merely the stimulation of pectic enzyme synthesis rather than actual induction.

The production of pectic enzymes in the synthetic medium by both A. solani and A. tenuis was found to be greatly influenced by the pH of the medium. Both and PMG in both the organisms were actively produced in low pH range (3-6 pH); the pH 4 was found to be optimum for both the enzymes. However, the alkaline pH(8-9) favoured the formation of PGTE and PMTE in both the organisms, whereas pH below 8 and above 9 was proved to be unfavourable for both the transeliminases in both the pathogens. With regard to the fungal growth, it was
observed that pH 4 was optimum for both the fungi.

The production of pectic enzymes by both the organisms in the synthetic medium was found to be very much influenced by the addition of various organic compounds, including carbohydrates, amino acids, plant growth regulators, phenolic substances, and fungicides in the medium.

The synthesis of PG in case of *A. solani* was found to be strongly inhibited by rhamnose, tyrosine, kinetin (100 ppm), IPA (100 ppm), ferulic acid (200 ppm), m-hydroxybenzaldehyde (200 ppm), phloroglucinol (200 ppm), blitane, thiram, blimix, thiovit and brassicol. Amongst these compounds ferulic acid at 200 ppm was found to be most effective as it was able to cause the complete inhibition of this enzyme.

In case of *A. tenuis* however, the formation of PG was very much decreased by serine, kinetin (100 ppm), IBA (100 ppm), phloroglucinol (200 ppm), ferulic acid (200 ppm), vanillin (200 ppm), brassicol, blitox and blimix. None of the various compounds caused any significant stimulation of PG synthesis in either organisms excepting lysine which was found to increase the production of this enzyme in case of *A. solani*.

The secretion of another glycosidase (PMG) was greatly suppressed by starch and tyrosine in both the organisms, while IBA (100 ppm), kinetin (100 ppm), vanillin (200 ppm), m-hydroxybenza-
ldehyde (200 ppm), brassicol and cuman in case of *A. solani* and IBA (10 ppm), GA (10 ppm), ferulic acid (200 ppm), sultaf and blitox in case of *A. tenuis* were very effective inhibitors for the production of this enzyme.

The inhibitory effects of leucine, lysine, kentin (10 and 100 ppm), IAA (100 ppm), IBA (100 ppm), GA (100 ppm), vanillin (200 ppm) and phloroglucinol (200 ppm) on the production of PMG by *A. tenuis* were, however, most remarkable as almost all of these compounds exhibited a complete control of enzyme production in this organism, while in *A. solani* none of these compounds could completely check the production of this enzyme.

The production of both the transaliminases in both the pathogens was even more strongly inhibited by various effector substances. The PGTE enzyme formation was completely checked by starch in case of *A. solani* and kentin (10 and 100 ppm), in *A. tenuis*. GA (100 ppm), IBA (100 ppm) and vanillin (200 ppm) were found to inhibit the production of this enzyme in both the organisms. Lactose, phenyl alanine, leucine, tyrosine, GA (100 ppm), kentin (100 ppm), ferulic acid (200 ppm), blitox and sultaf in case of *A. solani* and galactose, tyrosine, ferulic acid (200 ppm), Cosan and sultaf were found to cause a substantial decrease in the production of PGTE in *A. tenuis*. The enzyme PMTE
was found to be even more sensitive than PGTE. Alanine, IAA (100 ppm) IBA (100 ppm), kinetin (100 ppm), ferulic acid (200 ppm), m-hydroxybenzaldehyde (200 ppm) and vanillin (200 ppm) in case of *A. solani* and leucine, IBA (100 ppm), kinetin (10 and 100 ppm), GA (10 and 100 ppm), phloroglucinol (200 ppm), thiram, blitox and sulfa in *A. tenuis* showed most remarkable inhibitory effects by causing a complete control of this enzyme in the culture. However, starch, lactose, phenyl alanine, IPA (100 ppm), GA (100 ppm), phloroglucinol (200 ppm), cumar, brassicol and blitane in case of *A. solani* and sucrose, raffinose, serine, thercnine, IPA (100 ppm), vanillin (200 ppm), and m-hydroxybenzaldehyde (200 ppm) were also found to be very effective inhibitors for the production of PMTE in case of *A. tenuis*.

In general, there appeared to be no clear cut and specific correlation between the production of various pectic enzymes and fungal growth of both the organisms in presence of various organic substances used in the present study excepting few instances where inhibition of fungal growth and suppression of certain pectic enzymes appeared to be some what correlated.

Some of the effector compounds were also found to influence the enzyme activity after its formation in the culture.
For this purpose only the PG activity was examined and it was observed that the enzyme of *A. tenuis* was comparatively more sensitive than that of *A. solani*. However, lactose, starch, tyrosine, serine, IBA (100 ppm), GA (100 ppm), kinetin (100 ppm) ferulic acid (200 ppm), m-hydroxybenzaldehyde (200 ppm) and vanillin (200 ppm) in case of *A. tenuis* and vanillin (200 ppm), ferulic acid (200 ppm), and m-hydroxybenzaldehyde (200 ppm) in *A. solani* proved to be most effective substances for causing the inhibition of polygalacturonase activity.

Due to the recognized importance of cellulose degrading enzymes in pathogenesis caused by fungal pathogens and their applied value for converting native cellulosic materials into utilizable sugars, the detailed study of the production and activity of cellulolytic enzymes by both *A. solani* and *A. tenuis* was made during the course of present investigation.

Both *A. solani* and *A. tenuis* produced an active cellulase system (C x type) in culture and in the diseased fruits. Though, the cellulase activity was also observed in healthy tomato fruits but the amount of enzyme activity present in diseased fruits was very much higher than that of healthy ones. It was, therefore,
concluded that cellulolytic enzymes were actively involved in the pathogenesis and in the development of fruit rot.

To determine the optimum cultural conditions for the production of cellulolytic enzymes, the effects of various culture media, incubation period, native carbon sources and pH, were investigated. During these studies it was observed that synthetic medium at 12 day incubation period showed a good overall performance. On the basis of results obtained with various native carbon sources, such as cotton filter paper and CMC it was concluded that both *A. solani* and *A. tenuis* were capable of producing both C₁ and Cₓ type of cellulase and therefore, both the fungal species could be termed as true cellulolytic organisms. The experimental data also suggested that the cellulolytic enzymes were being secreted constitutively rather than inductively in both the pathogens. In both the organisms the maximum cellulase production was observed at pH 4 and pH range below 4 and above 8 appeared to be quite unfavourable for the synthesis of enzyme in both the fungi.

The production of cellulase by test fungi in the culture was also found to be affected significantly by various organic substances. Lysine and leucine in case
of *A. tenuis* were found to show the most remarkable inhibitory effects on cellulase synthesis, because in presence of these compounds the synthesis of enzyme was completely checked. Starch, rhamnose, lysine, tyrosine, IBA(100 ppm), kinetin (100 ppm), ferulic acid (200 ppm), vanillin (200 ppm), cuman and blimix in case of *A. solani* and serine, histidine, kinetin (100 ppm), GA (100 ppm), IPA (100 ppm), vanillin (200 ppm), phloroglucinol (200 ppm), blimix and cosan in case of *A. tenuis* also showed strong inhibitory effects on the formation of C₅ type of cellulase enzyme. On the basis of these results also, no correlation could be found between the fungal growth and the production of cellulolytic enzymes in presence of the various organic substances added in the culture medium.

Some of the effector substances were also found to inhibit the activity of preformed cellulases of both the test fungi. In this connection lactose and IPA (100 ppm) caused a more significant inhibition of cellulase activity of *A. solani*, while in case of *A. tenuis*, lactose, fructose, histidine, alanine, kinetin (100 ppm), IBA (100 ppm), vanillin (200 ppm), and phloroglucinol (200 ppm) proved to be more effective inhibitors of cellulase activity than other compounds.
To understand the biochemical alterations as a result of post infection changes, the extracts of healthy and diseased tomato fruits infected with *A. solani* and *A. tenuis* were analysed chromatographically. It was revealed by the chromatographic analysis that the pathogen infection resulted into the marked changes in the composition of sugars, amino acids, organic acids, and phenolic substances. Out of the four sugars (glucose, fructose, maltose, sucrose) present in the healthy fruits two namely, glucose and maltose were found to disappear as a result of pathogenesis. With regard to the changes in the composition of free amino acids pool, it was observed that five new amino acids including cystine, tyrosine, valine, leucine / isoleucine and asparagine were secreted in the diseased tissues as result of post infection changes, caused by both the organisms. The visual observations of developed chromatograms revealed that the concentrations of glutamic acid and methionine were increased in the diseased fruits. No significant alteration in the organic acid composition was observed excepting that during pathogenesis, both the pathogens induced the synthesis of succinic acid in the diseased fruits. Only one phenolic substance with Rf. value 0.32 was detected in the healthy and diseased
tomato fruits infected with both *A. solani* and *A. tenuis*. The amount of this substance was, however, found to decrease with the increase in the incubation periods in both the organisms.

Certain fungicides and phenolic substances were tried to control the tomato fruit rot caused by both the species of *Alternaria*. Although none of the compounds could control the disease completely, the extent of rot was, however, greatly reduced by thiram, brassicol, blitox, sultaf, vanillin and m-hydroxybenzaldehyde.
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Chromatogram of aminoacids developed on Whatman no. 1 filter paper.

R = Ripe fruit
S = Alternaria solani
T = Alternaria tenula

4, 8, 12 numbers stands for the days of incubation period.
A colony of *Alternaria solani* and *A. tenuis* growing on tomato agar medium.

Diseased tomato fruit infected by *A. solani*.

Diseased tomato fruit infected by *A. tenuis*. 