CHAPTER-VII

SUMMARY AND CONCLUSION
Chickpea (*Cicer arietinum* L) commonly known as gram is a versatile crop amongst the grain legumes and rank first among pulses, both in hectare and production. The major protein component of the Indian diet comes from pulses. Chickpea wilt is the most important and widespread disease in chickpea growing areas of the six continents. Chickpea is generally cultivated, as rainfed crop in rabi season in Vindhyan Plateau of M.P. It is grown in 26.60 Lac hectares with 19.88 Lac tones yield production, approximately. The yield of Chickpea is very low due to wilt disease caused by *Fusarium oxysporum* f. *sp. ciceri*. *Fusarium* wilt of chickpea is considered as the most important, devastating and a challenging one. Wilt disease have a great economic importance loosing 10-15% each year, regularly. However, during severe epidemic, crop losses go up to 60-70% in the country. These losses are much higher than other crops. Significant advances have been made in the management of chickpea wilt in developing countries but this area of research has received scant support and attention in India. Today, when human population is crossing the limits, the destruction caused by microorganisms is posing serious problems before farmers, scientists and administration. Fungal infection of plant gives an ugly appearance and affects the nutritive value due to changes in the physiology of the host.

On looking to the uses, production and importance of the chickpea crop, it was thought desirable to investigate the pathogens responsible for causing wilt disease of chickpea in Vindhyan Plateau area. The present investigation was also undertaken to evaluate some control measures under *in vitro* and *in vivo* conditions so as to find out some economical and feasible means for combating this malady.

*Fusarium oxysporum* f. *sp. ciceri* (Padwick) Snyd. & Hens. was isolated from diseased chickpea (*Cicer arietinum* L) plants from the five districts (Bhopal, Raisen,
Sagar, Sehore, Damoh) of the Vindhyan Plateau zone. Bhopal and Sehore district showed a highest frequency (58.66%) of the test pathogen. The isolated pathogen was tested for its pathogenicity and confirmed by the test tube seedling symptoms and pot experiments. From 35 samples of diseased plants collected from five districts (Bhopal, Raisen, Sagar, Sehore, Damoh), 20 isolates obtained and out of which 15 isolates proved pathogenic on chickpea variety JG-62 and designated as *Fusarium oxysporum. f. sp. ciceri*. Pathogenicity test showed pre-emergence symptoms on the chickpea plants and thus showing that the pathogen could get entry through the roots causing wilting in chickpea plants.

Various metabolites of the host tissues get affected due to infection of microorganisms, thus bringing down the nutritional value of the host. In the present study, certain noteworthy changes in the amino acids, sugars, organic acids and phenolic contents of healthy as well as infected chickpea plants with *Fusarium oxysporum. f. sp. ciceri* have been observed. A total of 17 and 9 amino acids were recorded in healthy and diseased chickpea plants tissues, respectively. The quantity of amino acids in extracts of healthy plants was found more in comparison to diseased plants tissues. Generally, the intensity of amino acids was found gradually decreased with increasing the intensity of infection of the pathogen in diseased plants. Cystine, glutamic acid, glutamine, glycine, iso-leucine, leucine, proline, serine, tyrosine and valine were totally absent in diseased chickpea plants tissues indicating the complete utilization of these amino acids by the pathogen for its growth.

Four sugar’s (glucose, fructose, lactose and sucrose) were found present in healthy chickpea plant tissues. The concentration of these sugars was decreased considerably after wilt incitation by the pathogen, *Fusarium oxysporum. f. sp. ciceri*. 
The analysis of healthy and diseased chickpea plant extracts revealed the presence of five organic acids. Citric acid, malic acid, succinic acid, oxalic acid and ascorbic acid were detected from the extracts of healthy plants while in diseased plant tissues, oxalic acid and ascorbic acid were absent and the intensity of other organic acids was quite low, thus indicating its utilization in pathogenesis.

Five phenols Ph₂ (Rf 0.45), Ph₅ (Rf 0.45), Ph₁ (Rf 0.35), Ph₄ (Rf 0.20), and Ph₃ (Rf 0.15), present in healthy chickpea plant extract. Phenol Ph₄, and Ph₅ were found totally absent in diseased chickpea plant extracts thus showing its involvement in the metabolism. Other phenols contents were decreased in the later stages of pathogenesis in diseased chickpea plants.

Amongst various cell wall degrading enzymes, pectolytic and cellulolytic enzymes which are usually known as chemical weapons of the pathogens, play an important role in the development of diseases. Fusarium oxysporum f. sp. ciceri was found capable of producing pectolytic (exo PG, exo PMG, endo PG and endo PMG) and cellulolytic (exo- β 1, 4 glucanase and endo- β 1, 4 glucanase) enzymes in vitro as well as in vivo conditions.

Three varieties of chickpea plants JG- 62 and JG- 74 both susceptible and L- 550 (resistant) were selected for detailed enzymological studies. The activity of pectolytic (exo PG, exo PMG, endo PG and endo PMG) and cellulolytic (exo- β 1, 4 glucanase and endo- β 1, 4 glucanase) enzymes could be recorded in healthy and diseased chickpea plants variety JG- 62. Healthy chickpea plants variety JG- 74, did not show the presence of these enzyme while the diseased plants of the same variety showed the presence of these enzymes. Variety L-550 could not produce these enzyme in vivo as they were complete resistant against the Fusarium oxysporum. The lower amount of some enzymes in healthy and higher amount in diseased plant tissues.
elucidates the fact that the main role of these enzymes in pathogenesis involves the breakdown of host tissues and in the development of disease in chickpea crop. It appears that these enzymes play a significant role in the pathogenesis of chickpea plants.

*Fusarium oxysporum. f. sp. ciceri* was capable of producing cell wall degrading enzymes *in vitro*. Different cultural conditions affect the production of these enzymes. In the present studies seven different culture media (Asthana and Hawker’s, Czapek’s, Glucose asparagine, Host extract, Potato dextrose, Richard’s and Sabourd’s) were tested for the production of pectolytic and cellulolytic enzymes. Amongst these, Czapek’s and Potato dextrose medium were proved to be the best for the production of pectolytic and cellulolytic enzymes by the test pathogen. However, other media were moderate in this regard. A positive correlation could be drawn between enzyme production *in vitro* and virulence of the pathogen. However no definite correlation could be drawn between biomass, pH and enzyme secretion in all the media tested.

Five carbohydrates (fructose, glucose, lactose, manitol and sorbitol) were tested for the pectolytic and cellulolytic enzyme production *in vitro*. Maximum secretion of pectolytic and cellulolytic enzymes was observed with glucose, lactose and fructose. Maximum production of exo PG and exo β-1, 4 glucanase enzyme was found with glucose while exo PMG enzyme was higher in medium containing lactose and sucrose (control). Endo PG, endo PMG and endo β-1, 4 glucanase enzyme production was favoured by fructose and sucrose (control). However, other carbohydrates were moderate in this regard. No definite correlation could be drawn between biomass, pH and enzyme secretion in all the media tested.
Four phenolic compounds (α-naphthol, salicylic acid, pyrocatechol and pyrogallol) with three concentrations (100, 250 and 500 ppm) were tested for the production of pectolytic and cellulytic enzyme/s by *Fusarium oxysporum f. sp. ciceri in vitro*. Pyrocatechol (100 ppm), pyrogallol and α-naphthol both (500 ppm) completely inhibited the production of exo PG enzyme, while exo PMG enzyme was completely inhibited by pyrocatechol (250 ppm) and pyrogallol (500 ppm). None of the phenolic compounds could check the production of endo PG, endo PMG and endo β-1, 4 glucanase enzyme, completely. Pyrogallol, pyrochatechol both (100 ppm), α-naphthol (250 ppm) and salicylic acid (500 ppm) completely checked the production of exo β-1, 4 glucanase enzyme by *Fusarium oxysporum*. However, other phenolic compounds were moderate in this regard. Generally, the enzyme secretion was found to be decreased by increasing the concentration of each phenolic compound in the culture media.

Seven medicinally important plant extracts (*Azadirachta indica, Aloe barbadense, Cassia fistula, Curcuma domestica, Emblica officinalis, Ocimum sanctum and Zinziber officinalis*) with three concentrations (50, 100 and 250 ppm), were tested for the radial growth of the *Fusarium oxysporum f. sp. ciceri*. Results obtained in the present study indicated that *Emblica officinalis* (50 ppm), *Curcuma domestica* (100 ppm), *Azadirachta indica* and *Aloe barbadense* (250 ppm) were able to check the radial growth of the test organism completely. *Cassia fistula* (89.02%), *Ocimum sanctum* (84.96%) and *Zinziber officinalis* (80.46%) were able to inhibit the mycelia growth, while rest of the plant extracts showed good inhibition of radial growth of the test pathogen. Data revealed that by increasing the concentration of each plant extract, a progressive decrease in the radial growth of the test organism was recorded.
Production of pectolytic and cellulolytic enzymes was also affected by all the plant extracts. *Emlica officinalis*, *Zinziber officinalis* both (50 ppm) and *Curcuma domestica* (100 ppm) inhibited the production of exo PG enzyme, whereas *Curcuma domestica*, *Emlica officinalis* both (50 ppm) and *Aloe barbadense*, *Zinziber officinalis*, *Azadirachta indica* all (250 ppm) inhibited the exo PMG enzyme, completely. None of the plant extracts could show the inhibition of endo PG and endo PMG enzyme synthesis completely, while higher concentration of each plant extract showed maximum inhibition of these enzymes *in vitro*. The exo β-1, 4 glucanase enzyme was inhibited by *Ocimum sanctum, Azadirachta indica, Emlica officinalis, Zinziber officinalis* all (50 ppm), while complete inhibition of endo β-1, 4 glucanase enzyme production was recorded with *Azadirachta indica* (100 ppm), *Emlica officinalis* and *Curcuma domestica* both at 250 ppm concentration. Other plant extracts also showed inhibition of these enzymes at higher concentration. The production of pectolytic and cellulolytic enzyme was found to be decreased by increasing the concentration of each plant extract, *in vitro*.

Four fungicides (bavistin, quanatiol, SAAF and thiram) with three concentrations (100, 250 and 500 ppm) were tested for the production of pectolytic and cellulolytic enzyme/s by *Fusarium oxysporum*. Thiram (100 ppm) and bavistin (500 ppm) checked the exo PG enzyme secretion completely, while exo PMG enzyme was completely checked by bavistin (500 ppm). None of the fungicides could check the endo PG, endo PMG and endo β-1, 4 glucanase enzyme secretion completely, however, thiram and bavistin (500 ppm) could control these enzymes production, significantly. Bavistin (100 ppm), thiram (250 ppm) and SAAF (500 ppm) completely checked the secretion of exo β-1, 4 glucanase enzyme. However, other fungicides
were moderate in this regard. It was observed that the enzyme secretion was decreased by increasing the concentration of each fungicide in the culture media.

Seven fungicides (mancozeb 63 WP, carbendazim 50 WP, carboxin 75WP, thiram 75 DS, bitertanol 25 WP, hexaconazole 5 EC and iprodine 50 WP) with three concentrations (100, 250 and 500 ppm) were evaluated for their effectiveness on the radial growth of *Fusarium oxysporum f. sp. ciceri in vitro*. Present results indicated that hexaconazole 5 EC (500 ppm) could complete check the radial growth completely, while carboxin 75 WP, mancozeb 63WP, bitertanol 25 WP, thiram 75 DS, iprodine 50 WP and carbendazim 50 WP could also reduce the radial growth of the test pathogen, substantially.

Seed treatment with conventional fungicides does not provide adequate control of wilt disease because of many pathogens are involved. Since this crop is grown principally in rainfed areas, many of the known conventional chemical methods have not been found in wide adaptation. During the past few years, the concept of biological control of plant pathogens has emerged as a potential means of controlling plant diseases. Recently, an attractive and promising approach to combat plant disease is by integration of biological methods with low non hazardous dosage of fungicides and other Integrated Pest Management (IPM) components in which if one component is failed, then the other components take care in controlling the disease.

For control studies, seven fungicides (mancozeb 63 WP, carbendazim 50 WP, carboxin 75WP, thiram 75 DS, bitertanol 25 WP, hexaconazole 5 EC and iprodine 50 WP) were tested under glass house and field conditions. Chemical control in glass house condition showed that hexaconazole 5 EC at 5 g/ Kg dosages was the best treatment which could control (70.15%) the chickpea wilt disease, while other
fungicides were moderate in this regard. In combination of fungicides, hexaconazole 5 EC + carbenazim 50 WP gave a significant control (83.75%) of the disease. Other fungicidal combinations were also found quite effective in controlling the disease. On the basis of these results it can be concluded that fungicidal combinations are much better than the fungicides alone.

The results of present experiments under glass house conditions were very significant as the *Fusarium oxysporum f. sp. ciceri* affecting chickpea crop could be controlled, by soil application having *Trichoderma viride*, *Trichoderma harzianum* and *Gliocladium virens*, successfully. The soil application of each antagonist/s (*Trichoderma viride*, *Trichoderma harzianum* and *Gliocladium virens*) at the rate of 3, 6, 9, 12, 15 and 30 g/2 Kg soil gave an excellent control of chickpea wilt disease in two growth cycles. Present result indicated that 30 g/2Kg soil application was the best. The level of disease control found increased with increasing the dosages of antagonists (*Trichoderma viride*, *Trichoderma harzianum* and *G. virens*) indicating a positive relationship between the two.

The combination of antagonist with fungicide/s gave beneficial and improved degree of control. Combination of hexaconazole 5 EC and mancozeb 75 WP with *Trichoderma harzianum*, *Trichoderma viride* and *G. virens* was found as the best treatment in controlling the wilt disease of chickpea followed by combination of thiram 75 DS and carbenazim 50 WP with antagonists. *Trichoderma viride*, *Trichoderma harzianum* and *G. virens* are also known to produce certain growth regulating factors which enhanced the growth of seedling in the present study. Increase in height, fresh weight and dry weight of chickpea seedling was found with increasing the dosage of *Trichoderma* spp. and *G. virens*. 

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Under field conditions, the effective disease control was achieved only when the seeds were treated with the combination of more than one fungicide. Seeds treated with a combination of hexaconazole 5 EC+ carbendazim 50 WP gave good disease control (42.89%) of chickpea wilt followed by hexaconazole 5 EC + carboxin 75 WP (40.83%), carbendazim 50 WP+ mancozeb 63 WP (39.28%), iprodine 25% + carbendazim 25 WP (38.75%) and carbendazim 50 WP + thiram 75 DS (36.18%). It is clear from the present results that the combination of fungicides could give better results than to fungicides alone. In field conditions, however the degree of disease control was increased significantly when these treatments were integrated with the antagonists (Trichoderma viride, Trichoderma harzianum and G. virens). Combination of hexaconazole 5 EC + Trichoderma harzianum gave disease control (59.84%) of chickpea wilt followed by Hexaconazole 5 EC + G. virens (52.17%) and Hexaconazole 5 EC+ T. viride (46.76%). Integration of chemicals with antagonists had an additive effect on disease control because of chemical eliminating or reducing soil saprophytes that compete with the antagonists for nutrients. Yields obtained in the integrated treatments were also significantly higher than in the chemical control treatments alone.

CONCLUSION

- In the present study it was found that Fusarium oxysporum f. sp. ciceri is present in Vindhyan Plateau zone (Bhopal, Raisen, Sagar, Sehore, Damoh) in rich condition which could cause wilt disease of chickpea severely. It is also reported that Fusarium oxysporum f. sp. ciceri showed variability in nature.
- Metabolic changes showed that wilt disease is responsible for reduction of the nutritive quality of chickpea yield.
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Summary and conclusion

- Present results indicated that cell wall degrading enzymes play an important role in wilt disease development thus responsible for the reduction of chickpea yield.

- Control measure studies clearly, indicate the tremendous potentials of *Trichoderma harzianum* followed by *Gliocladium virens* and *Trichoderma viride* as a biological control agent against wilt causing pathogen. It can also be successfully exploited either alone or in combination with fungicides to combat *Fusarial* wilt of chickpea.