INVESTIGATIONS ON PATHOGEN, HOST AND THEIR INTER-RELATIONSHIP IN THE CORYNESPORA LEAF DISEASE OF *HEVEA BRASILIENSIS*

THESIS SUBMITTED TO

THE MAHATMA GANDHI UNIVERSITY, KOTTAYAM

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN BOTANY

UNDER THE FACULTY OF SCIENCE

BY

SURESH KUMAR K. K., M. Sc. B.Ed.

MYCOLOGY AND PLANT PATHOLOGY DIVISION

RUBBER RESEARCH INSTITUTE OF INDIA

KOTTAYAM – 686 009

KERALA, INDIA

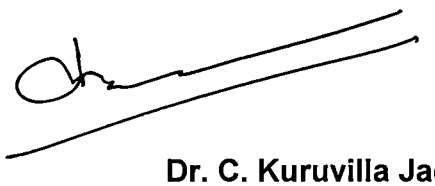
MARCH 2010

THE RUBBER RESEARCH INSTITUTE OF INDIA

(Rubber Board, Ministry of Commerce and Industry, Govt. of India)

Certificate

This is to certify that the thesis entitled **Investigations on pathogen, host and their inter–relationship in the Corynespora leaf disease of *Hevea brasiliensis*** is an authentic record of the research work carried out by Mr. Suresh Kumar K. K. under my scientific supervision and guidance at the Rubber Research Institute of India, Kottayam, in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** of the Mahatma Gandhi University, under the faculty of Science and no part thereof has been presented for the award of any degree, diploma or associateship in any University.



Dr. C. Kuruvilla Jacob

(Supervising Teacher)

Director of Training Centre

Rubber Research Institute of India

Kottayam

March 2010

Declaration

I hereby declare that this thesis entitled **Investigations on pathogen, host and their inter–relationship in the Corynespora leaf disease of *Hevea brasiliensis*** has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar title for recognition.

Kottayam

March 2010

Suresh Kumar K. K.

Acknowledgement

I am extremely grateful to express my deep sense of gratitude and appreciation to my supervising teacher Dr. C. Kuruvilla Jacob, Director of Training Centre, Rubber Research Institute of India, Kottayam, for his valuable guidance, constant encouragement, constructive criticism and periodical scrutiny of work at every stage, which paved the way for the genesis of this successful work.

I am thankful to Dr. N. M. Mathew, former Director, Dr. James Jacob, Director of Research, RRII, Kottayam for providing all the necessary facilities to conduct the research work and also for their encouragement.

I offer my most sincere thanks to Dr. Jacob Mathew, Dr. Shaji Philip, Dr. Annakkutty Joseph, Dr. Kochuthresiamma Joseph, Dr. C. Bindu Roy, Smt. Shylajadevi Mr. Thomson, Miss. Geetha Jose and all other officers and staff in the Mycology and Plant Pathology Division, RRII, for their valuable helps and suggestions.

I would like to acknowledge the invaluable help rendered from the members of RRII Library and in special Smt. Accamma Korah, Sr. Librarian, for the references and data collection.

I find no words to acknowledge my friends Joby Joseph, Sunil Varghese, Shyju Joseph, Cina Ann Zachariah, Jinu and Roshni Susan Elias for their valuable help and co-operation.

On a personal note, I wish to express my heartfelt gratitude to my wife Smt. Anupama K. for her encouragement, assistance and moral support. My daughter Arpitha S. Kumar deserves special mention for her sacrifices during the period of work. Mere words will not be enough to express my feelings to them. I am also thankful to my parents, sisters, brother and all other members of the family for their support and inspiration.

Above all, I thank God Almighty who helped me along my thesis work to come out successfully by his blessings in spite of some difficulties.

Suresh Kumar K. K.

INVESTIGATIONS ON PATHOGEN HOST AND THEIR INTER-RELATIONSHIP IN THE CORYNESPORA LEAF DISEASE OF *HEVEA BRASILIENSIS*

Abstract

The interaction of host and pathogen of Corynespora leaf disease of *Hevea brasiliensis* was studied with special reference to meteorological, nutritional and physiological requirements of the pathogen, *Corynespora cassiicola*. The fungal isolates used were collected from Kerala and Karnataka. All the six *C. cassiicola* isolates showed variations in nutritional requirements for their growth and sporulation. The involvement of offence and defense related enzymes and secondary metabolites in resistance of *H. brasiliensis* were also studied. The extracellular enzymes, cellulose and protease play a major role in the penetration of *C. cassiicola* and the development of disease. Changes in defence – related enzymes and secondary metabolites were evaluated after *C. cassiicola* infection on *H. brasiliensis* clones RRII 105, PB 260, RRIM 600 and GT 1. The former two clones show susceptible and the later two resistant reactions against *C. cassiicola* used. The time course of accumulation of defense enzymes triggered by pathogen inoculation showed greater activity in RRIM 600 and GT 1. In susceptible clones the defense mechanism starts functioning very slowly and reaches its effective level only after few days. The pathogen takes advantage of this delay for establishment of infection. The study helps in understanding the various events in *C. cassiicola- H. brasiliensis* interaction which is a key factor for developing proper management of Corynespora leaf disease of *H. brasiliensis*.

Chapter 1

EFFECT OF METEOROLOGICAL FACTORS ON *CORYNESPORA CASSIICOLA*

1. Introduction

Plant diseases occur due to the interaction of the pathogen, host and environment. Environment can influence the host, the pathogen or both. The environmental factors like light, temperature, humidity and pH affect disease development through their influence on the growth and/or susceptibility of host, on the multiplication and activity of the pathogen, or on the interaction of host and pathogen and its effect on the severity of symptom development (Pathak *et al.* 1996).

Corynespora cassiicola (Berk & Curt) Wei is a facultative fungus (Peries and Liyanage, 1987) which affects more than one hundred hosts all over the world. This fungus causes root (Raffel *et al.*, 1999), leaf (Peterson, 2002), stem and fruit (Tsay and Kuo, 1991; Kwon *et al.* 2005) diseases on many economically valuable crops. During the past decade, the pathogen has caused extensive damage to rubber tree plantations and may become a potential limiting factor in rubber yield in Asia (Breton *et al.*, 2000; Jacob, 2006a). Corynespora leaf disease has been originally reported from India as a minor disease of *H. brasiliensis* affecting nursery plants (Ramakrishnan and Pillay, 1961). This disease appears in the mature plantations when the trees refoliate after wintering. The most common symptom observed in India is the presence of circular or irregular amphigenous spots which measures 1-10 mm in diameter (Ramakrishnan and Pillay, 1961, Rajalakshmi and Kothandaraman, 1997). These spots sometimes may coalese to form enlarged lesions with a dark brown or papery center surrounded by a dark brown ring and yellow halo. Severe disease incidence leads to shriveling of leaves, blackening of veins forming fish bone appearance, blighting of lamina, defoliation, die back and ultimately death of tree (Jayasinghe *et al.*, 1998, Edathil *et al.*, 2000; Jacob, 2006b). Severity of the disease in different rubber growing regions varies greatly. High atmospheric humidity, temperature range of 28 to 30 ^{0}C and cloudy weather are reported to favour the disease. However, very little information on the effect of different

environmental factors such as temperature, light, humidity and pH on *C. cassiicola* is available. A knowledge of the critical levels of their factors for the growth and sporulation of *Corynespora cassiicola* would lead to better prediction of disease risk and more efficient use of disease management programmes to protect the crop. Hence the aim of this study was to understand the effect of meteorological parameters on growth and sporulation of the selected *C. cassiicola* isolates (from different geographical areas).

2. Review

As the information on influence of environmental parameters on growth and sporulation of *C. cassiicola* is scanty, the review of other (related) fungal species is also included whenever relevant.

2.1 Light

The effects of various forms of solar radiation on development and reproduction in fungi have been given considerable emphasis over the years (Stone and Scally, 2003). Light has been shown to stimulate, inhibit or have no effect on the growth of certain species. Most reports on the effect of light on the fungi are related to reproduction (Pardo and Forchaissin, 1993; Roger and Tivoli, 1996; Sanchez-Murillo *et al.* 2004). According to Giles *et al.* (2002) aerial pathogens are more light sensitive than soilborne pathogens in relation to spore production.

According to Oke (1990) alternate 12 hours of light and darkness favoured the sporulation of *C. cassiicola* isolate from tobacco plant. Breton *et al.* (2000) pointed out that exposing a 7-day-old *C. cassiicola* culture for three days at 28^0C to continuous light induced sporulation. Onesirosan *et al.* (1975) observed that sporulation of *C. cassiicola* was more under continuous light and that there was considerable variation between isolates. Pereira *et al.* (2003) considered *C. cassiicola* f. sp. lantanae as a potential biocontrol agent from Brazil for *Lantana camara* and reported that the isolate sporulates well under continuous light treatment for 15 days.

2.2 Temperature

Among the external factors that influence the growth of fungi, temperature plays an important role. Temperature affects almost every function of the fungi and fungi differ markedly in their temperature optima.

Onesirosan *et al.* (1975) studied the temperature requirement of *C. cassiicola* isolates and stated that the minimum temperature for the growth of isolates on PDA was 12°C and the growth rate increased with the increasing temperature. They also pointed out that at 32 and 36^{0}C, growth was much slower and colonies had irregular edges. Jinhyeuk *et al* (2001) indicated that the optimal temperature for mycelial growth and conidial growth of *C. cassiicola* is 30^{0}C and 25-30^{0}C respectively. According to Kwon *et al.* (2001) the optimal temperature for mycelial growth and conidial germination of *C. cassicola* causing leaf spot in pepper was 30^{0}C and 25-30^{0}C, respectively.

Kwon *et al.* (2005) studied Corynespora leaf spot of balsam pear caused by *Corynespora cassiicola* in Korea and reported 30^{0}C as the optimum temperature for mycelial growth of the pathogen. Wataru *et al.* (2005) found the optimum temperature as 27.5-30 for the mycelial growth of *C. cassiicola* infecting *Perilla* plants.

2.3 Humidity

The moisture requirement of fungi differs from species to species. Most species in nature live on substrates which are not saturated with water. The low moisture content of a substrate also is often a factor which limits the growth of fungi. Madan and Thind (1998) reported that *Ceratostomella pilifera*, a wood-staining fungus, does not grow on pine wood having a moisture content of 23 per cent but grows when it is 24.5 per cent. Narayanan and Sharma (2004) reported a humidity range of 69-80 per cent for the *in vitro* conidial germination and mycelial growth of casuarina wilt pathogen (*Trichosporum vesiculosum*). The growth and reproduction of fungi causing powdery mildew are generally inhibited by decreasing relative humidity (Kenyon *et al.*, 2002). Abiko and Ishii

(1988) observed conidial germination of *Corynespora melongenae* at 45-100% relative humidity.

2.4 pH

Fungi in general are more tolerant to acidic ions (H^+) than to basic ions (OH^-). However, most of the fungi grow at pH between 4 and 8 (Bilgrami and Verma, 1992). A pH range of 5.0 to 6.0 is optimum for the growth of majority of the fungi. pH of the medium exerts profound influence upon the availability of metallic ions, nitrogen, cell permeability, and enzyme activity (Hommes *et al.*, 1989). Agnihothrudu (1952) found that *Macrophomina phaseoli* from cotton tolerated a wide range of pH from 3.04 - 7.97 and the optimum for the growth was between pH 5.17 – 8.04. The optimum pH ranges for *Blastocladia pringsheimii*, *Allomyces arbuscula* and *Blastocladiella simplex* are rather narrow (Emerson and Cantino, 1948). Most of the pH optima reported in the literature are less than 7.0. Volz and Beneke (1969) reported pH 3.0 to the optimum for *Lenzites saepiaria*, *Fomes roseus, Merulius lacrymans* and *Coniophora cerebella.* The lower pH limits reported to vary from 5.3 to 0.5 for *B. simplex* and *Acontium velatum* (Emerson and Cantino, 1948).

3. Materials and Methods

3.1 Glassware and chemicals

Borosil brand glassware and analytical grade chemicals (Sigma, SRL and Bangalore Genei) were used throughout the study. The glassware were first cleaned with detergent, then immersed in cleaning solution (80 g potassium dichromate, 300 ml distilled water and 400 ml concentrated sulphuric acid) for 12 h, washed thoroughly with tap water; air dried and finally dried in a hot air oven.

3.2 Sterilization

Media and glassware other than Petri plates were sterilized in an autoclave at 1.05 kg/cm^2 pressure for 20 minutes. Petriplates were sterilized at 150 °C in hot air oven for five hours. The thermolabile compounds were filter sterilized using sintered glass filter (G5).

3.3 Preparation of fungal cultures

Six isolates of *C. cassiicola* namely Cc 02, Cc 03, Cc 04, Cc 05, Cc 08 and Cc 33 were used in this study. These were originally collected from different locations in Kerala and Karnataka. The isolates Cc 02, Cc 03, Cc 04 and Cc 33 were collected from the susceptible rubber clone RRII 105, and the isolates Cc 05 and Cc 08 were collected from infected leaves of clones PB 260 and GT 1 respectively. Of these clones, GT 1 is reported to show a high level of tolerance to the disease (Sinulingga, 1995). Further details regarding the origin of isolates are shown in Appendix – 1. The fungal cultures used were subcultures from a single celled conidium isolated from different clones of *H. brasiliensis*. The isolates remained virulent throughout the study as evidenced by inoculation tests on tender leaves of *Hevea* at various intervals. Those, which showed low virulence in inoculation studies, were transferred to toxin medium or passed through the plant to induce virulence as and when required.

3.4 Growth characteristics

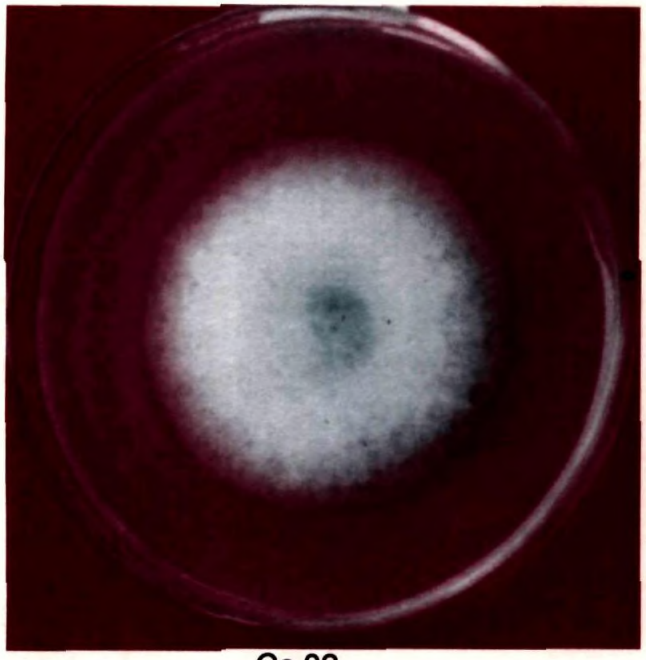
The growth characteristics of *C. cassiicola* isolates were studied *in vitro* in terms of the mycelial growth on different media. Petri plates containing 20 ml of medium were inoculated with 7 mm mycelial discs and incubated at 25 ± 2^0C. The radial growth of the fungus was measured after three days of inoculation and was continued up to eight-day of inoculation. Spore suspensions were prepared and a drop of this was placed on a glass slide and observed under microscope at one hundred times magnification to study the size, shape, number of septa in conidium and conidiophore. Two hundred and fifty conidia were observed for each isolate.

3.5 Light, temperature and humidity

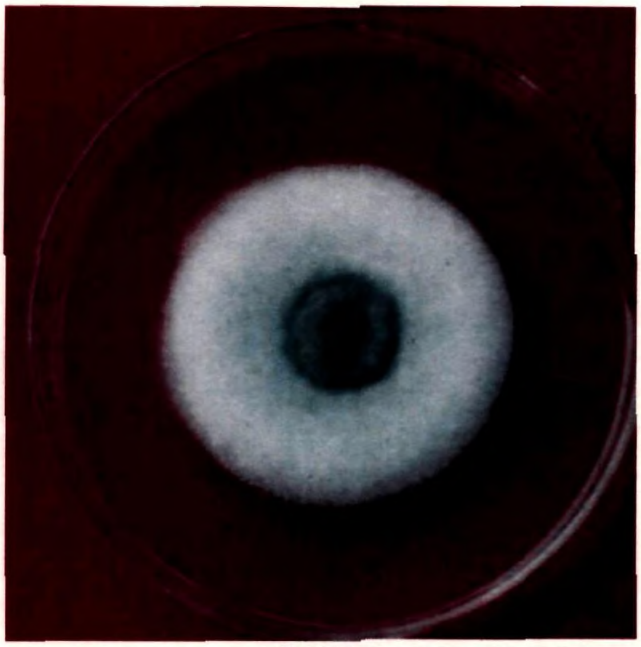
Effect of light on growth of *C. cassiicola* was studied on potato dextrose agar (PDA) medium with pH adjusted between 6.0 to 6.5. About 20 ml of the medium was poured into each of the six 9 cm. petriplates for each light period tested (continuous light, continuous dark, 4, 5, 6, 7 and 8 hours of bright light alternating with darkness). Inoculum discs of 5 mm diameter from the margin of 7 day-old cultures were placed

Plate - 1

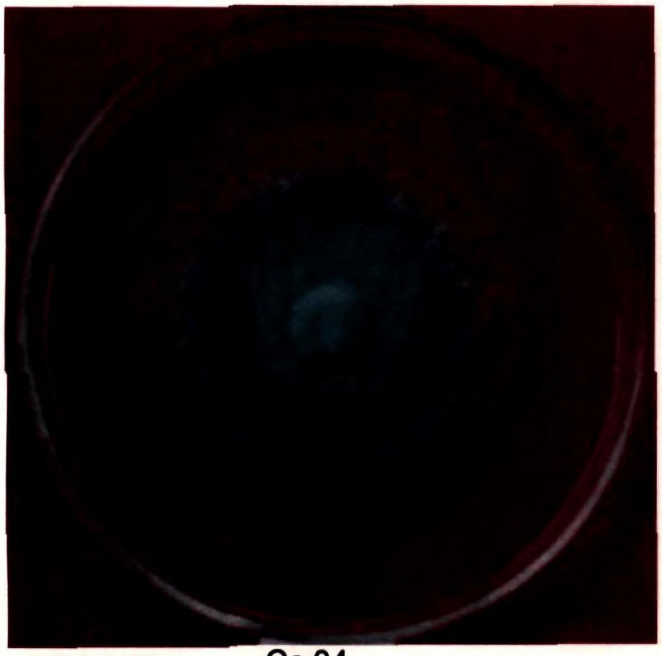
Corynespora cassiicola isolates



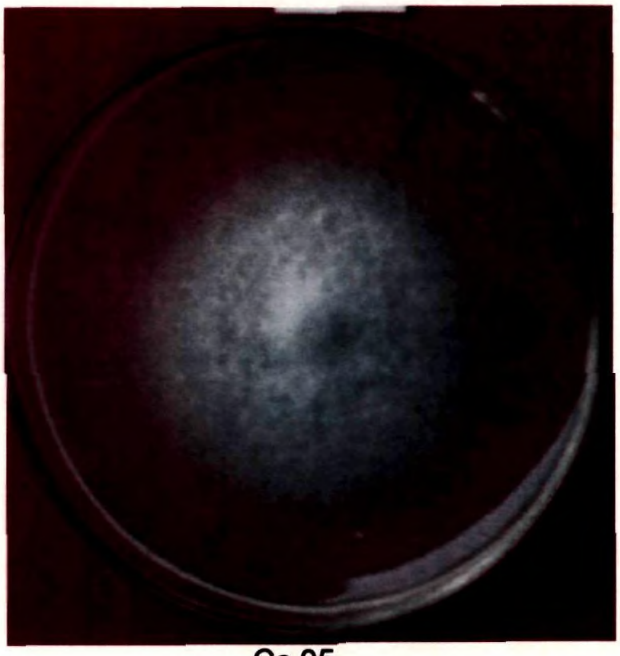
Cc 02



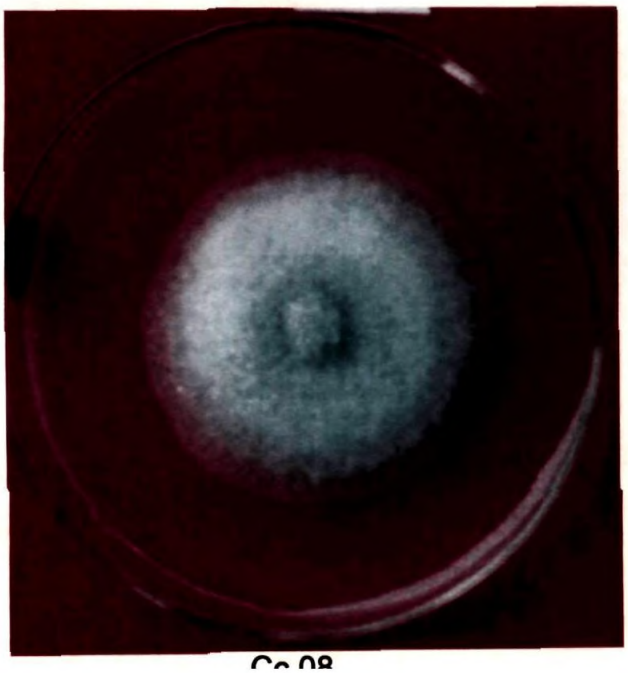
Cc 03



Cc 04



Cc 05



Cc 08

Cc 33

invertedly on the surface of the medium and were incubated at 25 ± 2^0C. Colony diameter was measured to compare the growth. The observations were recorded after incubation for three days and were continued upto 7 days. The conidia suspension was obtained by brushing the conidia and collecting them in 2 ml sterile water. Sporulation was measured as the number of conidia per ml of spore suspension obtained. The sporulation intensity after 7 and 12 days on the medium was assayed with a haemocytometer. The materials and methods adopted to study the temperature and humidity on the growth and sporulation of the pathogen were similar to those used in light except as noted below. Three petriplates at each temperature and humidity level were used for the study. The temperature levels tested were 15, 20, 23, 25, 27, 30 and 35 °C. The humidity levels studied were 50, 70, 80, 90 and 100 % relative humidity. The experiment on effect of temperature was repeated for temperatures of 30 to 35°C to ascertain the maximum favourable temperature for growth.

3.6 pH

Richard's medium was used to study the effect of pH on growth of the pathogen. The pH of the medium was adjusted by using stock solution (A) 0.1 M citric acid (19.2 g / l) and stock solution (B) 0.2 M Na_2HPO_4 (28.4 g / l). Fifty ml of the medium was used in 100 ml conical flasks and sterilized. Mycelial discs (5 mm) of six isolates of *C. cassiicola* from 7 day old cultures were inoculated in three replicates per pH levels *viz.* 3, 4, 5, 6, 7, 8 and 9. After the incubation for 15 days at 25 °C, the mycelial mats were washed with distilled water and dried to constant weight at 60°C. In all the experiments biomass (dry weights) of mycelial mat were recorded for comparing the treatments.

4. Result

4.1 Light

After three days of incubation, growth of *C. cassicola* isolates was measurable on the PDA medium for all the light periods studied. All the six isolates showed similar mycelial growth at 4 hours of bright light (Table 1.1). At 5 and 6 hours of bright light all the isolates showed better growth rate except Cc 33, which showed low growth at 6 hours

of bright light treatment. *C. cassiicola* isolate Cc 04 showed more growth at all the bright light hours studied including 7 and 8 hours of bright light, but the other five isolates exhibited moderate growth at these treatments. Continuous light and darkness promoted growth, but sporulation was very low. *C. cassiicola* isolates prefer four hours of daily light exposure for their maximum growth and 5 to 6 hours of light alternating with darkness for their maximum sporulation.

The period of incubation required for sporulation was 5 days for all the isolates of *C. cassiicola* tested. The isolates Cc 04, Cc 08 and Cc 33 showed good sporulation at all the light periods. Isolates Cc 05 and Cc 02 showed moderate spore production. However, isolate Cc 03 was very poor in sporulation. Low spore production was noticed in cultures incubated under continuous light or darkness even after 12 days of incubation.

4.2 Temperature

The optimum temperature range for the mycelial growth of *C. cassiicola* isolates was 25-27 °C (Table 1.2). Measurable growth of the fungus occurred at temperature between 20 to 30 °C. At 15°C growth was noticed but not measurable even after 7 days of incubation. At 35 °C no growth was observed. The maximum temperature for the growth of *C. cassiicola* is 32 ±2 °C. In temperature studies also the isolate Cc 04 showed more growth at all the temperature levels studied.

Maximum sporulation was observed at 25 to 27 °C. At 23 °C sporulation occurred only after 12 days of incubation. At 25 °C, the isolate from GT 1 produced spores even after 7 days of incubation. The isolate Cc 03 did not sporulate at 20, 23, 25 and 30 °C but it sporulated at 27 °C. All the six isolates of *C. cassiicola* showed very low growth and sporulation at 20 °C, but after 12 days of incubation the isolates Cc 04, Cc 08 and Cc 33 produced spores.

4.3 Humidity

Very good mycelial growth was noticed at all the relative humidity levels studied (Table 1.3). 90% humidity level was most favourable for all the six isolates of *C. cassiicola.*

Sporulation was noticed in 80, 90 and 100% relative humidity levels. The isolate Cc 04 was dominant with maximum growth rate at all humidity levels. Isolate Cc 03 showed low sporulation at all the humidity levels except 90% RH.

4.4 pH

The growth (biomass) of *C. cassiicola* isolates Cc 02 and Cc 04 was significantly more in medium with pH 6 and isolate Cc 03 with pH 7 (Table1.4). All the other isolates (Cc 05, Cc 08 and Cc 33) grew significantly better at both 6 and 7 pH levels. Significantly poor growth was observed for all the isolates at pH 3 and 9. At pH 5 the growth of isolate Cc 33 was significantly poorer than the rest of the isolates. At pH 8 all the isolates showed similar growth rate but it was not more than at pH 5, 6 and 7.

Table 1.1 Effect of light on radial growth (mm) of *C. cassiicola* isolates

Isolate	Light hours						
	4	5	6	7	8	24	0
Cc 02	52	61	62	47	53	54	52
Cc 03	55	55	58	43	48	52	52
Cc 04	58	74	74	64	64	62	61
Cc 05	52	63	70	48	53	53	51
Cc 08	53	62	65	44	48	58	58
Cc 33	53	59	49	47	40	49	48
CD ($P \leq 0.05$)	2.0	3.80	2.82	3.94	5.12	3.76	3.75

Table 1.2 Effect of temperature on radial growth (mm) of *C. cassiicola* isolates

Isolate	Temperature (^{0}C)						
	15	20	23	25	27	30	35
Cc 02	-	28	30	59	60	59	-
Cc 03	-	24	25	60	60	58	-
Cc 04	-	30	38	68	72	64	-
Cc 05	-	26	28	59	60	54	-
Cc 08	-	26	29	61	61	58	-
Cc 33	-	24	26	52	58	54	-
CD (P≤ 0.05)	-	1.6	1.8	2.3	3.0	2.9	-

Table 1.3 Effect of relative humidity on radial growth (mm) of *C. cassiicola* isolates

Isolate	Relative humidity (%)				
	50	70	80	90	100
Cc 02	57	58	60	63	59
Cc 03	60	58	56	62	60
Cc 04	67	63	68	78	72
Cc 05	63	59	60	66	62
Cc 08	58	59	59	66	60
Cc 33	57	58	53	60	59
CD (P≤0.05)	2.0	1.9	3.2	2.7	2.3

Table 1.4 Effect of pH on biomass (mg) of *C. cassiicola* isolates

Isolate	pH						
	3	4	5	6	7	8	9
Cc 02	-	41	123	286	203	122	-
Cc 03	-	37	220	201	293	126	-
Cc 04	-	66	282	310	318	190	-
Cc 05	-	52	149	226	238	132	-
Cc 08	-	50	166	218	221	156	-
Cc 33	-	50	178	180	189	160	-
CD ($P \leq 0.05$)	-	12	19	33	27	24	-

5. Discussion

The results of the present study may partially explain why the disease of *H. brasiliensis* incited by *C. cassiicola* is a serious problem in all the rubber growing countries. The intensity of the plant disease epidemics are greatly influenced by meterorological and physiological factors. Five and six hours of daily exposure to bright light favours the growth and sporulation of *C. cassiicola* isolates. According to Chee (1988) cultures incubated for three days in the dark followed by three days in light favoured sporulation of *C. cassicola*. Breton *et al.* (2000) reported that sporulation was induced on a 7-day-old culture by exposing for three days, at 28 °C, to continuous light. Almeida and Yamashita (1976) suggested that growth and sporulation of *C. cassiicola* occurred on different media supplemented with V-8 or Gerber's baby food consisting of vegetables, when cultures were kept under continuous light. But on the contrary Peries and Liyanage (1988) observed that the fungus is not light sensitive, as far as growth and sporulation was concerned. The present results points out that *C. cassiicola* is light sensitive. The

fungus favoured five hours of light for growth while sporulation was favoured by alternate exposure to 4, 5 and 6 hours of light and darkness. Although continuous exposure to light and darkness was not detrimental to growth, sporulation was affected.

There are three cardinal temperatures, which affect the mycelial growth of a fungus i.e., the minimum, maximum and optimum temperature (Shamsuri *et al.*, 1997). The minimum temperature required for the measurable growth of *C. cassiicola* was 20 °C and the maximum temperature at which growth of the isolates was not adversely affected was 32 °C. The optimum temperature range for growth was found to be 25 to 27 °C. Though 28 to 30°C was earlier reported as the favorable, the temperature range now observed corresponds with the day temperature experienced in the disease affected areas in South Karnataka (Manju *et al.*, 2001) during March – May.

High relative humidity favoured sporulation and mycelial growth. It has been reported that high relative humidity due to intercultivation or heavy weed growth created ideal micro-climatic conditions for the development of pink disease in Eucalyptus (Seth *et al.*, 1978). High relative humidity is always present under rubber canopy (RH› 90%) whether on dry or wet months (Shamsuri *et al.*, 1997), which leads to increase in severity of the disease. The relative humidity recorded in the Corynespora leaf disease prone areas during peak disease period varied from 31% to 93%.

The present study indicated that the latent period of sporulation of *C. cassiicola* isolates was 5 days with exception. Latent period is important as it greatly influence the rate of disease spread (Vanderplank, 1968). Fungal pathogens with shorter latent period are known to cause epidemics at faster rate (Achuo, 2001). The latent period is also important in disease control strategies, which aim at reduction in the rate of spread of the disease.

4.1 pH

Lilly and Barnet (1951) stated that majority of the fungi grow satisfactorily between pH 5.0 and 6.0. The results of the present studies revealed that the optimum pH for the

growth of *C. cassiicola* ranged from 6 – 7. On the leaf surface, the leaf exudates and the activities of microorganisms influence the pH and effective manipulation of these factors may be beneficial in disease management.

5. Bibliography

Abiko, K. and Ishii, M. (1988). Influence of temperature and humidity conditions on the outbreak of egg plant black rot. *Bulletin of the National Research Institute of Vegetables, Ornamental Plants and Tea,* **2**: 93-98.

Achuo, E. A. (2001). *In vitro* evaluation of exotic *Hevea* genotypes for resistance to *Corynespora cassiicola. Journal of Rubber Research,* **4** (4): 255-269.

Agnihothrudu, V. (1952). Rhizosphere microflora of some of the important crop plants of South India. *Plant Sciences*, **37**: 1-13

Almeida, A. M. R. and Yamashita, J. (1976). Growth and sporulation of *Corynespora cassiicola* (Berk & Curt) Wei. In different cultural media. *Fitopatologia Brasileira*, **1** (3):203-206.

Bilgrami, K. S. and Verma, R. N. (1992). *Physiology of Fungi.* Villkas Publishing House Hvt. Ltd. London, pp: 70-71

Breton, F, Sanier, C. and Auzac, J. (2000). Role of cassiicolin, a host-selective toxin, in pathogenicity of *Corynespora cassiicola*, causal agent of a leaf fall disease of *Hevea. Journal of Rubber Research*, **3** (2): 115-128.

Chee, K. H. (1988). Studies on sporulation, pathogenicity and epidemiology of *Corynespora cassiicola* on *Hevea* rubber. *Journal of Natural Rubber Research*, **3**: 21-29.

Edathil, T. T., Jacob, C. K. and Joseph, A. (2000). Leaf Diseases. In: *Natural Rubber*: *Agromanagement and Crop Processing* (Eds. P.J. George and C. Kuruvilla Jacob). Rubber Rresearch Institute of India, Kottayam, pp. 273-291.

Emerson, R. and Cantino, E. C. (1948). Growth and metabolism of *Blastocladia* in pure culture. *American Journal of Botany*, **35**: 157-171.

Giles, I., Bailey, P. T., Fox, R., Coles, R. and Wicks, T. J. (2002). Prospects for biological control of cut leaf mignonette, *Reseda lutea* (Resedaceae), by *Cercospora resedae* and other pathogens. *Australian Journal of Experimental Agriculture,* **42** (1): 37-41.

Hommes, R. W. J., Postma, P. W., Tempest, D. W. and Neijssel, O. M. (1989). The influence of the culture pH value on the direct glucose oxidative pathway in *Klebsiella pneumoniae* NCTC 418. *Archives of Microbiology*, **151**: 261-267.

Jacob, C. K. (2006 a). Symptoms of Corynespora leaf disease in rubber (*Hevea brasiliensis*) In: *Corynespora leaf disease of Hevea brasiliensis: Strategies for management* (Ed. C. Kuruvilla Jacob). Rubber Research Institute of India, Kottayam. pp 17-25.

Jacob, C. K. (2006). Corynespora leaf disease of *Hevea brasiliensis*: A threat to natural rubber production. In: *Corynespora leaf disease of Hevea brasiliensis*: *Strategies for management* (Ed. C. Kuruvilla Jacob). Rubber Research Institute of India, Kottayam. pp 9-16.

Jayasinghe, C. K., Silva W. P. K. and Singhe, W. D. S. (1998). *Corynespora cassiicola*: a fungal pathogen with diverse symptoms on *Hevea* rubber. *Bulletin of the Rubber Research Institute of Sri Lanka*, **39**: 1-5.

Jinhyeuk, K., Soowoong, K., Jeongsoo, K. and Changseuk, P. (2001). First report on Corynespora leaf spot in pepper caused by *Corynespora cassiicola* in Korea. *Plant Pathology Journal*, **17**: 180-183.

Kenyon, D. M., Dixon, G. R. and Helfer, S. (2002). Effect of relative humidity and photoperiod on the colony development of *Erysiphe* sp. on *Rhododendron*. *Plant Pathology*, **51**: 103-108.

Kwon, J. H., Kang, S. W., Kim, J. S. and Park, C. S. (2001). First report of corynespora leaf spot in pepper caused by *Corynespora cassicola* in Korea. *Plant Pathology Journal*, **17**: 180-183.

Kwon, J., Jee, H. and Park (2005). Corynespora Leaf Spot of Balsam Pear Caused by *Corynespora cassiicola* in Korea. *Plant Pathology Journal*, **21**(2): 164-166.

Lilly, V. and Barnett, H.L. (1951). Physiology of Fungi. McGraw Bill Book Co., Inc. New York. pp. 464.

Manju, M. J., Idicula, S. P., Jacob, C. K., Vinod, K. K., Prem, E. E. Suryakumar, M. and Kothandaraman, R. (2001). Incidance and severity of Corynespora Leaf Fall (CLF) disease of rubber in coastal Karnataka and north Malabar region of Kerala. *Indian Journal of Natural Rubber Research*, **14** (2): 137-141.

Narayanan C. and Sharma J. K. (2004). *In vitro* conidial germination and mycelial growth of *Casuarina* wilt pathogen. *Journal of Mycology and Plant Pathology* 34: 133-135.

Oke, O. A. (1990). The germination, growth and sporulation of *Corynespora cassiicola* (Berk and Curt) Wei causing leaf spot disease of Tobacco. *Nigerian Journal of Botany*, **3**:

Onesirosan, P., Arny, D. and Durbin, R. D. (1975). Increasing sporulation of *Corynespora cassiicola. Mycopathologia*, **55**: 121-123.

Pardo, A. G. and Forchaissin, F. (1993). Effect of light and nutrition on fruiting of *Ascobolus biguttulatus. Current Microbiology*, **27**: 69-72.

Pathak, V. N., Khatri, N. K. and Pathak, M. (1996). Fundamentals of Plant Pathology. Agro Botanical Publishers (India). pp. 121-125.

Pereira, M., Barreto, R. W., Ellison, C. A. and Maffia, L. A. (2003). *Corynespora cassiicola* f. sp. lantanae: a potential biocontrol agent from Brazil for *Lantana camara. Biological Control*, **26**: 21-31.

Peries, O. S. and Liyanage, A. de S. (1987). Diseases of economic importance of *Hevea brasiliensis* and their control. In: *Review of Tropical Plant Pathology* (Eds. S.P. Raychandhuri and J.P. Verma). Today & Tomorrow's Printers and Publishers, New Delhi- 110005. (India).

Peterson, P. D., and Campbell, C. L. (2002). Prevalence and ecological association of foliar pathogens of cucumber in North Carolina, 1996–1998. *Plant Disease*, **86**:1094-1100.

Raffel, S. J., Kazmar, E. R., Winberg, R., Oplinger, E. S., Handelsman, J., Goodman, R. M. and Grace, C. R. (1999). First report of root rot of soybeans caused by *Corynespora cassiicola* in Wisconsin. *Plant Disease*, **83** (7): 696.

Rajalakshmy, V. K. and Kothandaraman, R. (1997). Current status of Corynespora leaf fall in India: The occurrence and management. *Proceedings, Workshop on Corynespora Leaf Fall Diseases of Hevea Rubber*, 16-17 December 1996, Medan, Indonesia, pp. 37-46.

Ramakrishnan, T. S. and Pillay, P. N. R. (1961). Leaf spot of rubber caused by *Corynespora cassiicola* (Berk & Curt) Wei. *Rubber Board Bulletin*, **5** (1): 32-35.

Roger, C. and Tivoli, B. (1996). Effect of culture medium, light and temperature on sexual and asexual reproduction of four strains of *Mycosphaerella pinodes*. *Mycological Research*, **100**: 304-306.

Sánchez-Murillo, R. 1., Torre-Martínez, M., Aguirre-Linares, J. and Herrera-Estrella, A. (2004). Light-regulated asexual reproduction in *Paecilomyces fumosoroseus*. *Microbiology*, **150**: 311-319.

Shamsuri, M. H., Omar, M. and Napi, D. (1997). Studies on epidemiology of pink disease and the effect of temperature on mycelial growth of *Corticium salmonicolor* on *Hevea* rubber. *Journal of Natural Rubber Research*, **12** (1): 58-66.

Sinulingga, W. (1995). Resistance characteristics of large scale recommended rubber clones for 1992-1995 on leaf anatomy infected by *Corynespora cassiicola*. *Indonesian Journal of Natural Rubber Research*, **13** (3): 265-276.

Stone, M. and Scally, J. (2003). Fungal bioterrorism threat gaining public interest, yet not biggest concern of fungal specialists, survey finds. *Mycologia*, **54**: 1-2.

Tsay, J. G., and Kuo, C. H. (1991). The occurrence of Corynespora blight of cucumber in Taiwan. *Plant Protection Bulletin*, **33**:227-229

Vanderpank, J. E. (1968). Disease Resistance in Plants. New York, Academic Press. pp. 205.

Volz, P. A. and Beneke, E. S. (1969). Nutritional regulation of basidiocarp formation and mycelia growth of Agaricales. *Mycopathologia*, **37:** 225-253

Wataru, H., Hideaki, Y., Tukuhiro, K. and Suzumi, M. (2005). Ecology and Control of Leaf Spot of *Perilla* Caused by *Corynespora cassiicola. Bulletin of the Oita Pretectural Agricultural Research Center,* **35**: 13-41.

Chapter 2

Nutritional and physiological studies on *Corynespora cassiicola*

1. Introduction

Parasitism of a pathogen involves a nutritional relationship with the host from where it derives its entire nourishment (Nair, 2004). The effect of mineral nutrition on fungal growth and sporulation are usually explained in terms of the function of these elements in fungal metabolism. However, mineral nutrition may also exert influences on the growth and sporulation of fungi by effecting changes in fungal morphology, anatomy and physiology. The nutritional factors are always an important component of disease development and it may either increase or decrease the resistance/tolerance of the plants to pathogen (Marschner, 1995).

2. Review

This review covers the nutritional requirements and physiology of fungi in general as there were very little information on *C. cassiicola* or related fungal species.

2.1 Media

Fungi derive food and energy from the substrate on which they grow. In order to culture fungi in the laboratory, it is necessary to furnish in the medium those essential elements and compounds they require for the synthesis of their cell constituents and for the operation of their life processes. Some fungi are unable to synthesize certain essential compounds which they must obtain from the medium upon which they grow. Although the essential elements required for growth are same, there is no universal natural substrate or artificial medium on which all fungi grow (Rani and Murthy 2004).

Potato Dextrose Agar has been reported as one of the best medium for growth and sporulation of *C. cassiicola* (Volin and Pohronezny (1989). Dung (1995) observed variation in colony colour and growth rates of four *C. cassiicola* isolates on artificial media. Silva *et al.* (1998) studied 27 isolates of *C. cassiicola* from rubber in Sri Lanka

and five isolates from diverse hosts in Australia and reported that the growth on PDA varied considerably among the isolates.

2.2 Carbon

Carbon is an element basic to the structure and function of all cells and is thus crucial for the survival of living organisms. Carbon sources serve two essential functions in the physiology of fungi and other heterotrophic organisms. They supply, in the first place, the carbon needed for the synthesis of critical constituents such as carbohydrates, proteins, lipids and nucleic acids. Secondly, their oxidation provides a source of energy for proper functioning of the essential life processes of fungi. Fungi as a group are capable of utilizing a large variety of carbon compounds for growth and/or development. These range from small molecules such as sugars, organic acids and alcohols to large polymers such as proteins, lipids, polysaccharides and lignin. However, fungi cannot utilize all carbohydrates equally well and are selective in their ability to utilize sugars and other nutrients. The nutritional requirements differ among species and sometimes even among strains of the same species. Knowledge of carbon nutrition, therefore, is fundamental to an understanding of the physiology of the fungi. Much valuable work has been done in this area (Maas, 1976; Singh and Devi, 1996; Terashima, 1999; Jonathan and Fasidi, 2001), but it is apparent that the data required for confident generalization are still lacking.

The most widely used carbon sources are sucrose and glucose, followed by fructose, lactose and starch. Other sources are less suitable for the growth and sporulation of majority of fungi. Kumari *et al.* (1998) studied the effect of carbon sources on the growth and sporulation of *Alternaria brassicae* and reported sucrose as the best source for the mycelial growth and sporulation followed by galactose and maltose. Shreemali (1973) found better utilization of glucose and sucrose by *Botryodiplodia theobromae* for growth and sporulation. The superiority of glucose over other hexoses for the growth and sporulation has also been reported for several imperfect fungi including *Colletotrichum lindemuthianum, C. falcatum* (Mathur *et al.*, 1950; Kalaimani, 1997) and *Helminthosporium rostratum* (Agarwal and Sinkhede, 1959).

Kapoor and Singh (1973) studied the similarities and differences in utilization of two monosaccharides (glucose and fructose), a disaccharide (sucrose) and a polysaccharide (starch) by nine isolates of *Colletotrichum gloeosporioides* pathogenic on citrus in India. Glucose was observed to be utilized by seven and fructose by four. But the growth on glucose and fructose showed much variation. All the nine isolates showed similarity in their mode of utilization of sucrose but two isolates utilized sucrose through a hydrolytic pathway, which is an indication that they are capable of producing sucrase enzyme in the sufficient quantity.

Bahl and Grewal (1973) reported maximum growth of *Operculella padwickii*, causal agent of foot rot of *Cicer arietinum* in a medium containing glucose as the sole source of carbon followed by starch, sucrose and sorbitol. They also found that cellobiose, xylose, glycerol and inulin are poor sources of carbon for *O. padwickii* and there was no sporulation when sugar alcohol was used as source of carbon.

Verma and Prasad (1975) studied the utilization of carbohydrates by three fungi imperfecti (*Pestalotiopsis adusta, Botryodiplodia theobromae* and *Colletotrichum gloeosporioides*) causing leaf spot diseases of mahogany and reported that these pathogens could attain good growth on monosaccharides and efficiently utilize oligosaccharides through hydrolytic pathway. Investigations carried out by various researchers (Semino and Robbins, 1995; Smaali *et al.* 2007; Deshpande *et al.* 2008; Kruszewska *et al.* 2008) proved that fungi are capable of not only causing rupture of glycosidic bond but also synthesizing new oligosaccharides by transglycosidation.

Of the alcohols, only glycerol proved to be a good source in a few cases, while sorbitol, mannitol (Omanor *et al.*, 2008), organic acids and polysaccharides such as chitin, cellulose or pectin (Clarke, 1966) appeared to be very poor sources, despite the fact that the latter two compounds are major components of host cell walls penetrated by these fungi. Comparative study of the isolates of *C. gloeosporioides* by Assis *et al.* (2001) showed starch as the best carbon source for the mycelial growth and sporulation.

The variation in the utilization of sugars by some isolates may have been influenced by the method of sterilization of the medium. McKeen (1956) has shown that a toxic substance is formed when glucose and any one of the amino acids, including asperagine are autoclaved together.

Cook and Schroth (1965) observed thatw certain species of *Fusarium* require exogenous nutrients for germination because of insufficient endogenous reserves. Role of carbohydrates in inducing/inhibiting spore germination is well known. Some of the sugars are reported to increase germination whereas others have an inhibitory action (Eckert, and Ratnayake, 1994). Investigation of Ogava *et al.* (1999) revealed that germination of spores of *Exserohilum monoceras* increased as the sucrose or glucose concentrations increased from zero to 0.3% and the fructose concentrations increased to 0.1%. They also reported decreased spore germination with higher concentrations of carbon sources.

2.3 Nitrogen

Nitrogen is indispensable for fungal growth and development. Fungi require nitrogen for the synthesis of a variety of critically important cellular constituents, including amino acids, proteins, purines, pyrimidines, nucleic acids, glucosamine, chitin and various vitamins.

In general, nitrate nitrogen is most favorable for mycelial growth of many fungi (Lilly and Barnett, 1951; Goyal, 1980; Ahmad and Mir, 1998). The only known metabolic route for the assimilation of nitrate nitrogen is by reduction to nitrite via the enzyme nitrate reductase and then to ammonia (Pateman and Kinghorn, 1976). Christie (1958) reported calcium nitrate as the best source of nitrogen for the growth of *Phytophthora cactorum* but it was found to be a poor source for the growth of *Phytophthora drechsleri*. Kumari *et al.* (1998) studied the effect of different nitrogen sources on the growth and sporulation of *Alternaria brassicae* and found potassium nitrate as the best source for the growth followed by sodium nitrate and asperagine.

Roncadori (1965) as well as Cameron and Milbrath (1965) reported ammonium nitrate as the best source of nitrogen for the growth of *P. drechsleri.* According to Koul (1987), calcium nitrate was the best source of nitrogen for the growth of *Botryodiplodia theobromae*. He also pointed out that the fungus is capable of utilizing nitrate, ammonia and organic forms of nitrogen.

The effect of different nitrogen compounds on shot hole disease of stone fruits caused by *Wilsonomyces carpophillus* was investigated *in vitro* by Ahmad and Mir (1998). They found asperagin as the best nitrogen source that supported vegetative growth followed by sodium nitrate, potassium nitrate and ammonium sulphate. Dar (1999) found that peptone followed by asperagine were significantly superior to other nitrogen sources studied for the growth of *Chladobotryum dendroides*, the causal agent of cobweb disease of cultivated mushroom (*Agaricus bisporus*) and The studies of Palarpawar and Ghurde (1997) showed remarkably good growth of *Colletotrichum capsici* when peptone was used as the nitrogen source. They also observed that ammonium oxalate and sodium nitrite were the poorest sources for mycelial growth and sporulation.

Although nitrite is utilized as a nitrogen source by some fungi (Agnihotri, 1968), it is toxic to many species (Bidari and Govindu, 1975; Goyal, 1980). Pateman and Kinghorn (1976) reported that the nitrite toxicity is due to its ability to deaminate amino acids and to its interference with sulphur metabolism because of its similarity with sulphur ion. Pal and Grewal (1975) reported that the growth of *P. drechsleri* was negligible on sodium nitrate, which in turn indicated that the fungus is unable to utilize nitrite nitrogen or fix atmospheric nitrogen for its growth. According to Agnihotri (1968) poor growth on nitrite may be related in part to an effect of pH. On the other hand, Tandon and Agarwal (1953) reported utilization of nitrite nitrogen by *Fusarium coeruleum.*

2.4 Sulphur

Sulphur is an essential macronutrient as it is one of the major components of proteins and vitamins. Kertesz, and Mirleau (2004) has made an exhaustive study of sulphur sources for soil microbs and reached the general conclusions that inorganic sulphur compounds containing oxidized sulphur are utilized, while sulphide and disulphide sulphur are not utilized. Of the organic compounds containing sulphur, the alkyl thioalcohols, sulphides and disulphides are not used. Alkyl sulphonates and sulphinates are excellent sources of sulphur.

Fungi vary considerably in their ability to utilize different sources of sulphur (Levic and Pencic, 1990). Singh and Shankar (1971) studied the effect seven sulphur sources on growth and sporulation of *Glomerella cingulata* and reported maximum growth for manganese sulphate followed by sodium thiosulphate, zinc sulphate, potassium sulphate, sodium bisulphate, ammonium sulphate and magnesium sulphate. Magnesium sulphate was reported as the best sulphur source for the growth of *Gliocephalotrichum bulbilium*, but the growth was poor with zinc sulphate and sulphites (Jamaluddin *et al.*, 1975).

Pestalotiopsis funera can use a variety of organic and inorganic sulphur sources for growth and sporulation as described by Upadhyay and Dwivedi (1979). Magnesium sulphate is the best source for both these processes while thiourea is not utilized at all. Many lower fungi, including all members of the Blastocladiales, are unable to use sulphate as a source, but can grow on reduced forms of inorganic sulphur as well as organic sources.

2.5 Phosphorus

Phosphorus is an integral component of important macromolecules such as DNA, RNA and phospholipids as well as smaller molecules such as NAD, FAD, thiamine pyrophosphate, pyridoxal phosphate, vitamin B_{12} and coenzyme A. In addition, as a component of nucleotides such as ATP, GTP, CTP and UTP, phosphates are intimately involved with the storage and transfer of energy in the cell. Zhang and Roehr (2002)

found phosphorus to be an essential element for *Aspergillus niger*. Omission of phosphate from the synthetic medium reduced the biomass yield approximately by 50 per cent. Lima *et al* (2003) studied the effect of phosphorus on polyphosphate accumulation by *Cunninghamella elegans* and found that at three days of growth the biomass consumed up to 100 and 95% of inorganic phosphate from the media.

Roomans *et al.* (1979) and Okorokov *et al.* (1975) reported that phosphate stimulates the transport of divalent cations such as calcium and magnesium. However, the uptake of certain monovalent cations is inhibited by phosphate (Roomans and Borst-Pauwels, 1977). Alterations in the phosphorus concentration of the medium often result in striking changes in many biochemical processes. Lowering the phosphate content decreases the rate of glycolysis in *Saccharomyces cerevisiae* (Borst-Pauwels, 1967) and alters the relative participation of the Embden-Meyerhof pathway and the hexose-monophosphate shunt in *Candida utilis* (Dawson and Steinhauer, 1977).

2.6 Other macro elements

Potassium is essential for all organisms. However the effects of potassium on fungi have not been studied extensively. The relation between the quantity of potassium in the medium and the weight of mycelium produced by *Aspergillus niger* was studied by Steinberg (1945). The enzymes in yeast maceration juice which ferment glucose are activated by either potassium or ammonium ions as observed by Lichstein (1952). Guimaraes and Stotz (2004) noted that a low potassium content of the medium resulted in increased synthesis of oxalic acid by *Sclerotinia sclerotiorum*. Papagianni (2004) observed that the chemical composition of fungal mycelium varies, depending upon the quantity of potassium in the medium. The loss of potassium results in the inhibition of a variety of metabolic processes, including glycolysis and respiration (Garraway and Evans, 1984). He also revealed that the major role of potassium in fungi is regulation of the cellular osmotic potential. The osmotic potential of the cell relative to that of the medium determines whether or not water will enter and thus bring about the turgor pressure necessary for growth.

Siegenthaler *et al.* (1967) and Sykes and Porter (1973) reported requirement of sodium by *Thraustochytrium* and *Labyrinthula* species. Most fungi, however, are inhibited by sodium at concentrations above a certain level (Gisi *et al.*, 1977; Jones and Jennings, 1965). Tresner and Hayes (1971) tested 975 fungal species for their tolerance to NaCl. High intracellular sodium concentrations have been shown to inhibit several biochemical processes including respiration and fermentation (Norkrans, 1968), the synthesis of aflatoxins (Uriah and Chipley, 1976), and cellulolytic activity (Malik *et al.*, 1980).

Magnesium is required by all fungi and has a wide variety of regulatory roles. The intracellular magnesium concentration varies widely depending on the species, the stage of growth and the composition of the culture medium. *A. niger* has been more carefully investigated with respect to the effects of magnesium than any other fungus. Within certain limits of magnesium, Steinberg (1945) and Okorokov *et al.* (1975) demonstrated that the growth of *A. niger, Endomyces magnusii* and *Penicillium chrysogenum* is proportional to the magnesium in the medium. Tabak and Cooke (1968) demonstrated that *Penicillium glaucum, Botrytis cinerea* and *Alternaria tenuis* failed to grow in the absence of magnesium. Almost all metabolic reactions involving the transfer of phosphate groups usually require either magnesium or manganese ion as a co-factor.

The growth and reproduction of many species of fungi are enhanced when calcium is added to the culture medium. Jackson and Heath (1993) studied the role of this element in the fungal hyphal tip and showed no growth in the absence of this element. Calcium stimulated the production of sporangia in *Phytophthora fragariae* (Maas, 1976), *P. cactorum* (Elliot, 1972) and *Saprolegnia diclina* (Fletcher, 1979). Several species of *Pythium* require calcium for oospore formation (Lenny and Klemmer, 1966). Jongbloed and Borst-Pauwels (1992) demonstrated a synergistic effect between manganese and calcium upon the growth of various species of *Lactarius,* an ectomycorrhizal fungus.

2.7 Micro elements

Zinc is a functional component of a variety of fungal enzymes ranging from those involved in intermediary metabolism to the synthesis of DNA and RNA. One of the most thoroughly studied zinc enzymes is alcohol dehydrogenase, which contains four zinc atoms. Replacing zinc with cobalt results in an 83% decrease in the enzyme activity (Sytkowski, 1977). In yeast RNA polymerase, zinc aids in attaching the entering nucleotide to the growing RNA chain (Lattke and Weser, 1977). Zinc affects the production of a variety of metabolites. In *A. niger*, maximum citrate production occurs with low concentrations of zinc in the medium as observed by Wold and Suzuki (1976). White and Johnson (1971) reported the production of pigment cynodontin by *Helminthosporium cynodontis* to be influenced by zinc.

Zinc is an essential element for *A. niger* (Santen *et al.* 1999). Angel *et al.* (2007) reported the importance of zinc for *Trychophyton interdigitale, Rhizopus nigricans* and *Saccharomyces cerevisiae.* Similarly, Levinskaite, (2004) found zinc to be essential for the efficient utilization of different carbon sources by *Penicillium.* Bau (1979) reported the effect of zinc on growth, pigmentation and antibacterial activity of *Monascus purpureus.*

Copper is essential for animals, green plants and fungi because of its role as a metal activator of several fungal enzymes, particularly oxidases. However, at supraoptimal concentrations copper is a potent inhibitor of fungal growth and is used as a fungicide. Thus, the concentration of copper in growth media is of critical concern. Kurtz and Champe (1981) observed that the conidia colour of *A. nidulans* depended upon the copper content of the medium. Several copper-binding proteins are enzymes requiring Cu^{2+} for maximal activity. In addition, copper has been shown to have mutagenic properties that induce the formation of yeast strains with increased resistance to copper (Antoine, 1965).

Iron is an essential element for growth of all fungi. The most obvious effect of suboptimal iron concentrations upon fungi is decreased growth due to limited amount of

iron-containing enzymes formed under these conditions. It was shown by Barnes (1999) that the amount of catalase produced by *Aeromonas salmonicida* sub sp *.salmonicida* increased with the quantity of iron in the medium.

Soccoll *et al.* (2006) showed that the iron concentration is an important factor in citric acid fermentation of *A. niger*. Iron deficiency alters the biochemical processes of the host. Thomas and Dawson (1978) reported decreased levels of ATP in iron-deficient cells. Iron has been shown to form complexes with RNA (Pezzano and Coscia, 1970) and iron may interact with DNA in a manner that switches developmental processes on and off (Hall and Axelrod, 1978).

Boron is reported to stimulate the growth of several fungi, such as *Cercospora dolichi* (Rawla *et al.*, 1977), *Myrothecium verrucaria, Stachybotrys atra* and *Alternaria tenuis* (Naplekova and Anikina, 1970).

Molybdenum is essential for the utilization of nitrate nitrogen fungi and the fixation of atmospheric nitrogen by bacteria (Zhang and Gladyshev, 2008; Marino *et al.*, 2003). Marzluf (1997) found that more molybdenum was required by *A. niger* for maximum growth in media containing nitrate nitrogen than in media with ammonium nitrogen. Marzluf expressed the opinion that molybdenum is essential for *A. niger* even when ammonium nitrogen is available. Additional studies on *A. niger* and other organisms (Zhang, and Gladyshev, 2008) indicated that an increased need for molybdenum is associated with nitrate utilization. It may be assumed that the enzymatic reduction of nitrate is carried out by enzymes which require molybdenum as an activator.

2.8 Vitamins

Vitamins are organic compounds needed for growth, development and metabolite production. Typically they function as coenzymes or constituents of coenzymes and consequently are needed in minute amounts by the fungal cell. While many fungi can synthesize their own vitamins from simple precursors others have one or more vitamins supplied exogenously.

Oluma and Amuta (1999) indicated maximum growth of *C. cassiicola* on thiamine supplemented media. Fungi in which thiamine inhibits growth and/or reproduction include *Rhizoctonia solani* (Khan and Azam, 1975), *Suillus variegates* (Langkramer, 1969) and *Helminthosporium nodulosum* (Hegde and Rangansthaiah, 1971). Repression of the synthesis of the carrier protein or protein with the intracellular accumulation of thiamine has also been reported (Iwashima *et al.*, 1979; Iwashima and Nose, 1976; Iwashima and Nishimura, 1979).

Highest sporulation of *C. cassiicola* was noticed on biotin supplemented medium (Oluma and Amuta, 1999). The addition of biotin to the culture medium has been shown to decrease the activities of enzymes such as isocitrate lyase (Nabeshima *et al.*, 1977), glucose-6-phosphate dehydrogenase and glutamine synthetase (Desai and Modi, 1977); to increase the content of DNA, RNA and protein (Aurich *et al.*, 1967); and decrease the concentration of organic acids (Kacchy *et al.*, 1972). Addition of oleic, palmitoleic or linolenic acid can partially overcome biotin deficiency (Mizunaga *et al.*, 1975).

Some fungal species including *Aspergillus sp.* (Shchelokova and Vorobeva, 1982) are capable of synthesizing biotin, and some such as *Ashbya gossypii* (Dietrich *et al.*, 2004) lack the genes necessary to synthesize biotin. Some fungal species that are incapable of synthesizing biotin still have portions of the biotin biosynthetic pathway and are capable of utilizing intermediates to synthesize biotin (Phalip *et al.*, 1999; Wu *et al.*, 2005).

2.9 Growth regulators

Growth regulators are reported to influence the growth of some fungal plant pathogens (Al-Masri *et al.*, 2002). Shanmugam and Govindaswamy (1973) reported maximum growth of *Macrophomina phaseoli* in NAA at 10 ppm concentration followed by IAA 10 ppm. Among the five growth regulators tried only NAA inhibited the growth at 50 ppm. Ganacharya and Wankar (1977) observed that *Fusarium oxysporum* responded well to the external supply of growth regulators. This fungus preferred maleic

hydrozide for mycelial growth followed by IAA, NAA and GA, which indicated that the isolate was lacking these growth regulators.

Singh and Singh (1996) studied the effect of some plant growth regulators on mycelial growth and sclerotium formation in *Sclerotinia sclerotiorum* and reported that all growth regulators increased the mycelial growth at lower concentrations but as the concentration increased there was inhibitory effect. Misra and Mahmood (1961), while studying the effect of growth regulators on *Colletotrichum capsici* found that lower concentrations of IBA and NAA were beneficial for the fungal growth. Addition of growth regulators increased the growth of *Alternaria burnsii* (Sankhla *et al.*, 1970), *Rhizoctonia bataticola* (Sankhla and Mathur, 1967).

2.10 Phenolics

Fungal infection of plants induces phenolic substances (Borva and Das, 2000). The relationship between the presence of toxic compounds and the extent of fungal growth in plant tissues has been well established (Soni *et al.*, 1992). Growth studies of different phenolic compounds indicated that growth of *Cercosporium personatum* decreased with increasing concentration. Among the phenolic compounds, phenol was reported to be most toxic to fungal growth (Mahadevan, 1982).

Venkatachalam and Jayabalan (1995) reported the increased fungal growth inhibiting potential of catechol. Similar observations were recorded by Soni *et al.* (1992) in case of *Fusarium oxysporum*. Xiujuan *et al.* (1995) showed inhibition of growth and development of *Colletotrichum musae* isolates from banana fruits and *C. gloeosporioides* from mango fruits when treated with suitable concentrations of tannin, resorcinol, catechol, chlorogenic acid and caffeine.

Purkait and Purkayastha (1996) studied the pectolytic enzyme activities of some foliar fungi isolated from mangrove plants and their response to tannin. They proved the inhibitory action of tannin on pectolytic enzymes and that the inhibitions vary with fungal

species. Appel, (1993) recorded the inactivation of enzymes by phenolics including tannin of host plant.

2.3. Materials and methods

2.3.1 Media

The following basal media were used in this study. The media were modified suitably to suite each experiment and treatment.

2.3.1.1 Potato dextrose agar (PDA)

Potato (peeled and sliced)	-	200.0 g
Dextrose	-	20.0 g
Agar	-	20.0 g
Distilled water	-	1000.0 ml
pH	-	6.5

Potato slices were steamed in 900 ml of distilled water for 30 minutes and the extract was filtered through a strainer. Agar was added to this and boiled till it dissolved completely. This preparation was made up to 1000 ml with distilled water after adding dextrose and the pH adjusted.

2.3.1.2 Potato sucrose agar

Potato sucrose agar was prepared as discussed in PDA by replacing dextrose with sucrose.

2.3.1.3 Czapek dox agar (CDA)/ Czapek dox broth (CDB)

$NaNO_3$	-	2.0 g
K_2HPO_4	-	1.0 g
$MgSO_4.7H_2O$	-	0.5 g
KCl	-	0.5 g
$FeSO_4.7H_20$	-	0.01 g

Sucrose	-	30.0 g
Agar	-	20.0 g
Distilled water	-	1000.0 ml
pH	-	6.8

In the case of CDB, agar was not added.

2.3.1.4 Richard's synthetic agar (RSA)/ Richard's nutrient solution (RNS)

KNO_3	-	10.0 g
K_2HPO_4	-	5.0 g
$MgSO_4.7H_2O$	-	2.5 g
$FeCl_3$	-	0.02 g
Sucrose	-	50.0 g
Agar	-	15.0 g
Distilled water	-	1000.0 ml
pH	-	5.4

In the case of RNS, agar was not added.

2.3.1.5 Martin's rose bengal agar

Peptone	-	5.0 g
K_2HPO_4	-	1.0 g
$MgSO_4.7H_2O$	-	0.5 g
Rose bengal	-	30.0 mg
Dextrose	-	10.0 g
Agar	-	15.0 g
Water	-	1000.0 ml
pH	-	5.5

2.3.1.6 Sabouraud agar

Peptone	-	10.0 g
Dextrose	-	40.0 g
Agar	-	15.0 g

Water	-	1000.0 ml
pH	-	5.6

2.3.1.7 Glucose asparagine agar

Asparagine	-	0.5 g
KH_2PO_4	-	1.5 g
$MgSO_4.\ 7H_2O$	-	0.5 g
Dextrose	-	10.0 g
Agar	-	15.0 g
Water	-	1000.0 ml
pH	-	6.0

2.3.2 Carbon and nitrogen utilization

For comparison of isolates based on carbon and nitrogen source assimilation, sources containing carbon (10g/L) and nitrogen (2g/L) were added to the C-dox media. The final pH of the medium was adjusted to 7. The chemically pure carbon sources evaluated were: dextrose, fructose, mannitol, sucrose, lactose, sorbitol, starch and cellulose. The nitrogen sources evaluated were potassium nitrate, calcium nitrate, ammonium nitrate, ammonium sulphate, sodium nitrate, asparagine, peptone and urea. Aliquots of the medium (50 ml) were dispensed in 100 ml conical flasks and sterilized. After the inoculation with three 5 mm mycelial discs of *C. cassiicola* and incubation for 15 days at 25 °C, mycelial mats were filtered out dried to constant weight to record the fungal growth as dry weight for each isolate of *C. cassiicola* on every carbon and nitrogen source employed.

In order to study the effect of carbon and nitrogen sources on spore germination, the spore suspensions from seven-day-old *C. cassiicola* maintained on potato dextrose agar medium were made in sterilized distilled water, incubated at 27°C with different carbon (1%) and nitrogen (1%) sources. Spore suspension in sterilized distilled water served as control. Observations on the germination of spores were recorded after 24

hours. Two hundred spores were counted for each observation and three repetitive sets were maintained for each treatment.

3.3 Sulphur and phosphorous

Richard's medium was used as the basal medium and the quantity of sulphur (2g/L) and phosphorus (2g/L) in the basal medium was substituted singly with equal quantity from the different sulphur (Magnesium sulphate, potassium sulphate, sodium sulphate, sodium sulphite, sodium thiosulphate, ammonium sulphate and zinc sulphate) and phosphorus (Magnesium phosphate, potassium phosphate, sodium pyrophosphate, ammonium biphosphate and disodium hydrogen orthophosphate) containing compounds separately. After 15 days incubation, the mycelial growth was collected by filtering through whatman's filter paper no. 42, dried to constant weight in oven and the dry weight recorded.

3.4 Effect of other macroelements

To determine the effect of macro elements on growth and sporulation of *C. cassiicola*, four macro elements were chosen, and the method described by Steinberg (1945) was followed. The basal solution (containing 30g sucrose, 2g Na NO_3, 1g KH_2 PO_4, 5g Mg SO_4. $7H_2O$, 2g Ca NO_3, Fe^{++} 10ppm) was substituted with $(NH_4)_2$ H_2 PO_4 instead of KH_2 PO_4 keeping the amount of H_2 PO_4 constant in order to test the effect of potassium. Similarly, $MgSO_4$ was substituted by $(NH_4)_2$ SO_4, Ca NO_3 by NH_4 NO_3 and Na NO_3 by NH_4 NO_3 respectively. The experiments were conducted at 27°C. Solid macronutrient media were also employed to study the sporulation of *C. cassiicola* isolates.

3.5 Effect of microelements

The effect of various trace elements on growth of isolates *in vitro* was studied separately using liquid and solid Richard's solution as the basal medium. The trace elements zinc, copper and iron were incorporated to the medium in the form of sulphates. Boron and molybdenum were added as boric acid and ammonium-molybdate respectively.

In all the experiments with liquid media, 50 ml aliquots of medium was poured in each 100 ml flasks and sterilized. After the inoculation of three 5 mm mycelial discs of *C. cassiicola* and incubation for 15 days at 27°C, mycelial mats were filtered out and the fungal growth recorded as dry weight. In solid media, mycelial discs of 5 mm diameter were placed invertedly to determine the radial growth and sporulation after seven days incubation.

3.6 Leaf wilt bioassay

Coloured culture filtrate after the growth of the *C. cassiicola* isolates in media containing the different trace elements were filtered through whatman No. 42 filter paper and biowilt assay was performed to study the toxin production by the six *C. cassiicola* isolates. In the leaf wilt bioassay, petioles of *C. cassiicola* susceptible *Hevea* leaflets (RRII 105) and resistant leaflets (GT 1) were excised from the stem under water and immediately transferred to 15 ml flasks containing 5 ml culture filtrate and incubated for 48 h and wilting percentage was calculated. To test the thermostable nature of the toxic principle, the culture filtrate was autoclaved at 1.05 kg/cm^2 pressure for 20 minutes and biowilt assay performed using leaflets of clones RRII 105 and GT 1. Appropriate controls were maintained using uninoculated media and sterile water.

3.7 Amino acids

To study the effect of amino acids on growth and sporulation of *C. cassiicola* isolates, different amino acids were separately sterilized at 1.05 kg/cm^2 pressure for 10 minutes and added aseptically to liquid and solid nitrogen free Richard's media and pH adjusted to 7.0 prior to inoculation of the fungal isolates.

3.8 Vitamins

The basal medium employed for the vitamin studies consisted of 10 g glucose, 2 g asperagine, 1 g KH_2PO_4, 0.5 g $MgSO_4$. $7H_2O$ and 1000 ml distilled water. 25 ml of the medium was apportioned in each 100 ml flasks and sterilized. The desired concentration

of each vitamin (thiamine, biotin, inositol, pyrodixin, ascorbic acid and nicotinic acid) was added separately prior to inoculation. The pH of the medium was adjusted to 6.0. The basal medium without vitamin served as the control.

3.9 regulators

The basal Richard's medium was supplemented with 100 ppm each of IAA, IBA, NAA and GA_3 separately. This was done after autoclaving the medium. To each flask having a specific growth regulator, 5 mm disc of the inoculum from the culture grown for seven days on PDA was added. These were then incubated at 27± 2^0C for 15 days separately. Three replications were maintained for each trial. Estimation of fungal dry weight was done as described earlier.

4 Results

4.1 Nutrition

4.1.1 Carbon

The dry weights of the fungus after its growth in various carbon sources are presented in Table 2.1. Among the different carbon sources tested, maximum growth of all the six isolates of *C. cassiicola* was obtained in medium with lactose as carbon source, which is followed by sucrose, mannitol, sorbitol, fructose and dextrose. Starch and cellulose supported significantly poor growth with all the isolates. Incubation of spores of *C. cassicola* in different carbon source (1%) for 24 hours at 25 °C showed that spore germination was observed with all the carbon sources tested. Slight increase in germination of spores in sorbitol and fructose as compared to control was also observed.

4.1.2 Nitrogen

Response of various nitrogen sources indicated that all the sources tried were utilized satisfactorily (Table 2.2). In the present studies, isolates Cc 02, Cc 03, Cc 05 and Cc 08 grew better in peptone-amended medium, whereas isolates Cc 04 and Cc 35

preferred potassium nitrate and calcium nitrate respectively for their maximum growth. All isolates of *C. cassiicola* showed low growth in urea-amended medium. Ammonium sulphate, ammonium nitrate, sodium nitrate and asparagine supported moderate growth of *C. cassiicola* isolates. Effects of nitrogen sources on spore germination revealed that all nitrogen sources help in the germination of spores, but only slight increase in germination as compared to control was observed in potassium nitrate, asparagine, urea, sodium nitrate and peptone.

Table 2.1 Effect of carbon sources on the growth of *C. cassiicola*

Carbon source	Average dry weight of mycelium (mg)					
	Cc 02	Cc 03	Cc 04	Cc 05	Cc 08	Cc 33
Dextrose	175	188	224	174	171	190
Fructose	160	196	179	192	173	179
Sucrose	217	229	188	175	190	201
Lactose	263	276	238	179	217	234
Mannitol	177	180	197	173	181	185
Sorbitol	174	175	224	171	192	176
Cellulose	36	68	39	50	39	38
Starch	23	29	27	27	31	25
Control	9	8	11	9	8	11
CD $P \leq 0.05$	12.36	10.62	13.54	11.86	9.22	10.76

CD $P \leq 0.05$ for isolates = 11.00; Carbon sources = 9.1; Interaction = 8.31

Table 2.2 Effect of nitrogen sources on the growth of *C. cassiicola*

Nitrogen Source	Average dry weight of mycelium (mg)					
	Cc 02	Cc 03	Cc 04	Cc 05	Cc 08	Cc 33
Potassium nitrate	780	451	296	313	347	340
Ammonium sulphate	583	275	193	528	443	459
Ammonium nitrate	339	371	247	201	303	281
Calcium nitrate	489	395	299	525	398	757
Sodium nitrate	394	389	239	286	304	306
Asparagine	495	485	310	259	334	353
Peptone	672	635	470	616	617	485
Urea	256	253	193	158	229	202
Control	31	34	20	16	16	16
CD $P \leq 0.05$	12.34	13.02	10.54	11.43	9.67	9.99

CD $P \leq 0.05$ for isolates = 13.88; nitrogen sources = 10.12; interaction = 15.2

4.1.3 Amino acid

Among the different amino acids tested, proline supported maximum growth and sporulation of all the six isolates of *C. cassiicola* on Richard's media (Table 2.3). Next to proline, glycine, monohydrochloride and methionine also promoted the growth and sporulation of *C. cassiicola* isolates. Glutamic acid was used efficiently by *C. cassiicola*

isolates Cc 05 and Cc 08. Isolate Cc 03 did not sporulate with any of the amino acids studied. In glycine amended medium, *C. cassiicola* isolates Cc 02, Cc 03, Cc 04, Cc 05, and Cc 33 showed reduced growth, while isolate Cc 08 showed good growth.

4.1.4 Sulphur

Corynespora isolates showed better growth in magnesium sulphate amended medium than the other sulphur sources studied (Table 2.4). Isolates Cc 03 and Cc 04 grew more in zinc sulphate medium than the rest of the isolates but the growth was less when compared to the growth in $MgSO_4$ amended medium. A lower growth was noticed in ammonium sulphate, sodium thiosulphate and sodium sulphate amended media although with exceptions. In sodium thiosulphate containing medium isolate Cc 03 had more growth than the rest of the isolates. Cc 05 showed lowest growth in medium containing ammonium sulphate as the sulphur source.

4.1.5 Phosphorus

All the *C. cassiicola* isolates showed higher growth in disodium hydrogen orthophosphate amended medium (Table 2.5). Cc 02, Cc 03 and Cc 04 grew better than the other isolates. Phosphorus sources like magnesium phosphate and potassium phosphate were also preferred for the good growth of the isolates, but Cc 05 and Cc 33 showed reduced growth in magnesium phosphate amended medium. *C. cassiicola* isolates also grew in sodium pyrophosphate and ammonium biphosphate containing media but the growth was comparatively less than the rest of the phosphorus sources studied. In the basal (control) media all the isolates showed a significantly poor growth than in the amended media.

Table 2.3 Effect of amino acids on growth and sporulation of *C. cassiicola*

Isolate	Radial growth and sporulation											
	Proline		Glutamic acid		Methionine		Glycine		Monohydrochloride		Control	
	RG	Sp	RG	Sp	RG	Sp	RG	Sp	RG	Sp	RG	Sp
Cc 02	60	++	57	-	51	-	59	++	60	++	20	-
Cc 03	65	-	42	-	56	-	54	-	58	-	19	-
Cc 04	81	++	50	+	61	+	56	+	61	++	23	-
Cc 05	77	++	71	++	61	+	53	++	62	+	24	-
Cc 08	67	++	64	+	63	++	65	++	64	+	19	-
Cc 33	63	++	55	+	59	++	52	++	62	+	22	-
CD $P \leq 0.05$	1.35	-	1.43	-	1.67	-	1.60	-	1.76	-	0.54	-

CD $P \leq 0.05$ for isolates = 1.18; amino acids = 1.24; interaction = 1.18

RG = Radial growth Sp = Sporulation

Table 2.4 Effect of sulphur sources on the growth of *C. cassiicola*

Sulphur source	Average dry weight of mycelium (mg)					
	Cc 02	Cc 03	Cc 04	Cc 05	Cc 08	Cc 33
Magnesium sulphate	318	333	346	306	313	306
Potassium sulphate	201	276	263	200	193	208
Sodium sulphate	211	207	206	223	210	232
Ammonium sulphate	166	183	189	149	163	158
Zinc sulphate	180	220	232	196	188	160
Sodium thiosulphate	178	306	191	206	209	273
Sodium sulphite	163	178	194	183	169	185
Control	110	123	120	104	100	137
CD $P \leq 0.05$	7.09	7.66	6.54	8.36	8.37	7.55

CD $P \leq 0.05$ for isolates = 8.88; sulphur sources = 8.56; Interaction = 9.21

Table 2.5 Effect of phosphorous sources on the growth of *C. cassiicola*

Phosphorous source	Average dry weight of mycelium (mg)					
	Cc 02	Cc 03	Cc 04	Cc 05	Cc 08	Cc 33
Sodium pyrophosphate	300	326	299	318	311	320
Ammonium biphosphate	324	347	347	318	313	329
Disodium hydrogen orthophosphate	574	566	574	493	472	499
Magnesium phosphate	421	437	433	326	418	319
Potassium phosphate	426	428	432	414	416	420
Control	136	126	130	119	120	141
CD $P \leq 0.05$	11.43	12.66	12.88	9.78	8.98	7.67

CD $P \leq 0.05$ for isolates = 11.78; phosphorous sources = 13.68; interaction = 11.78

4.1.6 Vitamin

Variation was noticed in vitamin utilization by the isolates of *C. cassiicola*. All isolates showed significantly higher growth in thiamine-supplemented medium except Cc 03 and Cc 08, which grew better in inositol and biotin amended media respectively (Table 2.6). The isolate Cc 08 utilized pyridoxine and biotin similarly and a reduction in growth was noticed in thiamine-amended medium with the growth being lower than the control. Cc 02 performed equally well both in thiamine and inositol containing media. Maximum growth of Cc 03 was observed in inositol followed by pyridoxine and thiamine. Cc 05 preferred biotin for their optimum growth followed by thiamine. *C. cassiicola* isolate Cc 33 grew well in all the vitamin sources studied except pyridoxine and nicotinic acid. In nicotinic acid supplemented medium, all the isolates except Cc 33 showed a reduction in growth, which was lower than their controls.

Table 2.6 Effect of vitamins on the growth of *C. cassiicola*

Vitamins	Average dry weight of mycelium (mg)					
	Cc 02	Cc 03	Cc 04	Cc 05	Cc 08	Cc 33
Thiamine	373	340	387	360	271	359
Pyridoxine	312	341	323	310	319	313
Biotin	333	338	284	374	315	366
Nicotinic acid	219	222	196	210	235	337
Ascorbic acid	329	298	310	349	284	352
Inositol	372	376	318	321	306	341
Control	256	265	270	300	279	295
CD $P \leq 0.05$	11.10	10.68	10.66	13.23	13.71	10.44

CD $P \leq 0.05$ for isolates = 10.73; vitamines sources = 11.28; interaction = 13.11

4.1.7 Growth regulators

Among the growth regulators tried maximum growth of *C. cassiicola* isolates were observed in GA_3 amended medium and the isolate Cc 02 showed better growth among the six isolates (Table 2.7). Growth enhancement of all the isolates was noticed in IAA and IBA supplemented media. Naphthalene Acetic Acid (NAA) inhibited the growth of all the isolates.

Table 2.7 Effect of growth regulators on the growth of *C. cassiicola*

Growth regulators	Average dry weight of mycelium (mg)					
	Cc 02	Cc 03	Cc 04	Cc 05	Cc 08	Cc 33
IAA	333	292	306	314	334	306
IBA	398	318	310	318	317	309
GA3	616	429	313	367	417	412
NAA	28	46	24	19	23	20
Control	256	265	270	300	279	295
CD $P \leq 0.05$	14.69	12.98	13.00	16.76	14.90	15.18

CD $P \leq 0.05$ for isolates = 15.79; growth regulators = 14.68; interaction = 13.38

4.1.8 Macroelements

The dry weights indicated that magnesium was most essential for the mycelial growth of *C. cassiicola* (Table 2.8). Only Cc 08 showed moderate growth in medium devoid of magnesium, all the other isolates showed retarded growth. Next to magnesium, calcium, sodium and potassium also were found necessary for growth. Isolate Cc 02 grew well in medium without potassium and the growth was exactly similar to that of the control. All the isolates of *C. cassiicola* showed morphological difference with different macro elements studied.

4.1.9 Microelements

The effect of various trace elements on the nutrition of *C. cassiicola* is presented in Table 2.9. All the trace elements studied were beneficial and increased mycelial growth was noticed over the control. Among the five trace elements studied, zinc supported the maximum growth of *C. cassiicola* isolates followed by copper, molybdenum, boron and iron. Of the six *C. cassiicola* isolates, Cc 02 and Cc 03 isolates showed better growth in the presence of all the five trace elements studied than the rest isolates.

4.1.10 Bio-wilt

It was also noticed that the colour of the original medium changed after the growth of the pathogen in most of the media. Marked differences were showed by all the six isolates of *C. cassiicola* in the colour of the culture filtrates. Isolate with more sporulation produced deep coloured culture filtrate and the intensity of the colour depended upon the sporulation of the isolates. *C. cassiicola* isolate Cc 03 did not sporulate in any of the cultural media studied and the culture filtrates was colourless.

Marginal necrosis of leaves, necrotic patches, drooping and wilting were the characteristic symptoms observed on leaves of clone RRII 105. For GT 1 leaflets all other symptoms were observed except necrotic patches, but the percentage of wilting was lower than RRII 105 (Table 2.10). Wilting was observed after 24 hours of

incubation for RRII 105 and after 48 hours for GT 1 leaflets. Wilting started first in culture filtrates containing the trace elements copper and Zinc followed by boron, iron and molybdenum. Intensity of wilting was more for copper and zinc containing culture filtrate.

Table 2.8 Effect of macroelements on growth and sporulation of *C. cassiicola*

Isolate	Mean dry weight and sporulation									
	Basal medium - Ca		Basal medium - Mg		Basal medium - K		Basal medium - Na		Basal medium	
	DW	Sp	DW	Sp	DW	Sp	DW	Sp	DW	Sp
Cc 02	215	+	121	+	275	+	223	+	520	+++
Cc 03	194	-	91	-	201	-	194	-	488	-
Cc 04	246	+	130	-	211	+	187	+	346	++
Cc 05	187	-	63	-	261	-	161	-	376	++
Cc 08	289	-	174	-	220	+	187	+	471	+++
Cc 33	179	-	95	-	209	-	198	+	411	++
CD P ≤ 0.05	4.45	-	6.98	-	4.94	-	4.88	-	5.08	-

CD P ≤ 0.05 for Isolates = 5.09; macroelements = 4.16; Interaction = 4.56

DW = Dry weight

Sp = Sporulation

Table 2.9 Effect of microelements on growth and sporulation of *C. cassiicola*

Isolate	Mean dry weight and sporulation											
	Boron		Copper		Iron		Molybdenum		Zinc		Control	
	DW	Sp	DW	Sp	DW	Sp	DW	Sp	DW	Sp	DW	Sp
Cc 02	671	++	842	+++	646	++	709	+++	849	++++	334	++
Cc 03	592	-	561	-	409	-	562	-	640	-	312	-
Cc 04	769	+	872	++	677	+	641	+	935	+++	366	-
Cc 05	470	++	623	+++	526	++	512	++	623	++	348	+
Cc 08	541	+++	570	++	303	+	444	++	570	+++	237	-
Cc 33	409	+++	634	++++	331	+	601	+	634	++++	298	+
CD P ≤ 0.05	5.86	-	4.23	-	4.76	-	5.11	-	6.79	-	4.98	-

CD P ≤ 0.05 for Isolates = 5.17; microelements = 4.72; Interaction = 4.47

DW = Dry weight

Sp = Sporulation

Table 2.10 Effect of microelements on toxin production by *C. cassiicola* isolates

Isolates	Wilting percentage as % of control					
	Clones	B	Cu	Fe	Mo	Zn
Cc 02	RRII 105	10	50	29	28	68
	GT 1	1	4	1	1	6
Cc 03	RRII 105	8	18	10	7	16
	GT 1	0	1	0	0	1
Cc 04	RRII 105	17	60	41	47	88
	GT 1	0	8	1	1	9
Cc 05	RRII 105	29	61	32	39	77
	GT 1	0	6	1	1	8
Cc 08	RRII 105	42	78	33	30	75
	GT 1	1	9	1	1	8
Cc 33	RRII 105	41	76	30	31	76
	GT 1	1	8	1	1	8

4.1.11 Media

Various synthetic media influenced the radial growth of all the *C. cassiicola* isolates studied (Table 2.11). The growth was highest in Richard's agar followed by GAA and RB. The isolate Cc 4 showed significantly superior growth in all the synthetic media studied except 'C' dox medium and the growth rate was also high for this isolate. The morphology of the isolates changed in certain media. Variation in substrate pigmentation also was observed.

All the semi-synthetic media supported for the growth of *C. cassiicola* isolates (Table 2.12). Isolate Cc 04 showed better growth on all the media studied except PSA. Reduced growth was seen for PSA except for Cc 02, which grew well on this medium. Isolates showed variation in growth on different semi-synthetic media studied.

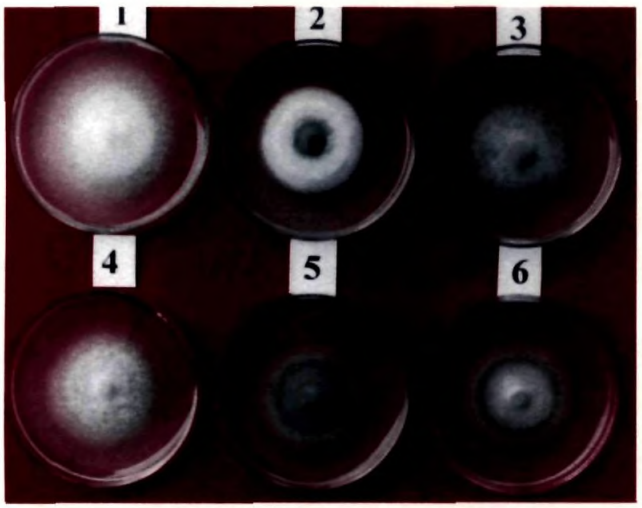
In liquid media, all the isolates grew well except Cc 04, which showed poor growth even after 10 days of incubation (Table 2.13). All the isolates grew optimally in Richard's broth followed by 'C' dox. The growth of isolates in PDB and PSB media were more or less similar with exception. In PDB, higher growth was exhibited by the isolate Cc 05.

4.1.12 Effect of phenolics on *C. cassiicola* isolates

Phenol, catechol and chlorogenic acid completely inhibited the growth of *C. cassiicola* isolates in all the three concentrations studied. Pyrogallole completely inhibited the growth of *C. cassiicola* isolates at higher concentrations (0.1 & 0.2%) but at 0.05% concentration all the six isolates showed some growth, though significantly lower than the controls. All the six *C. cassiicola* isolates grew well at all the three concentrations of tannin studied. Among the three concentrations of tannin, 0.1% induced the growth of isolate numbers Cc 04 and Cc 05 more than the controls. There was only very low inhibition of *C. cassiicola* in the presence of tannin in the medium.

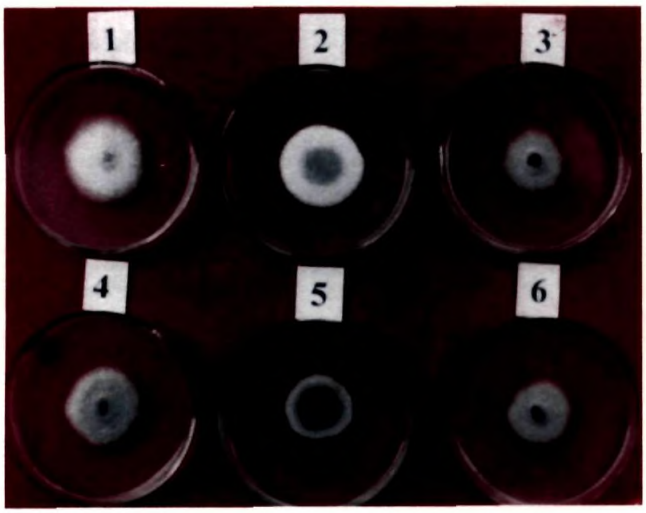
Plate - 2

Growth variation of *C. cassiicola* in different Solid media

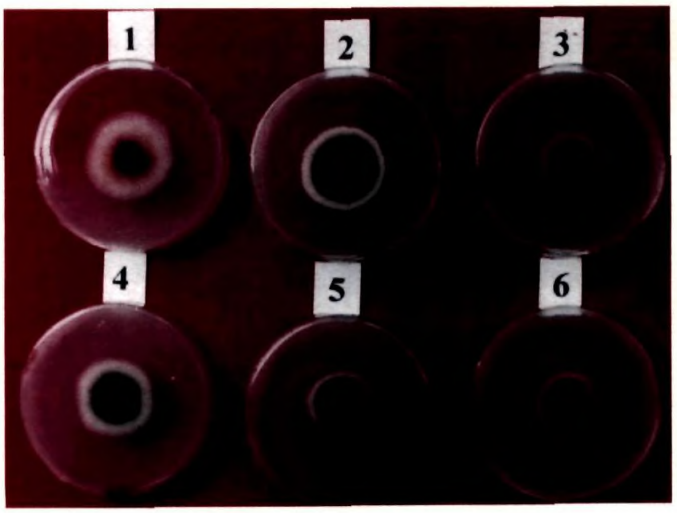


A

B



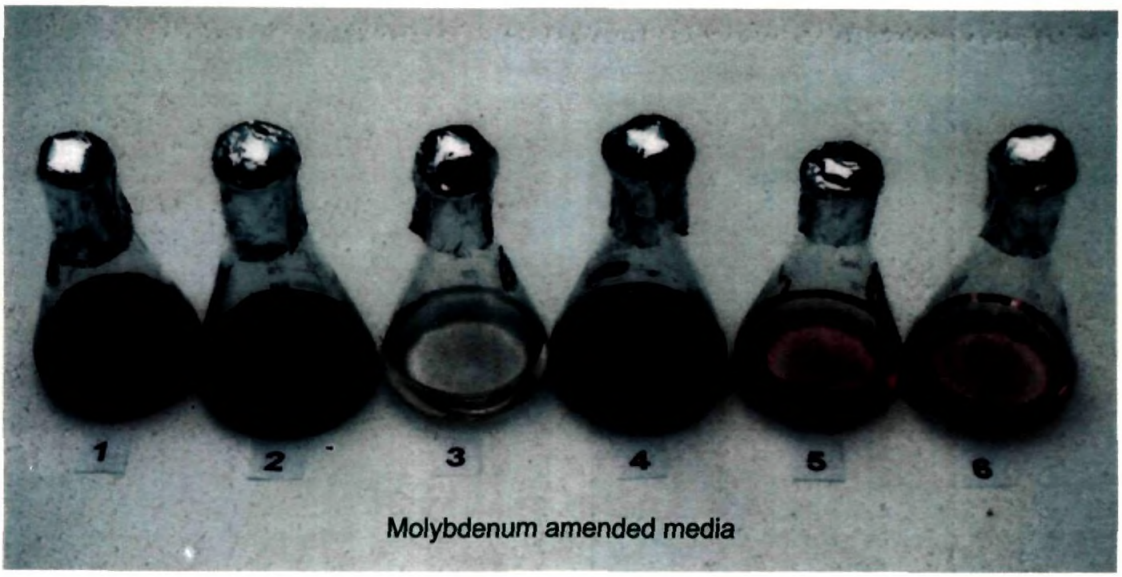
C



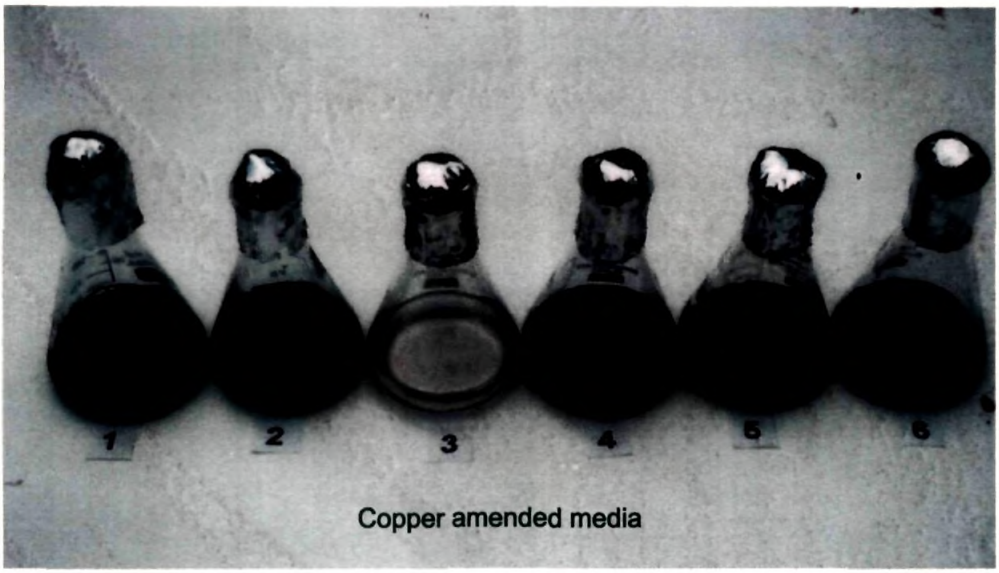
D

Plate - 3

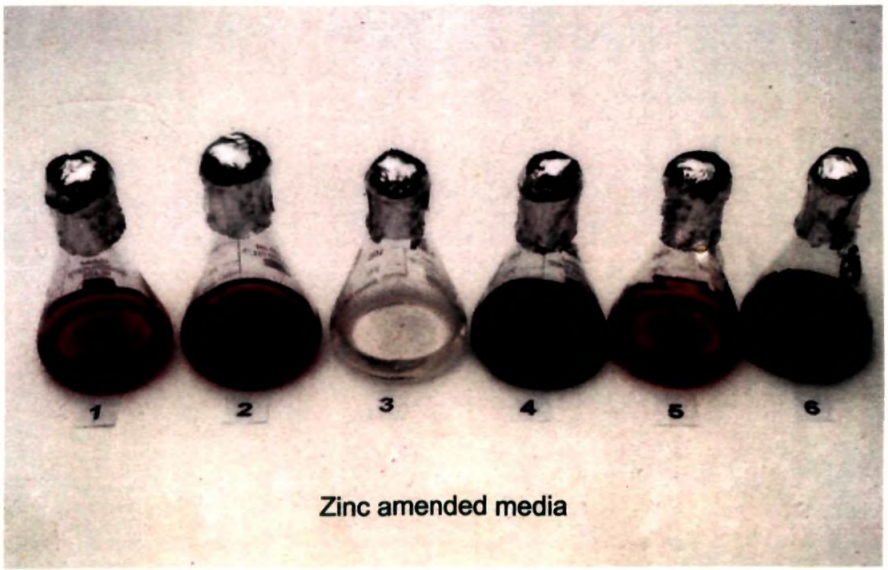
Coloured Culture filtrate of *C. cassiicola*



Molybdenum amended media



Copper amended media



Zinc amended media

Table 2.11 Effect of synthetic media on growth of *C. cassiicola*

Isolate	Media									
	Rose Bengal Agar		Sabraud's Agar		Cepadox Agar		Richard's Agar		Glucose Asperagine agar	
	RG	GR	RG	GR	RG	GR	RG	GR	RG	GR
Cc 02	67	6	58	7	54	6	61	9	60	9
Cc 03	58	7	56	6	50	5	62	9	62	9
Cc 04	67	12	66	10	55	7	86	17	77	13
Cc 05	62	9	59	7	54	5	80	14	69	10
Cc 08	57	6	54	6	50	5	69	10	61	9
Cc 33	62	8	51	6	51	5	64	9	59	8
CD P ≤ 0.05	1.41	-	1.11	-	1.43	-	1.24	-	1.26	-

CD P ≤ 0.05 for isolates = 1.27; microelements = 1.34; interaction = 1.17

RG = Radial growth

GR = Growth rate

Table 2.12 Effect of semi-synthetic media on growth of *C. cassiicola*

Isolate	Radial Growth of Mycelium (mm)							
	Carrot Dextrose Agar		Beetroot Dextrose Agar		Potato Dextrose Agar		Potato Sucrose Agar	
	RG	GR	RG	GR	RG	GR	RG	GR
Cc 02	59	5	64	7	63	6	62	6
Cc 03	62	6	70	8	71	8	55	5
Cc 04	79	11	71	9	72	9	53	5
Cc 05	62	6	64	7	70	9	59	6
Cc 08	60	5	60	6	66	8	51	4
Cc 33	62	6	61	6	60	6	56	6
CD P ≤ 0.05	1.12	-	1.41	-	1.23	-	1.18	-

CD P ≤ 0.05 for isolates = 1.09; nitrogen sources = 1.68; interaction = 1.22

RG = Radial growth

GR = Growth rate

Table 2.13 Effect of liquid media on growth of *C. cassiicola*

Isolate	Media			
	Potato Dextrose Broth	Potato Sucrose Broth	C'dox Broth	Richard's Broth
Cc 02	400	415	496	574
Cc 03	442	438	516	560
Cc 04	252	219	259	310
Cc 05	555	512	496	601
Cc 08	488	519	506	542
Cc 33	509	513	517	570
CD $P \leq 0.05$	10.90	13.56	11.46	9.99

CD $P \leq 0.05$ for isolates = 12.69; media = 10.48; interaction = 8.

Table 2.14 Effect of different coneentrations of phenolics on mycelial growth of *Corynespora cassiicola* isolates

Isolate	Phenolics (%)															Control
	Tannin			Phenol			Catechol			Chlorogenic acid			Pyrogallol			
	.05	0.1	0.2	.05	0.1	0.2	.05	0.1	0.2	.05	0.1	0.2	.05	0.1	0.2	
220	632	767	476	-	-	-	-	-	-	-	-	-	38	-	-	737
235	637	720	685	-	-	-	-	-	-	-	-	-	46	-	-	830
237	693	751	685	-	-	-	-	-	-	-	-	-	63	-	-	958
260	626	720	482	-	-	-	-	-	-	-	-	-	41	-	-	639
GT 1	780	983	603	-	-	-	-	-	-	-	-	-	73	-	-	1127
TP 1	633	693	545	-	-	-	-	-	-	-	-	-	64	-	-	858
CD P ≤ 0.05	30.45	20.67	24.78	-	-	-	-	-	-	-	-	-	9.98	-	-	46.87

CD P ≤ 0.05 for Isolates = 25.27; phenolics = 23.34; interaction = 25.27

5. Discussion

5.1 Carbon and nitrogen utilization

All the sugars except cellulose and starch (polysaccharides), supported good growth of *C. cassiicola* isolates. All the monosaccharides i.e. fructose, dextrose and mannitol were utilized better than the polysaccharides. Two disaccharides were tested, of which lactose was better utilized by all the isolates of *C. cassiicola* than sucrose. It is therefore probable that, the difference in utility of the disaccharides is due to the nature of monosaccharide units that constitute the disaccharides and their relative ease of hydrolysis into individual molecules. Thind (1977) reported fair growth of *Alternaria alternata* and *Gloeosporium fructigenum* on lactose. But on the contrary, lactose proved to be the poorest carbon source for the growth of other fungi like *Cladosporium cladosporoides* (Anilkumar and Sastry, 1980) *Colletotrichum* (Sahni *et al.*, 1975) *Penicillium crustosum* (Garcha and Singh, 1976). Lilly and Barnett (1951) reported that lactose is utilized by far fewer fungi than other saccharides.

Cellulose the main structural polysaccharide of plants and starch the storage sugar were less utilized among the carbon sources studied with *C. cassiicola*. The pathogen may not be able to utilize these polysaccharides directly. Cochrane (1958) reported that poor growth of *Helminthosporium sativum* on cellulose might be attributed to either non-availability of certain conditions affecting its utilization in the culture medium or absence of cellulose degrading enzymes.

All the nitrogenous compounds were significantly superior to control in supporting the growth of *C. cassiicola*. Out of the six isolates, four preferred peptone as the most favourable nitrogen source for their growth. Peptone is a complex mixture of peptides and aminoacids containing water soluble vitamins which may be utilized by *C. cassiicola* easily. Peptone supported the good growth of fungi like *Colletotrichum gloeosporioides*, *Cladosporium cladosporoide* and *Cercospora cruenta* (Purkayastha and Gupta, 1974; Anilkumar and Sastry, 1980; Gupta and singh, 1980). *C. cassiicola* isolates Cc 03 and Cc 33 grew well in potassium nitrate and calcium nitrate respectively. Amongst the nitrates, potassium nitrate and calcium nitrate supported higher growth of many fungi (Bakr and

Grewal, 1987; Singh and Prasada, 1973). Newton (1946) and Srivastava (1951) reported good growth of various species of *Alternaria* on potassium nitrate amended media. In general, nitrate nitrogen has been reported to be favourable for the mycelial growth of many fungi (Lilly and Barnett, 1951).

5.2 Vitamins

Vitamins are stimulatory or necessary in minute quantity for the growth of many fungi like *Septoria humuli* (Munjal and Gautham, 1977) *Pleurotus ostreatus* and *Volvariella volvacea* (Kundu, 2003). Some fungi have the ability to synthesis vitamins while others depend on exogenous supply. In the present study five isolates preferred thiamine for their growth indicating the role of thiamine in biomass generation of these fungi. Munjal and Gautham (1977) reported that *Septoria humuli* grew in medium incorporated with thiamine.

5.3 Media

The mycelial growth rate of isolates of *C. cassiicola* was significantly affected by culture media. *C. cassiicola* isolates preferred Richard's agar medium for their optimum growth. Richard's medium was found to be the best medium for the growth of various other pathogens like *Alternaria ajamopsidis* (Singh and Prasada, 1973), *Fusarium oxysporum* (Paulkar and Raut, 2004), *Macrophomina phaseolina* (Surichandraselvan and Seetharaman, 2003) and *Colletotrichum gloeosporioides* (Rani and Murthy, 2004).

5.4 Amino acid

The present study revealed that all the *C. cassiicola* isolates preferred proline for their good growth and sporulation. Several investigators pointed out the importance of proline for the growth and sporulation of fungi (Bahadhur *et al.*, 1976; Munjal and Gautham, 1977). But on the contrary, Bahl and Grewal (1973) reported poor growth and sporulation of *Operculella padwickii* in amino acids like proline and monohydrochloride.

5.5 Macroelements

All the macroelements studied were essential for the growth of *C. cassiicola* isolates, but magnesium was preferred by the isolates than calcium, potassium and sodium. Magnesium supported good growth of fungi like *Psathyerella atroumbonata*, *Endomyces magnussi* and *Penicillium chrysogenum* (Okorokov *et al.*, 1975), *Mortierella alpine* (Totani *et al.*, 2002). Purkayastha and Gupta (1974) observed that potassium was most essential for mycelial growth of *Colletotrichum gloeosporioides* followed by magnesium. The good growth of *C. cassiicola* isolate Cc 02 in potassium deficient medium appear to be an exemtion, as the role played by potassium in carbohydrate metabolism of fungi has been well documented (Ray and Purkayastha, 1977).

5.6 Microelements

Among the trace elements maximum growth of *C. cassiicola* isolates was recorded with zinc. This is in agreement with the findings of Chahal and Rawla (1977), Saini (1977) and Thind and Rawla (1967). The original medium was colourless which changed to vine red or grey or brown after the growth of *C. cassiicola* isolates and the current results demonstrated a positive correlation between sporulation and the intensity of pigmentation by *C. cassiicola* isolates.

5.7 Effect of phenolics on *C. cassiicola*

Growth studies of different phenolic compounds indicated that growth of *C. cassiicola* inhibited completely by phenol, catechol and chlorogenic acid. Among the phenolic compounds phenol and catechol, widely known antiseptics was mostly toxic to fungal growth and pyrogallole was inhibitory at higher concentrations (Venkatachalam and Jayabalan, 1995). The present study indicated enhanced growth of *C. cassiicola* isolates in all the three concentrations of tannin studied. Tannin enhanced the growth of fungi like *Aspergillus flavus, A. nidulans, A. niger and Penicillium sp* (Sivaswamy, 1982). Increase in mycelial dry weight of *Curvularia lunata* in presence of wattle tannin was also reported (Sambandam, 1983). The increased growth of *C. cassiicola* at lower concentrations of tannin may be due to the utilization of tannin as nutrient source by the fungus.

The relationship between enzymes and other biochemicals to growth of the pathogen *C. cassiicola* can be very interesting for the explanation of reciprocal physiological and biochemical effects of these enzymes in plant resistance. Biochemical studies in many plant pathogen interactions indicate that the infected plants had higher post infection total phenols, OD phenols, and proteins levels and showed higher oxidase activity compared to healthy plants (Rahayuningsih, 1990; Faize *et al.*, 2004; Christopher *et al*, 2007). It is believed that the increased biosynthesis of phenolics and stimulated polyphenol oxidase activity are responses to infection leading to greater accumulation of toxic quinones at the site of infection (Farkas and Kiraly, 1962; Basha and Chatterjee, 2007). Similarly increased peroxidase activity might have resulted in enhanced lignification and thus reduced the infection frequency by the pathogen (Angelini *et al.*, 1993; Rea *et al.*, 1998).

6. Bibliography

Agarwal, G. P. and Shinkhede, G. G. (1959). Physiological studies on *Helminthosporium rostratum* Drechs. *Oyton*, 13: 45-54.

Agnihotri, V. P. (1968). Effect of nitrogenous compounds on sclerotium formation in *Aspergillus niger. Canadian Journal of Microbiology*, **14**: 1253-1258.

Ahmad, S. and Mir, N. A. (1998). Effect of different carbon and nitrogen sources on the growth and sporulation of *Wilsonomyces carpophilus. Plant Disease Research*, **13**: 147-148.

Al-Masri, M. I., Ali-Shtayeh, M. S., Elad, Y., Sharon, A., Tudzynski, P. and Barakat, R. (2002). Effect of Plant Growth Regulators on White Mould (*Sclerotinia sclerotiorum*) on Bean and Cucumber. *Journal of Phytopathology*, **150**: 481

Angel, M. M., Oumaima, L., Rocio, V., Jorge, A., Patrick, A., Fernando, L., Jean-Paul, L. and Antonio, C. J. (2007). The regulation of zinc homeostasis by the ZafA transcriptional activator is essential for *Aspergillus fumigatus* virulence. *Molecular Microbiology*, **64**: 1182-1197.

Angelini, R., Bragaloni, M., Federico, R., Infantino, A. and Portapuglia, A. (1993). Involvement of polyamines, diamine oxidase and peroxidase in resistance of chickpea to *Ascochyta rabiei*. *Journal of Plant Physiology*, 142: 704-709.

Anilkumar, T.B. and Sastry, M.N.L. (1980). Effect of carbon, nitrogen sources and temperature on the growth of *Cladosporium cladosporoides*. *Indian Journal of Mycology and Plant Pathology*, 10 (1): 122-123.

Antoine, A. (1965). The resistance of yeast to copper ions. III. *Saccharomyces cerevisiae*, yeast foam, nature of two resistant forms. *Experimental Cell Research*, **40**: 570-584.

Appel, H. M. (1993). Phenolics in ecological interactions: The importance of oxidation. *Journal of Chemical Ecology*, **19**: 1521-1552.

Assis, T. C. Menezes, M., Andrade, D.E.G.T., Coelho, R. S. B. and Oliveira, S. M. A. (2001). Comparative study of *Colletotrichum gloeosporioides* isolatesas to effect of carbohydrate nutrition on the growth, sporulation and pathogenicity on fruits from three mango varieties. *Summa Phytopathologica*, **27**: 208-212.

Aurich, H., Neumann, W. and Kleber, H. P. (1967). Nucleic acids and proteins in *Neurospora* in biotin and pyridoxine deficiency, *Acta Biol. Med. Ger.* **19**: 221-229.

Bahadur, P., Sinha, V. C. and Upadhaya, Y. M. (1976). Effect of certain sugars and nitrogen sources on the growth of *Sclerotium rolfsii*. *Indian Phytopathology*, **29**: 426-427.

Bahl, N. and Grewal, J. S. (1973). Studies on physiology of *Operculella padwickii* causal organism of foot rot of *Cicer arietinum*, *Indian Phytopathology*, **26**: 622-630.

Bakr, M.A. and Grewal, J.S. (1987). Effect of various nitrogen sources on growth and sclerotia formation of *Sclerotinia sclerotiorum*. *Indian Journal of Mycology and Plant Pathology*, **17** (2): 150-153.

Barnes, A. C., Bowden, T. J., Horne, M. T. and Ellis, A. E. (1999). Peroxide-inducible catalase in *Aeromonas salmonicida* sub sp. *salmonicida* protects against exogenous hydrogen peroxide and killing by activated rainbow trout, *Oncorhynchus mykiss* L., macrophages. *Microbial Pathogenesis*, **26**: 149-158.

Basha, S. A. and Chatterjee, S. C. (2007). Activation of phenylalanine ammonia lyase contributes to non-host resistancein *Tritichum aestivum* against *Sclerotinia sclerotiorum*. *Indian Phytopathology*, **60**: 442-449.

Bau, Y. and Wong, H. (1979). Zinc Effects on Growth, Pigmentation and Antibacterial Activity of *Monascus purpureus*. *Physiologia Plantarum*, **46**: 63-67.

Bidari, V. B. and Govindu, H. C. (1975). Comparative studies on the physiology of leaf, neck and earhead isolates of *Helminthosporium sativum* pam., King and Bakke parasite on wheat crop in Karnataka. *Mysore Journal of Agriculture Science*, **9**: 421-426.

Borst-Pauwels, G. W. F. H. (1967). A study of the factors causing a decrease in the rate of phosphate uptake during phosphate accumulation. *Acta Bot. Neerl.* **16**: 115-131.

Borua, I. and Das, D. (2000). Changes in activities of polyphenol oxidase, acid phosphatase and phenol content in developing chilli varieties susceptible and resistant to *Colletotrichum capsici*. *Crop Research*, **19**: 230-234.

Cameron, H. R. and Milbrath, G. M. (1965). Variability in the genus *Phytophthora* I. Effect of nitrogen source and pH on growth. *Phytopathology*, **55**: 653-657.

Chahal, S. S. and Rawla, G. S. (1980). Trace element and vitamin requirement of *Leptosphaerulina trifolii*. *Indian Journal of Mycology and Plant Pathology,* **10**: 151-154.

Christie, T. (1958). Nutritional studies of *Phytophthora caetorum* (Lele and Cohn.) schroet. Part I. Utilization of nitrogen and carbon compounds. *New Zealand Journal of Science*, **1**: 83-90.

Christopher, D. J. Suthinraj, T. and Udhayakumar, R. (2007). Induction of defense enzymes in *Trichoderma viride* treated blackgram plants in response to *Macrophomina phaseolina* infection. *Indian Journal of Plant Protection*. **35**: 299-303.

Clarke, D. D. (1966). Factors affecting the development of single zoospore colonies of *Phytophthora infestans*. *Trans British Mycological Society*, **49**: 177.

Cochrane, V.W. (1958). Physiology of Fungi. John Wiley and Sons, New York, pp. 524.

Cook, R. J. and Schroth, M. N. (1965). Carbon and nitrogen compounds and germination of chlamydospores of *Fusarium solani* f. *phaseoli*. *Phytopathology*, **55**: 254-256.

Dar, G. M. (1999). Carbon and nitrogen nutrition of *Chladobotryum dendroides*, the causal fungus of cobweb disease of cultivated mushroom (*Agaricus bisporus). Applied Biological research*, **1**: 57-61.

Dawson, P. S. S. and Steinhauer, L. P. (1977). Radiorespirometry of *Candida utilis* in phased culture under nitrogen-, carbon-, and phosphorus – limited growth. *Canadian Journal of Microbiology*. **23**: 1689-1694.

Desai, J. D. and Modi, V. V. (1977). Growth, glucose metabolism and melaninformation in biotin deficient *Aspergillus nidulans*. *Folia Microbiologia*, **22**: 55-60.

Deshpande, N., Wilkins, M. R., Packer, N. and Nevalainen, H. (2008). Protein glycosylation pathways in filamentous fungi. *Glycobiology,* **18** (8): 626-637.

Dietrich, F. S., S. Voegeli, S. Brachat, A. Lerch, K. and Gates (2004). The *Ashbya gossypii* genome as a tool for mapping the ancient *Saccharomyces cerevisiae* genome. *Science,* **304:** 304–307.

Dung, P.T. and Hoan, N.T. (1999). Corynespora leaf fall on rubber in the year 1999 (Vietnam): New Record. *Proceedings of IRRDB Symposium*, 1999, China, pp. 24-25.

Eckert, J. W. and Ratnayake, M. (1994). Role of volatile compounds from wounded oranges in induction of germination of *Penicillium digitatum* conidia. *Phytopathology*, **84**: 746-750.

Elliot, C. G. (1972). Calcium chloride and growth and reproduction of *Phytophthora cactorum*. *Trans. British Mycological Society*, **58**: 169-172.

Faize, M., Faize, L., Ishizaka, M. and Ishii, H. (2004). Expression of potential defense responses of Asian and European pears to infection with *Venturia nashiqola. Physiology and Molecular Plant Pathology*, **64**: 319-330.

Farkas, G. L. and Kiraly, Z. (1962). Role of phenolic compounds in the physiology of plant disease and disease resistance. *Phytopathological Z.*, **44**: 105-150.

Fletcher, J. (1979). An ultrastructural investigation into the role of calcium in oospore-initial development in *Saprolegnia diclina. Journal of Gen Microbiology*, **113**: 315-326.

Ganacharya, N. M. and Wankar, B. N. (1977). Effect of trace element and growth regulators on Fusarium oxysporum, the cause of potato wilt. *Indian Journal of Mycology and Plant Pathology*, **7**: 81-82.

Garcha, H.S. and singh, V. (1976). Effect of various carbon and nitrogen sources on the sporulation of *Penicillium crustosum*. *Indian Journal of Mycology and Plant Pathology*, **6** (1): 104-105.

Garraway, M. O and Evans, R. C. (1984). Metabolism. In: *Fungal Nutrition and Physiology*. A Wiley-Intescience publication, pp: 293-314.

Gissi, U., Oertli, J. J. and Schwinn, F. J. (1977). Wasser-und Salsbeziehungen der sporangien von *Phytophthora cactorum* (Leb. Et Cohn). Schroet in vitro. *Phytopathological Z.*, **89**: 261-284.

Goyal, M. K. (1980). Studies on nutritional requirements of *Penicillium atramentosum*. *Indian Journal of Mycology and Plant Pathology*, **10**: 83-85.

Guimaraes, R. L. and Stotz, H. U. (2004). Oxalate Production by *Sclerotinia sclerotiorum* Deregulates Guard Cells during Infection. *Plant Physiology*, **136**: 3703-3711.

Gupta, R.P. and Singh, D.V. (1980). Effect of different carbon, nitrogen sources on the growth of *Cercospora cruenta*. *Indian Journal of Mycology and Plant Pathology*, **10** (2): 183.

Hall, N. E. L. and Axelrod, D. E. (1978). Sporulation competence in *Aspergillus nidulans*: A role for iron in development. *Cell Diff.* **7**: 73-82.

Hedge, R. K. and Ranganathaiah, K. G. (1971). Vitamin requirements of two isolates of *Helminthosporium nodulosum* of Ragi. *Mysore Journal of Agricultural Science*, **5**: 101-104.

Iwashima, A. and Nishimura, H. (1979). Isolation of a thiamine-binding protein from *Saccharomycetes cerevisiae*. *Biochim. Biophys. Acta*. **577**: 217-220.

Iwashima, A. and Nose, Y. (1976). Regulation of thiamine transport in *Saccharomycetes cerevisiae*. *Journal of Bacteriology*, **128**:855-857.

Iwashima, A., Nishimura, H. and Nose, Y. (1979). Soluble and membrane-bound thiamine-binding protein from *Saccharomycetes cerevisiae*. *Biochim. Biophys. Acta*. **577**: 460-468.

Jackson, S. L. and Heath, I. B. (1993). Roles of calcium ions in hyphal tip growth. *Microbiol Mol Biol Rev*, **57**(2): 367-382.

Jamaluddin, M. P., Tandon and Tandon, R. N. (1975). Effect of different sulphur sources on growth and sporulation of *Gliocephalotrichum bulbilium*. *Indian Phytopathology*. 28: 550-551.

Jonathan, S. G. and Fasidi, I. O. (2001). Effect of carbon, nitrogen and mineral sources on growth of *Psathyerella atroumbonata* (Pegler), a Nigerian edible mushroom. *Food Chemistry*, **72**: 479-483.

Jones, E. B. G. and Jennings, D. H. (1965). The effect of cations on the growth of fungi. *New Phytol*, **64**: 86-100.

Jongbloed, R. H. and Borst-Pauwels, G. W. F. H. (1992). Effect of aluminium and pH on growth and potassium uptake by three ectomycorrhizal fungi in liquid culture. *Plant and Soil*, **140**: 157-165.

Kacchy, A. N., Media, A. M., Modi, V. V. and Parekh, N. (1972). Octadecanol metabolism in *Candida tropicalis*. *Indian Journal of Experimental Biology*, **10**: 246-248.

Kalaimani, T. (1997). Effect of carbon, nitrogen, vitamin and amino acid sources on growth and sporulation of six isolates of *Colletotrichum falcatum* went. *Cooperative Sugar*, **28**: 607-616.

Kapoor, I. J. and Singh, G. R. (1973). Utilization of carbohydrates by nine isolates of *Colletotrichum gloeosporioides* from citrus. *Indian Phytopathology*, **26**: 279-283.

Kertesz, M. A. and Mirleau, P. (2004). The role of soil microbes in plant sulphur nutrition. *Journal of Experimental Botany*, **55**: 1939-1945.

Khan, M. W. and Azam, F. F. (1975). *In vitro* respose of cauliflower isolate of *Rhizoctonia solani* to certain growth factors. *Indian Phytopathology*, **28**: 202-205.

Koul, A. K. (1987). Effect of different sources of carbon and nitrogen on the growth and sporulation of *Botryodiplodia theobromae* causing blight of sunflower. *Indian Journal of Mycology and Plant Pathology*, **17**: 212-213.

Kruszewską, J. S., Perlinska-Lenart, U., Gorka-Niec, W., Orłowski, J., Zembek, P. and Palamarczyk, G. (2008). Alterations in protein secretion caused by metabolic engineering of glycosylation pathways in fungi. *Acta Biochimica Polonica*, **55**: 447-456.

Kumar, R. and Singh, S. B. (1996). Changes in biochemical constituents of sunflower leaves in relation to Alternaria blight development. *Indian Journal of Mycology and Plant Pathology*, **26**: 234-236.

Kumari, A., Jha, A. K., Ainha, S. K. and Ojha, K. L. (1998). Effect of different sources of carbon, nitrogen and vitamins on the growth and sporulation of *Alternaria brassicae* (Berk) Sacc. *Journal of Applied Biology*, **8**: 100-103.

Kundu, R. B. (2003). Stimulatory effect of different vitamins on mycelial biomass and protein production of *Pleurotus ostreatus* and *Volvariella volvacea* grown on agroindustrial wastes. *Journal of Mycopathological Research*, **41**: 237-240.

Kurtz, M. B. and Champe, S. P. (1981). Dominant spore color mutants of *Aspergillus nidulans* defective in germination and sexual development. *Journal of Bacteriology*, **148**: 629-638.

Langkramer, O. (1969). Effect of some vitamins on the growth of *Suillus variegates* (Sev. Ex Fr.) O. Kuntze. *Cezka Mykol.* **23**: 53-60.

Lattke, H. and Weser, U. (1977). Functional aspects of zinc in yeast RNA polymerase B. *FEBS Lett.* **83**: 297-300.

Lenny, J. F. and Klemmer, H. W. (1966). Factors controlling sexual reproduction and growth in *Pythium graminicola*. *Nature*, **209**: 1365-1366.

Levic, J. and Pencic, V. (1990). Utilization of carbon, nitrogen and sulphur compounds by *Kabatiella zeae* Narita et Hiratsuka. *Journal of Phytopathology*, **128**: 321-332.

Levinskaite, L. (2004). Zinc influence on the ability of *Penicillium* Link. Genus fungi to use natural C sources. *Ekologija*, **3**: 1-5.

Lichstein, H. C. (1952). Metabolism of microorganisms. *Annual Review of Microbiology*, **6**: 1-28.

Lilly, V. and Barnett, H.L. (1951). Physiology of Fungi. McGraw Bill Book Co., Inc. New York. pp. 464.

Lima, M. A. B., Nascimento, A. E., Souza, W., Fukushima, K. and Campos-Takaki, G. M. (2003). Effects of phosphorus on polyphosphate accumulation by *Cunninghamella elegans*. *Brazilian Journal of Microbiology,* 34: 363-372.

Maas, J. L. (1976). Stimulation of sporulation of *Phytophthora fragariae*. *Mycologia*, **68**: 511-522.

Mahadevan, A. (1982). Biochemical aspects of disease resistance Part I. performed inhibitory substances prohibitions. *Today and Tomorrow's Printers and Publishers, New Delhi, India*, pp. 425.

Malik, K. A., Kauser, F. and Azam, F. (1980). Effect of sodium chloride on the cellulolytic ability of some *Aspergilli*. *Mycologia*, **72**: 229-243.

Marino, R., Howarth, R. W., Chan, F., Cole, J. J. and Likens, G. E. (2003). Sulfate inhibition of molybdenum-dependent nitrogen fixation by planktonic cyanobacteria under seawater conditions: a non-reversible effect. *Hydrobiologia*, **500**: 277-293.

Marschner, H. (1995) Rhizosphere pH effects on phosphorus nutrition. In: *Genetic manipulation of crop plants to enhance integrated nutrient management in cropping systems – 1*, Phosphorus: proceedings of an FAO/ICRISAT Expert Consultancy Worshop. (Eds J C Lee, K K Sharma, G V Subbaro and E A Kueneman). ICRISAT Asia Center, India. Pp: 107–115.

Marzluf, G. A. (1997). Genetic regulation of nitrogen metabolism in the fungi. *Microbiology and Molecular Biology Review*, **61**, 17–32.

Mathur, R. S., Barnett, H. L. and Lilly, V. G. (1950). Factors influencing growth and sporulation of *Colletotrichum lindemuthianum* in culture. *Phytopathology*, **40**: 104-114.

McKeen, W. E. (1956). An interaction product of glycine and dextrose toxic to *Phytophthora fragariae*. *Science*, **123**: 509.

Misra, A. P. and Mahmood, M. (1961). Effect of vitamins and hormones on growth and sporulation of *Colletotrichum capsici* (Syd.) Butler & Bisby. *Indian Phytopathology*, **14**: 20-29.

Mizunaga, T., Kuraishi, H. and Aida, K. (1975). Mechanism of unbalanced growth and cell death of biotin-deficient yeast cells in the presence of aspartic acid. *Journal of Gen. Applied Microbiology*, **21**: 305-316.

Munjal, R. L. and Gautam, S. R. (1977). Studies on physiology of *Septoria humuli*. *Indian Phytopathology*, **30**: 513-517.

Nabeshima, S., Tanaka, A. and Fukui, S. (1977). Effect of biotin on the level of isocitrate lyase in *Candida tropicalis*. *Agr. BIol. Chem.* **41**: 281-285.

Nair, S. (2004). Bacterial Associations: Antagonism to Symbiosis. In: *Marine Microbiology: Facets & Opportunities*, (Ed. Ramaiah, N.), National Institute of Oceanography, Goa, pp; 83-89

Naplekova, N. N. and anikina, A. P. (1970). Assimilation of boron bycellulose decomposing microorganisms. *Mikrobiologiya*, **39**:547.

Newton, W. (1946). The growth of *Sclerotinia sclerotium* and *Alternaria solani* in simple nutrient solution. *Science in Agriculture*, **26** (7): 303-304.

Norkrans, B. (1968). Studies on marine occurring yeasts: Respiration, fermentation and salt tolerance. *Arch. Microbiology*, **62**: 358-372.

Ogawa, A., Shibayama, H. and Gohbara, M. (1999). Effects of sugars and amino acids on germination of spores of *Exserohilum monoceras*. *Report of the Kyushu Branch of the Crop Science Society of Japan*, **65**: 36-38.

Okorokov, L. A., Lichko, L. P., Kholodenko, V. P., Kadomtseva, V. M., Petrikevich, S. B., Zaichkin, E. I. and Karimiva, A. M. (1975). Free and bound magnesium in fungi and yeasts. *Folia Microbiologia*, **20**: 460-466.

Oluma, H. O. and Amuta, E. U. (1999). *Corynespora cassiicola* leaf spot of pawpaw (*Carica papaya* L.) in Nigeria. *Mycopathologia*, 145:23-27.

Omanor, I. B., Eziashi, E. I. and Adekunle, A. A. (2008). Carbon nutrition in relation to growth of three *Monascus* species isolated from decaying date fruits. *African Journal of Microbiology Research*, **2**: 153-155.

Pal, M. and Grewal, J. S. (1975). Utilization of different nitrogen sources by *Phytophthora drechsleri* var. cajani. *Indian Phytopathology*, **28**: 499-501.

Palarpawar, M. Y. and Ghurde, V. R. (1997). Influence of different nitrogen sources on growth and sporulation of *Colletotrichum capsici* and *Colletotrichum curcumae*. *Journal of Mycology and Plant Pathology*, **27**: 227-228.

Papagianni, M. (2004). Fungal morphology and metabolite production in submerged mycelial processes. *Biotechnology Advances*, **22**: 189-259.

Pateman, J. A. and Kinghorn, J. R. (1976). Nitrogen metabolism. In: *The filamentous fungi*, (eds. J. E. Smith and D. R. Berry), New York: Wiley, **2**: 159-237.

Paulkar, P. K. and Raut, B. T. (2004). Variability among the isolates of *Fusarium oxysporum* f. sp. *ciceri*. *Journal of Mycology and Plant Pathology*, **34**(1): 20-23.

Pezzano, H. and Coscia, L. (1970). The presence of iron and vanadium in ribonucleic acid from yeast. *Acta Physiol. Latino. Amer.* **20**: 402-420.

Phalip, V., I. Kuhn, Y. Lemoine and Jeltsch, J. M. (1999). Characterization of the biotin biosynthesis pathway in *Saccharomyces cerevisiae* and evidence for a cluster containing *BIO5*, a novel gene involved in vitamer uptake. *Gene,* **232:** 43–51.

Purkait, R. and Purkayastha, R. P. (1996). Pectolytic enzyme activities of some foliar fungi isolated from mangrove plants and their response to tannin. *Indian Phytopathology,* **49**: 366-372.

Purkayastha, R. P. and Gupta, M. S. (1974). Studies on *Colletotrichum gloeosporioides* inciting anthracnose of Jute. *Indian Phytopathology*, **28**: 454-458.

Rahayuningsih, S. A. (1990). Peroxidase activities and their relationship to resistance to pepper plant to *Phytophthora palmivora. Industrial Crops Research Journal*, **3**: 18-22.

Rani, G. S. and Murthy, K. V. M. K. (2004). Cultural and nutritional characteristics of *Colletotrichum gloeosporioides*, the causal organism in cashew anthracnose. *Journal of Mycology and Plant Pathology*, **34**: 317-318.

Rani, G. S. and Murthy, K. V. M. K. (2004). Cultural and nutritional characteristics of *Colletotrichum gloeosporioides*, the causal organism in cashew Anthracnose. *Journal of Mycology and Plant Pathology,* **34**: 317-318..

Rawla, G. S., Rewal, H. S. and Chahal, S. S. (1977). Organic factors and trace elements for *Cercospora dolichi* and their gross effects on growth. *Indian Phytopathology*, **30**: 189-194.

Ray, C. and Purkayastha, R. P. (1977). Some physiological studies on *Colletotrichum corchorum* causing anthracnose of jute. *Indian Phytopathology*, **30**: 189-194.

Rea, G., Laurenzi, M., Tranquilli, E., D'Ovidio, R., Federico, R. and Angelini, R. (1998). Developmentally and wound-regulated expression of the gene encoding a cell wall copper amine oxidase in chickpea seedlings. *FEBS Letters*, 437: 177-182.

Roncadori, R. W. (1965). A nutritional comparison of some species of *Phytophthora. Phytopathology*, **55**: 595-599.

Roomans, G. M. and Borst-Pauwels, G. W. F. H. (1977). Interaction of phosphate with monovalent cation uptake in yeast. *Biochimica et Biophysica (BBC) – Biomembranes*, **470**: 84-91.

Roomans, G. M., Theuvenet, A. P. R., van den Berg, T. P. R. and Borst-Pauwels, G. W. F. H. (1979). Kinetics of Ca2+ and Sr2+ uptake by yeast. Effect of pH, cations and phosphate. *Biochim. Biophys. Acta.* **551**: 187-196.

Sahni, S.S., Chahal, D.S. and Aujila, S.S. (1975). Effect of carbon, nitrogen and temperature on the growth of different species of *Colletotrichum*. *Indian Journal of Mycology and Plant Pathology*, **5** (2):194-196.

Saini, S. S. (1977). Trace element studies on fungi –II optimum requirements of essential trace elements for the growth of four species of *Colletotrichum*. *Indian Phytopathology*, **30**: 399-402.

Sambandam,T. (1983). Ph.D. Thesis, University of Madras, Madras, pp: 141.

Sankhla, H. C. and Mathur, R. L. (1967). Response of *Rhizoctonia betaticola* to growth regulators, trace elements and vitamins. *Indian Phytopathology*, **20**: 142-145.

Sankhla, H. C., Masih, B. and Mathur, R. L. (1970). Effect of trace elements and growth regulators on *Alternaria burnsii*, incitant of blight of Rumin. *Indian Phytopathology*, **23**: 533-537.

Santen, Y. V., Benen, J. A. E., Schroter, K., Kalk, K. H., Sylvie, A., Visser, J. and Diikstra, B. W. (1999). 1.68-Å Crystal Structure of Endopolygalacturonase II from *Aspergillus niger* and Identification of Active Site Residues by Site-directed Mutagenesis. *Journal of Biology and Chemistry*, **274**: 30474-30480.

Semino, C. E. and Robbins, P. W. (1995). Synthesis of "Nod"-like chitin oligosaccharides by the Xenopus developmental protein DG 42. Proceedings of the National Academy of science, USA. 92: 3498-3501.

Shanmugam, N. and Govindaswamy, C. V. (1973). Physiological studies on *Macrophomina phaseoli* causing groundnut root rot. *Indian Journal of Mycology and Plant Pathology*, **3**: 1-4.

Shchelokova, E., and L. Vorob'eva, (1982). Biotin formation by the fungus *Rhizopus delemar*. *Prikl. Biokhim. Mikrobiol.* **18:** 630–635.

Shreemali, J. L. (1973). The effect of carbon and nitrogen sources on the growth and sporulation of six different isolates of *Botryodiplodia theobromae*. *Indian Phytopathology*, **26**: 220-224.

Siegenthaler, P. A., Belesky, M. and Goldstein, S. (1967). Phosphate uptake in an obligately marine fungus: A specific requirement of sodium. *Science*, **155**: 93-94.

Silva, W. P. K., Deverall, B. J. and Lyon, B. R. (1998). Molecular, physiological and pathological characterization of Corynespora leaf spot fungi from rubber plantations in Sri Lanka. *Plant Pathology*, **47**: 267-277.

Singh, K. P. and Singh, U. P. (1996). Effect of some plant growth regulators on mycelial growth and sclerotium formation in *Sclerotinia sclerotiorum*. *Indian Journal of Mycology and Plant Pathology*, **26**: 23-29.

Singh, M. I. and Devi, R. K. T. (1996). Effect of different carbon sources and amino acids on growth and sporulations of *Fusarium* species causing sheath rot of rice. *Plant Disease Research*, **11**: 139-142.

Singh, R. A. and Shankar, G. (1971). Effect of physical factors and nutrition on growth and perithcial development of *Glomerella cingulata in vitro*. *Indian Phytopathology*, 24: 728-734.

Singh, S.D. and Prasada, R. (1973). Studies on physiology and control of *Alternaria cyamopsidis*, the incitant of blight disease of gaur. *Indian Journal of Mycology and Plant Pathology*, **3**: 33-39.

Sivaswamy, N. (1982). Ph.D. thesis, University of Madras, Madras, pp: 229.

Smaali, I., Maugard, T., Limam, F., Legoy, M. and Marzouki, N. (2007). Efficient synthesis of gluco-oligosaccharides and alkyl-glucosides by transglycosylation activity of β-glucosidase from *Sclerotinia sclerotiorum*. *World Journal of Microbiology and Biotechnology*, **23**: 145-149.

Soccoll, C. R., Vandenberghe1, L. P. S., Rodrigues, C. and Pandey, A. (2006). New Perspectives for Citric Acid Production and Application. *Food Technology and Biotechnology*, **44**: 141-149.

Soni, G. L., Sedha,R. K., Khanna, P. K. and Garcha, H. S. (1992). Growth inhibition of *Fusarium oxysporum* by phenolic compounds. *Indian Journal of Microbiology*, **32**: 45-49.

Srivastava, J.P. (1951). Effect of various nitrogen compounds on the growth of *Alternaria tenuis*. *Journal of Botanical Society*, **30**: 108-112.

Steinberg, R. A. (1919). A study of some factors on the chemical stimulation on the growth of *Aspergillus niger*. *American Journal of Botany*, **6**: 330-372.

Steinberg, R. A. (1945). A dibasal (minimal salt, maximum yield) solution for *Aspergillus niger*, acidity and magnesium optimum. *Plant Physiology*, **20**: 600- 608.

Steinberg, R. N. (1941). Sulphur and trace elements nutrition of *Aspergillus niger*, *Journal of Agricultural Research*, **43**:109-127.

Suriachandraselvan, M. and Seetharaman K. (2003). Effect of culture media on growth and sclerotial production of different isolates of *Macrophomina phaseolina* infecting sunflower. *Journal of Mycology and Plant Pathology*, **33**(2): 226-229.

Sykes, E. E. and Porter, D. (1973). Nutritional studies of Labyrinthula sp. *Mycologia*, **65**: 1302-1311.

Sytkowski, A. J. (1977). Metal stoichiometry, coenzyme binding, and zinc and cobalt exchange in highly purified yeast alcohol dehydrogenase. *Arch. Biochem.. Biophys.* **184**: 505-517.

Tabak, H. H. and Cooke, W. B. (1968). The effects of gaseous environments on the growth and metabolism of fungi. *The Botanical Review*, **34**:126-252.

Tandon, R. N. and Agarwal, G. P. (1953). Nitrogen nutrition of *Fusarium coerulium* I. Role of nitrites and ammonium salts. *Proceedings of the national Academy of Science, India, Section B*, **23**: 179.

Terashima, Y. (1999). Carbon and nitrogen utilization and acid production by mycelia of the ectomycorrhizal fungus *Tricholoma bakamatstake in vitro*. *Mycoscience*, **40**: 51-56.

Thind, K. S. and Rawla, G. S. (1967). Trace element studies on six species of *Helminthosporium*. *Proceedings of Indian Academy of Science*, **66B**: 250-265.

Thind, T.S. (1977). Studies on nutritional requirements of *Alternaria alternata* and *Gloeosporium fruitigenum*. *Indian Journal of Mycology and Plant Pathology*, **7** (2): 197-199.

Thomas, K. C. and Dawson, P. S. S. (1978). Relationship between iron-limited growth and energy limitation during phased cultivation of *Candida utilis*. *Canadian Journal of Microbiology*, **24**: 440-447.

Totani, N., Yamaguchi, A., Yawata, M. and Ueda, T. (2002). The role of morphology during growth of *Mortierella alpina* in arachidonic acid production. *Journal of Oleo Science*, **51**: 531-538.

Tresner, H. D. and Hayes, J. A. (1971). Sodium chloride tolerance of terrestrial fungi. *Applied Microbiology*, **22**: 210-213.

Upadhyay, R. K. and Dwivedi, R. S. (1979). Effect of vitamins and hormones on the growth of *Pestalotiopsis funera* causing leaf spot of *Eucalyptus globules*. *Procedings of the Indian National Science Academy*, **45**: 37-44.

Uriah, N. and Chipley, J. R. (1976). Effects of various acids and salts on growth and aflatoxin production by *Aspergillus flavus* NRRL 3145. *Microbios*. **116**: 275-278.

Venkatachalam, P. and Jayabalan, N. (1995). Inhibitory effects of some phenolic compounds on *in vitro* culture of *Cercosporidium personatum*. *Indian Phytopathology*, **48**(2): 166-170.

Verma, R. A. B. and Prasad, S. S. (1975). Utilization of carbohydrates by three fungi. Imperfecti causing leaf spot diseases of Mahogany, *Indian Phytopathology*, **28**: 317-321.

Volin, R. B. and Pohronezny, K. (1989). Severe spotting of fresh tomato fruit incited by *Corynespora cassiicola* after storm-related injury. *Plant Disease*, **73**: 1018-1019.

White, J. P. and Johnson, G. T. (1971). Zinc effect on growth and cynodontin production of *Helminthosporium cynodontis*. *Mycologia*, **63**: 548-561.

Wold, W. S. M. and Suzuki, I. (1976). The citric acid fermentation of *Aspergillus niger*: regulation by zinc of growth and acidogenesis. *Canadian Journal of Microbiology*, **22**: 1083-1092.

Wu, H., K. Ito and Shimoi, H. (2005). Identification and characterization of a novel biotin biosynthesis gene in *Saccharomyces cerevisiae*. *Applied Environvironmental Microbiology*, **71**: 6845–6855.

Xiujuan, L., ShengMing, H., YeTong, Y. and YiXian, X. (1995). Effects of phenols on growth and reproduction of *Colletotrichum corda* in latent infection. *Acta Mycologica Sinica*, **14**: 277-282.

Zhang, A. and Roehr, M. (2002). Effects of Varied Phosphorus Concentrations on Citric Acid Fermentation by *Aspergillus niger*. *Acta Biotechnologica*, **22**: 383 – 390.

Zhang, Y. and Gladyshev, V. N. (2008). Molybdoproteomes and Evolution of Molybdenum Utilization. *Journal of Molecular Biology*, **379**: 881-899.

Chapter 3

Offence and defence related enzymes in the Corynespora leaf disease of *Hevea brasiliensis*

3.1 Introduction

Plant cell wall is the first barrier and penetration of the cell wall appears to be the first requirement for pathogenesis of fungal pathogens (Moerschbacher *et al.*, 1990; Kumar *et al.*, 2007; Samia and El-Khallal, 2007). The cell walls are complex amalgamations of carbohydrates (cellulose, hemicellulose and pectic polysaccharides), proteins, lignin and encrusting substances such as cutin, suberin and certain inorganic compounds (Gupta *et al.*, 1995; Hammerschmidt, 1999; Das *et al.*, 2003; Zhao *et al.*, 2007; Jayaraj *et al.*, 2008). The pathogen may recognize the cell wall composition of its host and secrete suitable enzymes to degrade the host cell wall. An array of different enzymes may be necessary to degrade the cell wall (Herrera *et al.*, 2004). Extracellular enzymes are usually capable of digesting insoluble materials such as cellulose, protein and starch, and the digested products are transported into the pathogen cell where they are used as nutrients for growth (Gibb and Strohl, 1987; Oh *et al.*, 2000).

Biotic stresses from a battery of potential pathogens can alter the host metabolisms. Host plants have evolved mechanisms to perceive such attacks and to translate such perception into adaptive responses through enzyme activity (Dangl and Jones, 2001). In fungal plant pathogenesis, enzymes play a crucial role in external and internal interactions (Lebeda *et al.*, 2001). To restrict the development of fungal pathogenesis, the plants form many defence mechanisms. They build mechanical barriers of lignin, suberin, callose and produce antimicrobial compounds with low molecular weight like phenols, chinons and alkaloids. Enhanced production of some enzymes and their increased activity are important plant defence mechanisms. These enzymes occur frequently in many isoforms and are involved in synthesis of defence substances or have direct antimicrobial activity.

3.2 Review

3.2.1 Fungal enzymes in offense

Plant pathogenic fungi produce many types of cell wall degrading enzymes both *in vitro* (Farley and Ikasari, 1992; Chrzanowsk *et al.*, 1993; Fan-Ching and Lin, 1998; Aleksieva and Peeva, 2000) and *in vivo* (Ikotun and Balogun, 1987; Bahkali *et al.*, 1997). These enzymes have been shown to play a major role in the penetration of pathogen and development of several plant diseases (Mendgen *et al.*, 1996; Tucker and Talbot, 2001; Pegg *et al.*, 2009). As reports on specific activity of offence and defence related enzymes studied in the *Corynespora cassiicola* - *Hevea brasiliensis* interactions are scanty, a review on these enzymes involved in fungal pathogenesis on other crop plants is attempted here.

3.2.1.1 Cellulase

Cellulose is the major cell wall polysaccharide and is composed of glucose units in chain configuration, connected by β-1, 4-glycosidic bonds. The enzymatic hydrolysis of cellulose requires the action of several cellulolytic enzymes (Yazdi *et al.*, 1990). Several pathogens are known to produce cellulases *in vitro* and they have been detected in infected tissues also (Mendgen and Deising, 1993; Pushalkar *et al.*, 1995; Pardo, 1996; Ahmed *et al.*, 2009).

Evidence has been accumulating that a number of fungi produce several cellulolytic components (Pushalkar *et al.*, 1995; Wei *et al.*, 1996; Dariot *et al.*, 2008). Such a multiple-component cellulase system has been clearly demonstrated for *C. cassiicola* (Joseph, 1998). Culture filtrates of the fungus contain at least three cellulotytic components: endo-β-1,4 glucanase (Cx – cellulase), exo-β-1,4 glucanase (C_1 – cellulase), β - glucosidase (cellobiose). C_1-cellulase initiates hydrolysis of cellulose, splitting alternate bonds from the non-reducing end of cellulose yielding cellobiose. Endo-glucanase attacks only carboxymethyl cellulose and does not act on native cellulose. β – Glucosidases hydrolyze cellobiase, which is an inhibitor of exo-glucanase (Ahmed *et al.*, 2009). Cellulases are rarely constitutive, as most are induced by cellobiase and cellobiase can repress cellulase synthesis (Shallom and Shoham, 2003; Li *et al.*, 2006; Gao *et al.*, 2008).

Several studies have provided evidence to show the involvement of cellulolytic enzymes in fungal pathogenesis (Schafer, 1994; Huckelhoven, 2007). Histological studies using tobacco roots infected by *Phytophthora parasitica* var. *nicitianae* demonstrated the involvement of cellulases in fungal pathogenesis (Johri and Devi, 1998). Cellulases also play an important role in diseases that cause wilting. *Fusarium oxysporum* and *Verticillium albo-atrum* produce cellulase in culture and degrade cellulose *in vivo* (Bailey, 1995; Fradin and Thomma, 2006). The presence of Cx in wilt affected tomato plants infected with *Pseudomonas solanacearum* and *F. oxysporum f. lycopersici* has been demonstrated earlier and it has been suggested that in pathogenesis this enzyme plays a significant role by causing the dissolution of cell walls (Mehta and Mehta, 1993; Mehta *et al.*, 1993).

3.2.1.2 Protease

Structural proteins are also important components of the plant cell wall. Proteases may be involved in degradation of plant cell wall proteins (Rauscher *et al.*, 1995; Andrade *et al*, 2002). Efficient protease biosynthesis by fungi belonging to the genera *Aspergillus* (De Vries and Visser, 2001), *Penicillium* (Abbas *et al.*, 1989), *Humicola* (Aleksieva and Peeva, 2000) and some other species have been reported. Investigations on protease production by many fungal cultures have shown that the quantity of proteases produced varies greatly with the media used. Regulatory effects exerted by the carbon sources on protease production have been described (Guzman *et al.*, 2005).

Kannaiyan *et al.* (1975) observed the presence of proteolytic enzymes in the culture filtrates of *Claviceps microcephala.* Enzyme activity was maximum in casein and egg albumin culture filtrates on seventh day of incubation, while prolonged incubation increased the production of the enzyme in peptone and gelatin media too.

Ball *et al.* (1991) observed that the ultraviolet induced nonpathogenic mutant of *Pyrenopeziza brassicae* was deficient in protease production *in vitro.* They transformed the protease mutant with clones from the genomic library of *P. brassicae* and the transformant showed concomitant restoration of pathogenicity and proteolytic activity *in vitro.*

3.2.2 Host enzymes in defence

Biotic stresses from a battery of potential pathogens can alter the host metabolisms. Host plants have evolved mechanisms to perceive such attacks and to translate such perception into adaptive responses through enzyme activity (Dangl and Jones, 2001). In fungal plant pathogenesis, enzymes play a crucial role in external and internal interactions (Lebeda *et al.*, 1999). To restrict the development of fungal pathogenesis, the plants form many defence mechanisms. They build mechanical barriers of lignin, suberin, callose and produce antimicrobial compounds with low molecular weight like phenols, chinons and alkaloids. Enhanced production of some enzymes and their increased activity is an important plant defence mechanism. These enzymes occur frequently in many isoforms and are involved in synthesis of defence substances or have direct antimicrobial activity.

3.2.2.1 Oxidoreductase enzymes

Several authors reported the role of oxidative enzymes and their metabolic products in the plant defence (Weber *et al.*, 1967; Smith and Hammerschmidt, 1988; Chandra and Tyagi, 1993; Li and Steffens; 2002). Enzymes like oxidases, dehydrogenases and reductases are grouped under this. In some plant species, hypersensitivity and necrotic browning of tissues have been associated with increased activity of oxidative enzymes (Kosuge, 1969; Mayer and Harel, 1979; Rubin and Artsikhouskaya, 1964). Dehydrogenases catalyse the transfer of hydrogen atom from a substratum to a hydrogen acceptor. The activity of dehydrogenases especially in pentose-phosphate pathway and Kreb's cycle increase with the increase in a general metabolism of injured plants or of those attacked by pathogens (Malca and Zscheile, 1964). Dehydrogenase activity is reported to be generally higher in incompatible host-pathogen combinations than in the compatible ones (Madhukar and Reddy, 1990). Reductase plays a major role in nitrogen metabolism of diseased plants by the addition of hydrogen ion or an electron and removal of oxygen (A.del Rio *et al.*, 2006).

3.2.2.1.1 Peroxidase

Weber *et al.* (1967) studied the enzymatic changes associated with induced and natural resistance of sweet potato to *Ceratocystis fimbriata* and peroxidases activity was

well correlated with their resistance. Peroxidases (PO) have been implicated in a variety of defence–related processes, including the hypersensitive response, secondary cell wall biosynthesis by polymerizing hydroxy and methoxycinnamic alcohols into lignin and forming rigid cross–links between cellulose, pectin, hydroxyproline–rich glycoproteins and lignin, suberization and phytoalexin production (Nicholson and Hammerschmidt, 1992; Wojtaszek, 1997; Baysal *et al.*, 2003). PO is reported to have an important function in the induction of systemic resistance (Farkas and Lovrekovich, 1965). Jennings *et al.* (1969) studied PO activity associated with Helminthosporium leaf spot of maize and reported that the resistant host exhibited higher peroxidase activity when compared to the control.

In Thanjavur wilt affected coconut palms more PO activity who observed in the diseased tissues than the healthy ones, which enhanced with increase in disease severity (Karthikeyan and Bhaskaran, 1992). Fengming *et al.* (1997) documented the changes in activity of PO in different cellular locations of cotton seedlings on infection by *Fusarium oxysporum* f.sp. *vasinfectum* and their possible role in the resistance of cotton. The activity of PO in resistant cucumber leaves during infection with *Cladosporium cucumerinum* was significantly higher than those in susceptible cultivars (Baoju and fengyun, 1998). Kalia (1998) observed a marked increase in PO activity in both resistant and susceptible genotypes but the increase was more pronounced in resistant ones. Egea *et al.* (2001) reported increase in activity of PO in pepper variety resistant to *Phytophthora capsici.*

Saharan *et al.* (2001) observed that PO activity increased with increasing intensity of Alternaria blight in susceptible cluster bean variety Fs 277 while in another susceptible variety PNB, the activity declined. Mohammadi and Kazemi (2002) investigated the changes in peroxidase activity in resistant and susceptible wheat heads of flowering, milk, dough and ripening stages following inoculation with *Fusarium graminearum* and observed significant activity during the milk stage.

Breton *et al.* (1996) reported significant PO activity in resistant than in susceptible *Hevea* clones on *C. cassiicola* infection. Different levels of nitrogen application enhanced

PO activity in resistant and susceptible clones of *H. brasiliensis* infected by *C. cassiicola* (Joseph, 1998).

3.2.2.1.2 Polyphenol oxidase

Polyphenol oxidases (PPO) in plant cells are mainly compartmentalized in vesicles or plastids (Butt, 1980; Mayer and Harel, 1979) and in the cell wall. PPO catalyzing the oxygen dependent oxidation of phenols to quinines are ubiquitous among angiosperms and are assumed to be involved in plant defence against pests and pathogens (Li and Steffens, 2002). Jennings *et al.* (1969) reported that PPO might function as an alternate electron transport chain and serve as terminal oxidase in infected plant tissue.

Kalia (1998) investigated the enzymic association of powdery mildew resistance in garden pea and reported higher PPO activity in resistant than in susceptible genotypes in the pre-infection stage. Post inoculation increase in PPO activity was observed in both resistant and susceptible genotypes except for a few which either exhibited no change or decrease.

Higher PPO activity was observed in resistant cucumber cultivars infected with *Cladosporium cucumerinum* than in susceptible cultivars at the initial stages of infection (Baoju and Fengyun, 1998). There was no significant difference between them in later stages of infection. Similarly, Saharan *et al.* (2001) reported that the quantity of PPO increased with the increase in intensity of Alternaria blight up to 50% in highly susceptible cluster bean cultivars, compared to their respective healthy leaves. With the increase in disease intensity, enzyme activity started to decline. Saharan *et al.* (1999) reported that PPO activity in both resistant and susceptible varieties of cluster bean infected with *Alternaria* increased markedly in response to infection.

The specific activity of PPO and level of bound phenol in chickpea cultivars resistant and susceptible to *Ascochyta rabiei* were documented in one-month-old leaves after inoculation with two isolates of *A. rabiei* (Khirbat and Jalali, 1998). The specific activity of PPO remained higher in the resistant genotype in response to inoculation from

six to ten days with both the pathogen isolates. However, the activity sharply declined after ten days of inoculation with both the isolates in the susceptible genotype.

On inoculation of resistant and susceptible wheat heads with *Fusarium graminearum* maximum PPO activity was observed during the milk stage, which subsequently declined. The activity was three times higher in resistant cultivars than in the non-inoculated control plants (Mohammadi and Kazemi, 2002).

Arora and Bajaj (1985) investigated PPO associated with induced resistance of mung bean to *Rhizoctonia solani* and determined isozyme patterns at different stages of hypocotyls of mung bean infected with the pathogen. It was also indicated that ethephon induced resistance was related to PPO activity.

The variations of PPO activity have been investigated in tomato plants, the roots of which had been subjected to biotic and abiotic stress that induce resistance to *Fusarium oxysporium* f.sp. *lycopersici* (Gentile *et al.*, 1988). It was concluded that increased PPO activity is a systemic response to cellular injury caused in the root by stresses.

PPO usually accumulate upon wounding in plants and catalyse the biosynthesis of oxidative phenols (Avdiushko *et al.*, 1993). Vivekananthan (2003) found that the mango leaves, flowers and fruits treated with *Pseudomonas fluorescens* (FP7) and chitin bioformulations enhance the activity of PPO and thereby suppress anthracnose incidence. Sible (2003) recorded about sevenfold increase in PPO activity in treatment involving FP7 amended with chitin and fourfold on treatment with FP7 alone.

Gogoi *et al.* (2000) reported increased level of PPO activity in all the wheat genotypes irrespective of their resistant and susceptible reaction subsequent to inoculation with *Neovossia indica.*

3.2.2.1.3 Catalase

Catalase (CAT) is a tetrameric, heme-binding protein that catalyses the dismutation of H_2O_2 to H_2O and O_2. CAT occurs in almost all aerobically respiring organisms and was one of the first enzymes to be crystallized (Eventoff, *et al.*, 1976; Fita and Rossman, 1985). In plants, H_2O_2 is produced during the oxidative burst, which is often the first response of a resistant plant to an avirulent pathogen (Levin *et al.*, 1994). In leaf tissue CAT is localized in peroxisomes, to scavenge the H_2O_2 produced by glycolate oxidase in the C_2 photorespiratory cycle (Perl-Treves and Perl, 2002).

Changes in CAT activity as a result of fungal infection have been reported in various host pathogen interactions (Maxwell and Bateson, 1967; Fric and Fuchs, 1970; Sharma and Kaul, 1999; Bestwick *et al.*, 2001). Sarkar and Joshi (1977) reported decrease in CAT activity in MLO infected Poulownia witches broom. Saharan *et al.* (2001) observed reduction in CAT activity with the increase in Alternaria blight severity, compared to healthy leaves in highly susceptible cultivars of cluster bean. Rameshsunder and Vidhyasekaran (2003) investigated the induction of defence related biochemical changes by elicitors of red rot pathogen and a non – pathogen in sugarcane cell culture. They observed that the elicitor untreated suspension cultured cells recorded higher constitutive levels of antioxidant enzyme CAT, which suggest their possible role as suppressor mechanism in the sugarcane *Colletotrichum falcatum* interaction. CAT has been reported as a free radical scavenger and as a suppressor mechanism in a number of host-pathogen / elicitor interaction (Scandalios, 1993).

Vir and Grewal (1975) noticed significant increase in CAT activity in the leaves and stems of resistant gram varieties up to eighth day after *Ascochyta rabiei* inoculation which declined subsequently. Sharma and Kaul (1996) investigated the biochemical nature of resistance in apple to *Venturia inaequalis* causing scab and showed positive correlation of CAT with disease resistance. Fengming *et al.* (1997) studied the changes in activity of CAT in leaves, stems and roots of cotton seedlings on infection with *Fusarium oxysporum* f.sp. *vasinfectum* and observed no significant difference between resistant and susceptible cultivars. Although several studies establish an important role for CAT in plant disease

resistance, the precise functions of CAT in resistance signaling is not clearly understood (Chen *et al.*, 1993a; Chen *et al.*, 1993b; Conrath *et al.*, 1995; Durner and Klessig, 1996; Chen *et al.*, 1997).

3.2.2.1.4 Ascorbic acid oxidase

Ascorbic acid oxidase (AAO), one of the terminal oxidases, is a cell wall localized glycoprotein belonging to the family of blue copper oxidases those catalyses decrease in ascorbic acid content induced by severe stress. The decrease in ascorbic acid may be due to its direct destruction by O_2 and derived species (Ormaetxe *et al.*, 1998; Bartoli *et al.*, 1999). It was found that AAO activity increases in Acacia galls as compared to healthy tissues. The decrease in ascorbic acid content in galls was due to the increased activity of AAO and respiration (Dharmadhikari and Jite, 1996).

The aerobic oxidation of ascorbic acid to dehydro-ascorbic acid (DHA) is via a free radical semidehydro-ascorbic acid (Lin and Varner, 1999). Maxwell and Bateman (1967) studied the changes in activities of some oxidases in extracts of *Rhizoctonia* infected bean hypocotyls in relation to lesion maturation and observed no marked differences in activity of the diseased tissue extracts and healthy hypocotyl extracts at any of the three stages of lesion maturation. Increased oxidation of ascorbate was reported in *Pinus pinaster* and *Quercus rebur* as a result of stress (Schwanz and Polle (2001). The acid content in banana fruits infected with *Botryodiplodia theobromae*, *Helminthosporium speciferum* and *Aspergillus flavus* showed a gradual reduction as the incubation period progressed (Singh, 1993). Prasad *et al.* (1989) observed decrease in ascorbic acid content in rotting of tomato fruits caused by *Sclerotium rolfsii*.

Joseph (1998) studied biochemical changes in *H. brasiliensis* as influenced by nitrogenous fertilizer application and *C. cassiicola* inoculation. A significant reduction in ascorbic acid content was observed in rubber seedlings after infection. The increased levels of nitrogenous fertilizer application reduced the activity of AAO and the sampling period did not alter the activity of this enzyme.

3.2.2.1.5 Indole-3-acetic acid oxidase

Auxin levels in normal plants depends not only upon the rate of synthesis but also on the rate at which auxin is inactivated by oxidation of indole-3-acetic acid oxidase (IAAO). Arora *et al.* (1986) determined the activity of IAAO and its isozyme during the pathogenesis of pearl millet by *Sclerospora graminicola.*

Hashim *et al.* (1978) stimulated IAAO activities of preparations from *Hevea* leaves by 2, 4-dichlorophenol as well as by naturally occurring phenolics, p-coumaric acid, scopoletin, 4-methylumbelliferone and chlorogenic acid. Kaempferol and quercetin, which have both been associated resistance to the South American leaf blight disease of *Hevea,* were shown to function both as cofactors and as competitive inhibitors of IAA oxidase.

Jite and Tressa (1999) studied the biochemical changes in *Jasminum grandiflorum* infected by *Uromyces hobsoni* and observed a reduction in the activity of IAAO. They also pointed out that the increased levels of host phenols bring about a lag in IAAO activity, which accounts for increased amounts of IAA in *J. grandiflorum* after infection. Therefore, phenols have been designated as auxin-protectors.

An increase in IAAO has been reported in maize infected with *Helminthosporium maydis* and *H. turcicum* (Sukhwal and Purohit, 2003). IAAO activity rose up to 16-fold in resistant maize variety after infection with *H. maydis.* Srivastava and Van Huystee (1973) reported that IAAO activity was related to oxidase function of peroxidase.

3.2.2.1.6 Cinnamyl alcohol dehydrogenase

Cinnamyl alcohol dehydrogenase (CAD) catalyses the conversion of p-hydroxy-cinnamaldehydes to the corresponding alcohols and is considered a key enzyme in lignin biosynthesis (Blanco-Portales *et al.*, 2002). Lignin biosynthesis is probably controlled by two signal-transduction pathways. One is involved in the development of vascular tissue and the other in plant defence responses (Walter, 1992; Mitchell and Barber, 1994). CAD induction has been studied in relatively few plant defence responses, and in all of these

cases only coniferyl alcohol dehydrogenase activity was measured (Moerschbacher *et al.*, 1986; Grand *et al.*, 1987; Walter *et al.*, 1988; De Sa *et al.*, 1992).

The increased activity of CAD during the expression of resistance was noticed by Grand *et al.* (1987). Mitchell *et al.* (1994) examined the substrate-specific induction of wheat leaf CAD in relation to its role in regulating the composition of defensive lignin induced at wound margins. Treatments of wounds with partially acetylated chitosan hydrolysate or spores of the nonpathogen *Botrytis cinerea* elicited lignification at wound margins and invoked significant increase in CAD activity. Pillonel *et al.* (1992) partially purified multiple forms of CAD from healthy wheat, but none of these exhibited a preferential activity for sinapyl alcohol. Walter *et al.* (1988) suggested that early induction of CAD reduces the levels of hydroxyl cinnamyl acids which repress the expression of phenylpropanoid enzymes earlier in the pathway.

Moerschbacher *et al.* (1988) studied the lignin biosynthetic enzymes in stem rust infected resistant and susceptible near-isogenic wheat lines and reported increased CAD activity in infected plants of compatible and the incompatible interaction from 16 to 40 hours after inoculation. In resistant plants CAD activity continued to increase up to 7 days after inoculation. While in susceptible, the activity declined, 3-days after inoculation.

3.2.2.1.7 Nitrate reductase

Nitrate reductase (NR) is one of the first enzymes responsible for the biosynthesis of amino acids and a key regulator of influx of reduced nitrogen in plants. Nitrate reduction catalyzed by the enzyme nitrate reductase is considered as a rate limiting step in the overall process of nitrate assimilation (Srivastava, 1980).

Rao *et al.* (1992) studied the levels of NR in smut infected leaf tissues of sugarcane and reported that the activity was higher in the infected tissues than the healthy tissues at all the stages of infection. They recorded maximum NR activity at the initiation stage of infection which decreased there after. Investigations of Karthikeyan and Bhaskaran (1992) on Thanjavur wilt affected coconut palms revealed that NR was more in the diseased

tissues than in the healthy. They also observed that the activity of the enzyme enhanced with the increase in disease severity.

Investigations of Walters and Ayres (1980) on the effect of powdery mildew disease on the uptake and metabolism of nitrogen by barley plants pointed out that infection had no effect on the activity of either nitrate reductase or nitrite reductase. Robert and Walters, (1988) found a decreasing trend of NR, in *Allium porrum* leaves after 12 days of inoculation with *Puccinia allii* while there was little effect on nitrite reductase. Murray and Ayres (1986) observed a substantial reduction in NR activity in mildew infected barley seedlings.

3.2.2.1.8 Malate dehydrogenase

Malate dehydrogenase (MDH) is a homodimeric enzyme well known for the many cell compartment-specific isoenzymes. There is a mitochondrial MDH that functions in the tricarboxylic acid cycle which is usually NAD+ -dependent. There are two chloroplast enzymes in plants, one NADP+ -dependent and one NAD+ -dependent. Among other functions, these compartment-specific isoforms help to shuttle reducing equivalents in the form of malate/oxalacetate across membranes and into various cell compartments where they are needed. The NADP+ -dependent MDH from chloroplasts has a role in the mechanism for exporting reducing equivalents during photosynthesis (Strodtkotter *et al.*, 2009).

Qualitative and quantitative changes in the activity of certain dehydrogenases in the diseased plant tissue during pathogenesis have been reported (Chile and Vyas, 1983; Madhukar and Reddy, 1990). Scott and Smillie (1963) correlated the decrease in rate of photosynthesis in infected barley leaf segment with the increase in the activity of dehydrogenase enzyme. Scott (1965) reported slight reduction of malate dehydrogenase activity in barley leaves infected with *Erysiphe graminis*.

The changes in proteins and enzymes in susceptible bean leaves after infection by the bean rust fungus, *Uromyces phaseoli* revealed an increase in number of isozymes of malate dehydrogenase (Staples and Stahmann, 1964).

3.2.2.2 Hydrolases

Hydrolytic enzymes have a wide range of functions in plant metabolism (Christopher *et al.*, 2007). These enzymes catalise a variety of compounds and cause hydrolysis of ionic linkages which generally result in the cleavage of the substrate. The role of hydrolytic enzymes in host-pathogen interaction has not been studied in detail. Digestive enzymes like carbohydrases, esterases, proteases and phosphatases belong to this group.

3.2.2.2.1 Chitinase

Chitinases catalyses the hydrolysis of β-1,4 linkages of the N-acetyl-D-glucosamine polymer, called chitin, which is a major component of the cell walls of most of filamentous fungi except Oomycetes (Adams, 2004). Chitinases are divided into six classes, depending on their structural homologies (Chang *et al.*, 1995). The basic class I chitinases contain N-terminal cystein-rich domain with putative chitin-binding properties linked by a variable proline-rich hinge region, to a highly conserved catalytic domain. Most of these display a C-terminal extension required for vacuolar localization of the protein. Class II chitinases lack the terminal N-terminal cysteine-rich domain whilst class IV display four deletions in the catalytic domain, nevertheless these chitinases share high homology with the class I chitinases and are both extracellular. Class V chitinases are characterized by the duplication of the N-terminal chitin-binding domain. In contrast, class III chitinases are structurally unrelated to the other types of plant chitinases as they possess a domain with weak similarity to prokaryotic chitinases, and display lysozyme activity. Finally class VI chitinases are similar to bacterial chitinases from, for example, *Bacillus circulans*, *Serratia marcescens* or *Streptomyces plicatus*.

The activation profile of a bean chitinase promoter during fungal infection indicates that chitinase expression is intimately associated with the response of the plant to pathogen

invasion (Roby *et al.*, 1990). Although chitinase is generally found at low or basal levels in healthy plants, its expression is increased during pathogen attack (Kang and Buchenauer, 2002), and it accumulates intercellularly in the vacuole. When *Cladosporium fulvum* was inoculated on a susceptible tomato variety, chitinase activity increased slowly while in resistant variety there was rapid induction (Joosten and De wit, 1989). Induction of defence enzymes makes the plant resistant to pathogen invasion (Van Loon *et al.*, 1998).

A five-fold increase in chitinase activity has been reported in suspension-cultured rice cells 24 hours after *Rhizoctonia solani*-elicitor treatment (Velazhahan *et al.*, 2000). Metraux and Boller (1986) reported that the activity of chitinase increased up to 600-fold in the leaf infected with fungal, bacterial or viral pathogens and was induced about 10 times less strongly in uninfected areas of infected leaves. Jongedijk *et al.* (1995) observed that the synergistic activity of chitinase and β-1,3 glucanase enhances fungal resistance in transgenic tomato plants. Studies of Fernandez *et al.* (1998) revealed that chitinases constitute biochemical defence mechanisms in tomato plants against *Alternaria solani*. Caruso *et al.* (1999) also reported induction of chitinase in germinating wheat seeds infected with *Fusarium culmorum*.

Deborah *et al.* (2001) found differential induction of chitinase and β-1,3 glucanase in rice in response to inoculation with *Rhizoctonia solani* and *Pestalotia palmarum*, a non-pathogen. Attempts have been made to exploit these anti-fungal proteins to develop disease resistant transgenic crop plants (Broglie *et al.*, 1991; Lin *et al.*, 1995; Marchant *et al.*, 1998). Bokma *et al.* (2001) determined the sequences of cDNA and genomic DNA of hevamine, a chitinase from the rubber tree *H. brasiliensis*.

3.2.2.2.2 Lipase

Lipases are widely distributed in plants and microorganisms and are the most versatile biocatalysts endowed with the property of bringing about a variety of reactions that include hydrolysis, inter-esterification, esterification, alcoholysis, acidolysis and aminolysis (Kanwar *et al.*, 2004). Recent structural studies on several lipases have provided leads towards understanding of hydrolytic activity, interfacial activation and

stereoselectivity (Kim *et al.*, 1997). Most of the lipases are extracellular enzymes. Within the cell, they are not completely activated but exist in a pre-lipase form (Farell *et al.*, 1993; Ayora *et al.*, 1994; Kok *et al.*, 1995).

Changes in phospholipids content in cell organelles have been observed in several plants due to infection (Hoppe and Heitefuss, 1974; Sednina et al., 1981). The crude extract of *Erysiphe pisi* caused pea mesophyll protoplasts to lose their contents and the crude extract contained phospholipase (Faull and Gay, 1983). Phospholipase has been detected in extracts of *Thielaviopsis basicola* (Lumsden and Bateman, 1968), *Sclerotium rolfsii* (Tseng and Bateman, 1969) and *Sclerotium sclerotiorum* (Lumsden, 1970).

Phosholipases of the host are activated when the cells are treated with toxins produced by the pathogen (Vidhyasekaran, 1998). AF-toxin, produced by *Alternaria alternata* strawberry pathotype, induced a considerable decrease in phospholipids content in microsomes of strawberry protoplasts in parallel with a decline in cell viability (Lee *et al.*, 1992). Lipid degrading enzymes are involved in the signal transduction of plant cell in response to various stimuli (Scherer, *et al*, 1988, Martiny-Baron and Scherer, 1989, Palmgren and Sommarin, 1989, Scherer and Andre, 1989).

3.2.2.2.3 Phosphatase

General phosphatases are classified as alkaline phosphatases or acids based solely upon whether their optimal activity is above or below pH 7.0 (Vincent *et al.*, 1992). In plants, acid phosphatases (or acid phophastase activity) have been localized to many cellular compartments including vacuoles, chloroplasts, cell walls, membranes, Golgi complex, and cytoplasm (Duff *et al.*, 1994; Olmos and Hellin, 1997). Acid phosphatases are ubiquitous enzymes found in plants and other organisms that catalyse the dephosphorylation of a wide variety of substrates by hydrolysis of phosphate esters and are believed to have roles in energy transfer and metabolic regulation in plant cells (Duff *et al.*, 1994). Whereas plant alkaline phosphatases have been characterized as having specific metabolic roles, most plant acid phosphatases have been shown to hydrolyze a wide

variety of phosphorelated substrates *in vitro* making their cellular role(s) difficult to define (Duff *et al.*, 1994).

Beber *et al.* (2000) activated a putative barley acid phosphatase by the chemical inducers of systemic resistance. Jakobek and Lindgren (2002) studied the expression of a bean acid phosphatase cDNA and correlated it with disease resistance. A decrease in activity of phosphatase was noticed in susceptible bean leaves after infection by *Uromyces phaseoli* (Staples and Stahmann, 1964).

Jasmonic acid (JA) is an established wound signal that plays a role in plant-pathogen interactions. Kenton *et al.* (1999) studied the requirement for calcium and protein phosphatase in the jasmonate-induced increase in tobacco leaf acid phosphatase specific activity. They reported that the application of JA to tobacco leaf explants, seedlings or to intact leaves via the petiole resulted in an increase in the specific activity of acid phosphatase (AP) and a reduction in overall protein content. The AP activity increase was restricted to wounded tissue and HR lesions and was absent from unwounded or uninfiltrated tissue on the same leaf.

3.2.2.3 Lysases

Lyases are crucial enzymes in plant-pathogen interaction that favors the host plant by synthesizing defence barriers during the fungal invasion (Cahill and McComb, 1992). These enzymes result in a direct removal of groups from substrate nonhydrolytically.

3.2.2.3.1 Phenylalanine ammonia lyase

Phenylalanine ammonia lyase (PAL) is the first enzyme of phenyl propanoid metabolism in higher plants and has been suggested to play a significant role in regulating the accumulation of phenolics (Booker and Miller, 1998), phytoalexins (Kala and Chaudhary, 2001) and lignins (Bowles, 1990). Bayliss *et al.*, (2001) investigated the variability of PAL activity in leaves of subterranean cloves infected with *Kabatiella caulivora* and considered the post infectional increase of PAL as a general defence response. Green *et al.* (1975) noted more than two fold increase in PAL activity at four

hours after inoculation which reached a peak at 24 hours and then decreased sharply by 48 hours. In chickpea infected with *Ascochyta rabiei* maximum PAL activity was observed 12 to 24 hours after infection, and it coincided with the period of most rapid production of phytoalexins, which is a defence mechanism of (Sarwar *et al.* 2001).

PAL activity has also been reported to be involved in resistance of some graminaceous hosts to rust infections. In compatible and incompatible interactions of wheat and *Puccinia graminis* f.sp. *tritici*, higher PAL activities were measured 8-16 hours after inoculation, followed by another peak only in the resistant variety, coincident with haustoria formation and hypersensitive reaction (Moerschbacher, 1988).

Induction of PAL in cell suspension cultures of bean after exposure to an elicitor preparation from the cell walls of the phytopathogenic fungus *Colletotrichum lindemuthianum* was observed (Bolwell *et al.*, 1986). In tomato cell cultures, PAL activity was induced both at the enzyme and mRNA levels by *Verticillium albo-atrum* inoculation (Bernards and Ellis, 1991). PAL activity increased by more than ten fold in pine (*Pinus* sp.) cell cultures after treatment with a fungal elicitor at 24 hours after treatment, coinciding with the initiation of cell wall lignification (Campbell and Ellis, 1992).

Shiraishi *et al.* (1989) reported an increase in synthesis of cinnamic acid and increase in the level of PAL activity occurred by as early as 2 hours after inoculation in barley leaves infected with *Erysiphe graminis*. Shiraishi *et al.* (1995) observed increases in PAL activity at two different times in barley cultivars inoculated with *E. graminis*. The first increase in enzyme activity began at 3 hours after inoculation and this was followed by a second peak activity between 12 and 15 hours. The conidium produces a primary germ tube that attempts penetration beginning about 2 hours after inoculation and an appressorium that attempts to penetrate beginning 9-10 hours after inoculation. Thus it appears that the elevation of PAL enzyme levels by hosts is a direct response to attempted penetration by the fungus.

3.2.2.3.2 Tyrosine ammonia lyase

Tyrosine ammonia lyase (TAL) one of the key enzymes in the phenyl propanoid pathway was inducible in response to biotic and abiotic stress and was increasingly recognized to play an important role in disease resistance. Green *et al.* (1975) studied change in level of TAL and lignin in wheat inoculated with *Erysiphe graminis* f.sp. *tritici* and reported increased activity of TAL in all inoculated genotypes beginning 4 hours after inoculation that reached a peak at 24 hours, and decreased sharply by 48 hours.

Khan *et al* (2003) reported that the application of chitin and chitosan to soybean leaf tissues caused increased TAL activity. The elevation of the activity was dependent on the chain length of the oligomers and time after treatment. Maximum activity was noticed after 36 hours of infection. Increase in total phenolic content of soybean leaves following infection showed a positive correlation with enzyme activity.

Joseph (1998) reported that *C. cassiicola* inoculation on *H. brasiliensis* increased the activity of TAL in plants receiving 25 and 50% of recommended dose of nitrogen. However, in 100 and 200% nitrogen treated plants, inoculation of *C. cassiicola* considerably reduced the TAL activity. A marginal increase in TAL activity with time was also reported.

3.2.2.3.3 Phosphoenol pyruvate carboxylase

Walters and Ayres (1983) investigated the changes in nitrogen utilization and enzyme activities associated with CO_2 exchanges in healthy and powdery mildew infected barley and reported maximum stimulation of carboxylase activity after infection. They also found an increasing trend of carboxylase activity to a maximum around six days after inoculation, but the activity returned to healthy control levels by nine days after inoculation.

3.2.2.4 Synthetases

These enzymes catalyse the synthesis of different types of bonds such as C-C, C-N, C-S and C-O.

3.2.2.4.1 Glutamate synthase

Nitrogen is the major limiting nutrient in most plant species. Irrespective of the nitrogen source the reduced form of nitrogen ultimately available to higher plants for direct assimilation is ammonium. In most plants ammonium is assimilated into amino acids through the co-operative activity of two enzymes: glutamine synthetase (GS) and glutamate synthase (GOGAT) (Lea and Miflin, 1974). GS catalyses the incorporation of ammonium into glutamate, producing glutamine. GOGAT catalyses the transfer of the amide group of glutamine to 2-oxoglutarate, resulting in the formation of two molecules of glutamate. Glutamine and glutamate serve as nitrogen donors for the biosynthesis of many other compounds (Lea and Ireland, 1999; Temple *et al.*, 1998).

Roberts and Walters (1988) studied the nitrogen assimilation and metabolism in rust infected *Allium porrum* leaves before and after infection and reported an increase in activity of GOGAT after the infection by *Alternaria porri*. Walters and Ayres (1980) reported reduced activities of glutamate dehydrogenase, GS and GOGAT in powdery mildew disease of barley. Sadler and Shaw (1979) examined the effects of rust infection on enzymes of ammonium assimilation in flax cotyledons and observed a decrease in GOGAT activity.

3.3 Materials and methods

3.3.1 Fungal enzymes in offence

Richard's medium was used as the basal medium for the study of offence related enzymes in *C. cassiicola* isolates. The medium (50 ml) was dispensed in each 100 ml conical flask and the pH was adjusted to 6.5 before sterilization. Three 7 mm mycelial plugs were inoculated in each flask and incubated for 20 days at 25 ± 2^0C. The mycelial mat was separated by filtration and the culture filtrates obtained at different intervals *viz* (5, 10, 15 and 20 days) were used for the enzyme assay.

3.3.1.1 Cellulase

The production of reducing sugar (glucose) due to cellulolytic activity was measured by the dinitrosalicylic acid method (Gascoigne & Gascoigne, 1960). The reaction mixture consisted of 1 ml of 1% carboxymethyl cellulose (CMC) in 0.1M sodium citrate (pH 5.0), 0.5 ml of 0.1 M sodium citrate (pH 5.0) and 2.5 ml of enzyme extract. The reaction mixture was incubated at 55^0C for 30 mins. Later, 1 ml of dinitrosalicylic acid reagent (DNS) was added and the tubes were kept in boiling water bath for 5 mins. The DNS reagent was prepared by dissolving 1 g of DNS, 200 mg of crystalline phenol and 50 mg sodium sulphate in 100 ml of 1% NaOH. While still warm, 1 ml of 40% sodium potassium tartarate was added. Denaturated enzyme controls were maintained. After cooling, the colour was read at 540 nm in a shimadzu UV-160A, UV-Visible spectrophotometer. Results were expressed as mg glucose formed hr^{-1} ml^{-1} enzyme.

3.3.1.2 Protease

Amino acids released by the action of protease are measured by the reaction of α amino group with ninhydrin to give a coloured derivative, diketohydrindylidenediketohydrindamine, plus the aldehyde of the amino acid and CO_2. The coloured product of the reaction has a characteristic absorption peak at 570nm and the intensity of colour was related to the quantity of amino acid.

The reaction mixture consisted of 0.5ml of 0.1% casein in 0.1M sodium phosphate buffer, pH7.0, as substrate and 0.5ml of enzyme extract. This was incubated for 30 min at 30 ^{0}C and 0.5ml ninhydrin reagent was added and the tubes were heated in a boiling water bath for 20 min. Ninhydrin reagent was prepared by dissolving 0.8g stannous chloride in 5000 ml of 0.2M citrate buffer, pH 5.0 and mixing this solution with 20g of ninhydrin in 500ml of methyl cellosolve (2-methoxy ethanol) 5ml of diluent (n-propanol, water-1:1) was added while the tubes were still warm. Denatured enzyme controls were maintained. The colour was read at 570nm in a Shimadzu UV-160A, UV- visible spectrophotometer. A working curve was prepared using a Leu standard of 1-3 mg ml^{-1}. The enzyme activity was

expressed as mg amino acids released per hour per ml of enzyme. The procedures adopted were essentially that of Moore and Stein (1948).

3.3.2 Host enzymes

3.3.2.1 Peroxidase

One gram of plant tissue was crushed in 3ml of 0.1M-phosphate buffer (pH 7.0) by grinding with a pre-cooled mortar and pestle. The homogenate was centrifuged at 18,000g at 5 °C for 15 min. and the supernatant was used as the enzyme source.

The assay system contained 2.0ml guaiacol, 0.5ml citrate – phosphate buffer (pH 6.0) and 0.5ml enzyme extract. Peroxidase activity was determined by measuring the increase in absorbance at 470 nm for 1 min. after the addition of one drop of H_2O_2 (10 µmole) to the reaction mixture (Hashim *et al.*, 1980) and was expressed as unit change in absorbance (ΔA / min / g sample).

3.3.2.2 Polyphenol oxidase

One gram of the plant material was crushed with 4ml of chilled 0.1m sodium phosphate buffer at pH 7.1 in a previously chilled pestle and mortar. The extract was strained through two layers of cheese cloth and the volume was made up to 5ml with the buffer, centrifuged at 2100g for 30min and the supernatant was used as the enzyme source (Sridhar *et al.*, 1969).

The reaction mixture contained 2.0ml catechol, 0.5ml citrate phosphate buffer (pH 6.0) and 0.5ml of enzyme extract. Polyphenol oxidase activity were assayed by determining the absorbency increase at 470 nm and expressed as unit change in absorbance (ΔA / min / g sample).

3.3.2.3 Catalase

Plant tissues were homogenized in a blender with phosphate buffer at 1-4 °C and centrifuged. The sediments were stirred with cold phosphate buffer and allowed to stand in the cold with occasional shaking. The extraction was repeated twice. The supernatant was combined for assay. Enzyme (0.01-0.04 ml) was mixed with 3 ml of H_2O_2 – PO_4 buffer. The time Δt required for the decrease in absorbance from 0.45 to 0.4 was noted. Catalase activity was expressed as change in absorbance per minute.

3.3.2.4 Ascorbic acid oxidase

One part of plant tissue was macerated with five parts (w/v) of 0.1 M phosphate buffer (pH 6.5) in a homogenizer. The homogenate was centrifuged at 3000g for 15 min. and the supernatant was used as the enzyme source (Sadasivam and Manickam, 1996).

Pipetted out 3 ml of substrate solution prepared by dissolving 8.8 mg Ascorbic acid in 30 ml phosphate buffer pH 5.6) and 0.1ml of enzyme extract were added to it to get a positive increase in absorbance. The absorbance change was measured at 265 nm at 30 sec. intervals for 5 min. Ascorbic acid oxidase activity was expressed as the change in absorbance per minute.

3.3.2.5 Indole acetic acid oxidase

Five grams of the plant material were cut into small bits and was homogenized in two successive 20ml aliquots of cold acetone. The homogenate collected was air dried until free of acetone odour, the resulting dry powder was weighed and stored in freezer using cold containers.

One gram of acetone powder was ground in two successive 20ml aliquots of 25mM phosphate buffer (pH 6.2) in a mortar chilled in an ice bath. The extract was filtered

through Whatman No.1 filter paper after each grinding. The filtrate was combined and diluted to 50ml with phosphate buffer.

The assay mixture contained 2ml of phosphate buffer (pH 6.2), 1ml para-coumaric acid, 1ml of manganese chloride and 2ml of enzyme extract. To this 4ml of indole acetic acid solution was added to start the reaction. The reaction was incubated in dark with shaking at 30°C and 2ml of the mixture was withdrawn after zero and fifty minutes of incubation and 5.2ml of perchloric acid and 0.5ml ferric nitrate solution was added. It was then diluted to 10ml with water. The absorbance was measured at 535nm after the incubation of the reaction mixture in the dark for 60 min. IAA oxidase activity was expressed as μmole IAA destroyed per hour per μg enzyme protein.

3.3.2.6 Cinnamyl alcohol dehydrogenase

One gram of the leaf sample was ground in phosphate buffer (10 mM, pH 7.5) containing 4 mM mercaptoethanol. Particulate materials were pelleted by centrifugation (12,000 rpm for 10 min) and the supernatant was used for the assay. CAD activity was measured following the oxidation of appropriate hydroxyl cinnamyl alcohol at 30^0C.

The assay mixture contained 100 mM cinnamyl alcohol, 100 mM tris-HCl buffer (pH 9.3), 100 mM NADP and 200 μl of enzyme extract. CAD activity in pKat/g fresh weight was monitored at 400 nm using spectrophotometer (Heidi *et al.*, 1994).

3.3.2.7 Nitrate reductase

Leaf sample (1 g) was homogenized in 6 ml of grinding medium containing 1 mM EDTA, 20 mM cystein and 25 mM potassium phosphate adjusted to a final pH 8.8 with KOH. The extract was centrifuged for 15 min at 30,000g and the supernatant was used as the enzyme source. All the extraction procedures were carried out under ice-cold conditions.

The reaction mixture contains 0.5 ml phosphate buffer (pH 7.5), 0.2 ml potassium nitrate solution, 0.4 ml NADH solution and 0.7 ml water. The reaction was initiated by the addition of 0.2 ml enzyme extract to the reaction mixture. A blank was prepared in the same way using water instead of enzyme extract. This was incubated at 30^0C for 15 min and the reaction was terminated by the rapid addition of 1 ml sulphanilamide followed by 1 ml naphthyl ethylenediamine reagent. The absorbance of the sample was measured at 540 nm after 30 min. a standard graph was prepared by using sodium nitrite and the activity was expressed as micromole nitrite produced per min per mg protein.

3.3.2.8 Malate dehydrogenase

One gram of plant material was thoroughly ground with acid washed sand in a prechilled pestle and mortar in grinding medium(1ml/1g tissue) Containing 50mM Tris – HCl (pH 8.0), 50 mM $MgCl_2$, 5mM 2-mercaptoethanol and 1mM EDTA. The homogenate was filtered through four layers of cheese cloth and the filtrate centrifuged at 3000 g for 20 minutes at 5 °C. The supernatant was used as enzyme source.

Assay mixture contained 0.5 ml oxaloacetic acid, 0.5 ml magnesium chloride, 1.3 ml tris-HCl buffer (pH 7.8) and 0.2 ml enzyme extract. NaOH (0.5 ml) was added to the reaction mixture quickly and the absorbance (A) recorded every 30 seconds for three min. at 340 nm. Enzyme activity was calculated by using the following equation:

µmoles NaOH oxidized / min / 0.2 ml enzyme extract – decrease in absorbance / min X 0.1613 X 3.

3.3.2.9 Chitinase

Colorimetric assay of chitinase enzyme was carried out as per Boller and Mauch (1988) using colloidal chitin as substrate. The reaction mixture consisted of 10 ml of 0.1 M sodium acetate buffer (pH 4.0), 0.4 ml enzyme extract and 0.1 ml colloidal chitin (10mg). After incubation for 2 h at 37^0C, the reaction was stopped by centrifugation at 1000g for 3 min. An aliquot of the supernatant (0.3 ml) was pipetted into a glass reagent tube

containing 30 ml of 1 M potassium phosphate buffer (pH 7.0) and incubated with 20 ml 3% (w/v) snail gut enzyme for 1 h. the reaction mixture was held in a boiling water bath for 3 min and then rapidly cooled in ice-water bath. After the addition of 2 ml DMAB, the mixture was incubated for 20 min at 37^0C. The absorbance was measured immediately thereafter at 585 nm. N-acetylglucosamine was used as the standard.

3.3.2.10 Lipase

Five gram plant material was crushed with 10 ml of ice cold acetone in a pestle and mortar, filtered and the powder washed successively with acetone, acetone:ether (1:1) and ether. The powder was air dried and stored in refrigerator. One gram of powder was extracted in 20 ml ice cold water, centrifuged at 15,000 rpm for 10 min and the supernatant used as the enzyme source (Sadasivam and Manickam, 1996).

Twenty milliliters of the substrate was taken in 500 ml beaker and 5 ml of phosphate buffer (pH 7.0) was added to it. The beaker was placed on top of the magnetic stirrer cum hot plate and the content was stirred slowly. The temperature was maintained at 35 °C. The electrodes of a pH meter were dipped in the reaction mixture and the final pH adjusted to 7.0. To this 0.5 ml of enzyme extract were added and pH recorded immediately and a timer was set on to record the reaction time. NaOH (0.1 N) was added at frequent intervals to bring the pH to initial value. The titration was continued for 30-60 minutes period. Lipase activity was calculated by using the following formula:

$$\text{Activity meq/min/g sample} = \frac{\text{Volume of alkali consumed X Strength of alkali}}{\text{Weight of sample in gram X Time in min.}}$$

3.3.2.11 Phosphatase

Leaf samples (1g) were homogenized in 10 ml of ice-cold 50 mM citrate buffer (pH 5.3) in a pre-chilled pestle and mortar. The filtrate was centrifuged at 10,000g for 10 min and the supernatant was collected and used as the enzyme source.

3.3.2.12 Phenylalanine ammonia lyase

Leaf sample (500 mg) was homogenized in 5 ml of cold 25mM borate-HCl buffer (pH 8.7) containing 5mM mercaptoethanol (0.4ml/l). The homogenate was centrifuged at 12,000g for 20 min and the supernatant was used as the enzyme source.

PAL activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290nm as described by Dickerson *et al.* (1984). The reaction mixture consisted of 0.5ml borate buffer (pH 8.7) and 0.2ml of enzyme extract diluted to 2ml with water. The reaction was initiated by the addition of 1 ml of L-phenylalanine solution. After incubation at 32^0C for 30 min, the reaction was stopped by the addition of 0.5ml of 1M trichloroacetic acid and the absorbance was measured at 290 nm.

3.3.2.13 Tyrosine ammonia lyase

All the methods used to assay PAL were followed to measure TAL except that the substrate was replaced with tyrosine.

3.3.2.14 Phosphoenol pyruvate carboxylase

The samples were ground thoroughly in a pre-chilled pestle and mortar in grinding medium (1ml/g tissue) containing 50mM Tris-HCl (pH 8.0), 50mM $MgCl_2$, 5mM 2-mercaptoethanol and 1mM EDTA. The homogenate obtained was filtered and centrifuged at 3000g for 20 min at 5^0C and the supernatant was used as the enzyme source.

Enzyme extract (0.2ml) was added to the reaction mixture containing 0.8ml Tris-HCl buffer (pH 8.0), 0.5ml $MgCl_2$, and 0.5ml sodium bicarbonate and 0.5ml phosphoenol pyruvate. To this 0.5ml of NADH was added quickly, mixed well and recorded the initial absorbance at 340nm and was continued up to 3 min at every 30 sec intervals. A blank was also prepared as above, in which an additional 0.5ml of Tris-HCl buffer (pH 8.0) was added instead of NADH. Enzyme activity was calculated by using the following equation:

μmoles NaOH oxidized / min /0.2 ml enzyme extract – decrease in absorbance / min X 0.1613 X 3.

3.3.2.15 Glutamate synthase

One gram of the leaf sample was ground with 5 ml of phosphate buffer (pH 7.5) containing 1 mM disodium EDTA, 1 mM dithioerythritol and 1% polyvinyl pyrrolidone (PVP) and centrifuged at 10,000g for 30 min at 4^0C. The supernatant was collected and used as the enzyme source.

The reaction mixture consisted of 1 ml of glutamin (5 mM), 1 ml of 2-oxoglutarate (5 mM), 1 ml of NADPH (0.25 mM), 0.2 ml of enzyme extract and 1.8 ml of Tris-HCl buffer 50 mM (pH 7.6). In the blank 1 ml of buffer was added instead of 2- oxoglutarate. The reaction mixture was incubated for 20 min at 37^0C and the change in absorbance was recorded at 340 nm. The activity of the enzyme was expressed as nmol of NAD(P)H oxidized per min per mg protein.

3.4 Results

3.4.1 Fungal enzymes in offence

3.4.1.1 Cellulase

Cellulase activity increased with the increase in incubation period. Maximum cellulase activity was noticed after 20 days of incubation for all the isolates studied (Table 3.1). During the early stages of incubation *Corynespora* isolates Cc 03 and Cc 04 showed a two-fold increase in cellulase activity, while the activity was three-fold for Cc 33. The initial cellulase activity between 5 and 10 days after inoculation (DAI) was significant for all the isolates but the increase was comparatively less in Cc 02, Cc 04 and Cc 08. For the isolate Cc 02 cellulase activity increased steadily during incubation and the increase was up to seven-fold by 20 DAI. Cc 03 showed a nine-fold increase between 5 and 20 DAI and

it increased four times between 15 and 20 DAI. A seven-fold increase in cellulase activity was noticed in isolates Cc 04 and Cc 05 by 20 DAI, Isolates Cc 08 and Cc 33 showed five times increase in cellulase activity between 5 and 20 DAI. Among the isolates Cc 03 and Cc 33 showed much higher cellulase activity at 15 DAI and were on par. Cc 33 maintained significantly higher activity at 20 DAI.

Table 3.1 Cellulase activity (mg glucose formed hr^{-1} ml^{-1} enzyme) of *C. cassiicola* isolates during different growth periods

Age of culture (days)	Isolate					
	Cc 02	Cc 03	Cc 04	Cc 05	Cc 08	Cc 33
5	16.31	30.16	32.78	20.24	19.22	49.00
10	48.34	66.39	59.66	56.11	39.77	84.70
15	99.69	159	135.58	80.35	78.44	160.00
20	118.10	276	234.06	134.44	100.06	298.08
CD $P \leq 0.05$	12.36	16.37	18.28	19.33	15.19	19.49

CD $P \leq 0.05$ for isolates = 13.45; periods = 17.28; interaction = 18.55

3.4.1.2 Protease

Protease activity increased with the advancement of the colony growth in all the cases except Cc 05, which showed a reduction in activity at the initial stage of incubation (Table 3.2). Maximum protease activity was observed 20 DAI for all the isolates studied- the older the culture, the greater the activity. The aggressive isolates Cc 03, Cc 04 and Cc 33 showed superior protease activity at all the incubation periods studied, the latter two

showing significantly higher activity at 20 DAI though being themselves on par. Among the avirulent isolates, only Cc 08 showed significant increase in activity with respect to different time intervals.

Table 3.2 Protease activity (mg amino acids released hr^{-1} ml^{-1} enzyme) of *C. cassiicola* isolates during different growth periods

Age of culture (days)	Isolate					
	Cc 02	Cc 03	Cc 04	Cc 05	Cc 08	Cc 33
5	58.11	61.00	60.08	59.00	49.33	70.35
10	62.26	72.60	69.33	50.60	58.31	86.66
15	67.00	84.33	79.14	58.06	70.00	97.44
20	79.00	99.90	107.71	77.00	80.11	108.84
CD $P \leq 0.05$	3.26	4.30	3.56	3.11	3.19	2.99

CD $P \leq 0.05$ for isolates = 3.45; periods = 3.28; interaction = 4.95

3.4.2 Host enzymes in defense

3.4.2.1 Oxidoreductase enzymes

3.4.2.1.1 Peroxidase

Peroxidase activity considerably increased during the course of the defence reaction against invading pathogen. For GT 1 and RRIM 600 (resistant clones) enzyme activity

increased in leaves up to third day after infection and then it declined but remained more than double of that in the other two clones (Table 3.3). The increase in activity was greater in primary stages of pathogenicity for GT 1 than that of RRIM 600. Peroxidase activity reduced after the initial increase for PB 260 (susceptible clone). In RRII 105 (susceptible clone) increased activity was noticed at 24 h after infection and then it declined up to 72 h of infection thereafter reverting to the earlier levels at 96 h. *C. cassiicola* infected leaves showed higher peroxidase activity than the healthy plants in all the clones tested.

3.4.2.1.2 Polyphenol Oxidase

Polyphenol oxidase activity gradually increased with the increase in period of infection in all the susceptible and resistant clones studied except RRII 105 (Table 3.4). PPO activity increased up to 72 h of infection for RRII 105 and then it showed a slight reduction at 96 h of infection. However, high PPO activity was observed for RRII 105 than the other clones studied at the initial stage of infection. Higher enzyme activity was noticed for all the infected plants than respective healthy controls.

3.4.2.1.3 Catalase

Catalase activity increased in *C. cassiicola* infected leaves of *H. brasiliensis* clones and the maximum catalase induction was observed in GT 1 followed by RRIM 600 (Table 3.5). Inoculation of the resistant clones (GT 1 and RRIM 600) resulted in higher catalase activity over the susceptible and healthy controls of all the four clones. The increase was steady up to 96 h after inoculation in resistant clones, while there was a final reduction in activity for susceptible clones. For RRII 105 the reduction of catalase enzyme occurred after 48 h of infection and it declined to the levels lower than in uninoculated controls at 96 h of infection. For PB 260, catalase activity increased continuously from 24 h to 72 h of infection and then it declined at 96 hours after infection.

Table 3.3 The level of activities (unit/ min / mg protein) of peroxidase in diseased and healthy leaves (mean of three replications) from resistant and susceptible *H. brasiliensis* clones

Clone	Inoculated plants					Healthy controls				
	Sampling time (h)				Mean	Sampling time (h)				Mean
	24	48	72	96		24	48	72	96	
RRII 105	3.39	2.99	2.60	3.44	3.11	1.12	1.18	1.18	1.26	1.18
PB260	3.22	3.18	3.18	3.07	3.16	1.00	1.93	1.66	1.59	1.54
RRIM 600	3.43	5.76	7.20	7.11	5.88	1.55	1.67	1.88	1.88	1.74
GT 1	3.78	8.44	9.21	6.60	7.01	2.20	2.00	2.00	2.61	2.20
CD $P \leq 0.05$	0.12	0.22	0.16	0.19	-	0.09	0.08	0.08	0.08	-

CD $P \leq 0.05$ for clones = 0.15; periods = 0.15; interaction = 0.20

Table 3.4 The level of activities (unit/ min / mg protein) of polyphenol oxidase in diseased and healthy leaves (mean of three replications) from resistant and susceptible *H. brasiliensis* clones

Clone	Inoculated plants					Healthy controls				
	Sampling time (h)				Mean	Sampling time (h)				Mean
	24	48	72	96		24	48	72	96	
RRII 105	4.66	6.27	7.09	6.28	6.08	2.00	2.60	2.58	2.38	2.39
PB260	3.55	4.55	6.17	7.67	5.49	1.88	1.92	1.99	1.89	1.92
RRIM 600	2.81	4.48	4.78	5.81	4.41	1.34	1.38	1.40	1.38	1.38
GT 1	2.58	8.44	9.21	6.60	7.01	2.20	2.00	2.00	2.61	2.20
CD $P \leq 0.05$	0.16	0.19	0.23	0.26	-	0.09	0.08	0.10	0.10	-

CD $P \leq 0.05$ for clones = 0.23; periods = 0.23; interaction = 0.34

Table 3.5 The level of activities (unit/ min / mg protein) of catalase in diseased and healthy leaves (mean of three replications) from resistant and susceptible *H. brasiliensis* clones

Clone	Inoculated plants					Healthy controls				
	Sampling time (h)				Mean	Sampling time (h)				Mean
	24	48	72	96		24	48	72	96	
RRII 105	1.62	3.36	2.77	2.31	2.51	1.28	1.39	2.60	2.36	1.90
PB260	1.70	2.23	2.89	2.23	2.26	1.38	1.45	1.48	1.99	1.57
RRIM 600	2.67	4.32	5.76	8.10	5.21	2.00	2.10	2.06	2.41	2.14
GT 1	3.72	5.69	6.66	9.16	6.30	1.90	2.11	2.20	2.36	2.14
CD $P \leq 0.05$	0.13	0.14	0.12	0.10	-	0.08	0.09	0.09	0.09	-

CD $P \leq 0.05$ for clones = 0.12; periods = 0.12; interaction = 0.14

3.4.2.1.4 Ascorbic acid oxidase (AAO)

An increase in AAO activity was noticed with all the *H. brasiliensis* clones studied. But the increase was lesser in susceptible clones (Table 3.6). Infected leaves of resistant clones showed slight increase in AAO activity over the susceptible clones, RRIM 600 exhibited significantly higher AAO activity among the four *Hevea* clones studied. There was a remarkable increase in AAO activity in GT 1 during the initial stages of infection (at 48 h). Uninoculated controls did not show much variation of the AAO activity and it remained lower than the inoculated leaves.

3.4.2.1.5 IAA Oxidase

A well-marked change in activity of IAA oxidase occurred in resistant clones GT 1 and RRIM 600 (Table 3.7). The activity increased rapidly after the first day of inoculation and then a steady and slow increase was noticed up to fourth day after inoculation. GT 1 exhibited higher IAA oxidase activity among the four clones tested. For PB 260 and RRII 105, enzyme activity increased slightly from 24 h after inoculation which continued up to 96 hours after inoculation. IAA oxidase activity remained low throughout the experiment in healthy controls.

3.4.2.1.6 Cinnamyl alcohol dehydrogenase

CAD activity was accelerated after infection in *H. brasiliensis* clones (Table 3.8). The activity increased up to72h of infection for RRII 105 and PB 260 but declined at the later stages of infection. GT1 showed very high activity, which remained so even up to 96h. RRIM 600 also showed an increasing trend of CAD activity with time after inoculation. For both these clones, the activity continuously increased from 24 to 96h after infection. All the healthy controls showed low CAD activity throughout the period of observation.

3.4.2.1.7 Nitrate reductase

Nitrate reductase activity reduced after the infection of *C. cassiicola* in both the susceptible and resistant clones (Table 3.9). For RRII 105 and PB 260 the reduction was more prominent than for RRIM 600 and GT 1. The control plants of both susceptible and resistant clones had higher enzyme activity.

Table 3.6 The level of activities (unit/ min / mg protein) of Ascorbic Acid Oxidase in diseased and healthy leaves (mean of three replications) from resistant and susceptible *H. brasiliensis* clones

Clone	Inoculated plants					Healthy controls				
	Sampling time (h)				Mean	Sampling time (h)				Mean
	24	48	72	96		24	48	72	96	
RRII 105	1.88	1.92	2.16	2.17	2.03	1.66	1.66	1.69	1.78	1.69
PB260	1.96	2.11	2.28	2.61	2.24	1.58	1.51	1.60	1.61	1.57
RRIM 600	2.61	2.92	3.88	3.92	3.33	1.71	1.69	1.77	1.68	1.71
GT 1	2.08	3.11	3.44	3.49	3.03	1.86	1.89	1.87	1.86	1.87
CD $P \leq 0.05$	0.09	0.11	0.17	0.11	-	0.09	0.09	0.08	0.09	-

CD $P \leq 0.05$ for clones = 0.11; periods = 0.11; interaction = 0.22

Table 3.7 The level of activities (µmole IAA oxidized / min / mg protein) of IAA oxidase in diseased and healthy leaves (mean of three replications) from resistant and susceptible *H. brasiliensis* clones

Clone	Inoculated plants					Healthy controls				
	Sampling time (h)				Mean	Sampling time (h)				Mean
	24	48	72	96		24	48	72	96	
RRII 105	3.11	3.29	4.20	5.80	4.10	1.98	1.99	1.87	2.10	1.98
PB260	3.32	3.41	4.72	6.52	4.49	2.28	2.31	2.28	2.39	2.31
RRIM 600	3.61	7.69	8.88	9.67	7.46	2.60	2.58	2.91	2.82	2.72
GT 1	3.21	10.66	10.89	12.12	9.22	2.67	2.56	2.88	2.96	2.76
CD $P \leq 0.05$	0.18	0.18	0.22	0.20	-	0.09	0.07	0.08	0.09	-

CD $P \leq 0.05$ for clones = 0.21; periods = 0.21; interaction = 0.30

Table 3.8 The level of activities (pKat / g fresh weight) of cinnamyl alcohol dehydrogenase in diseased and healthy leaves (mean of three replications) from resistant and susceptible *H. brasiliensis* clones.

Clone	Inoculated plants					Healthy controls				
	Sampling time (h)				Mean	Sampling time (h)				Mean
	24	48	72	96		24	48	72	96	
RRII 105	1.4	1.9	2.6	1.5	1.9	0.6	0.6	0.6	0.6	0.6
PB260	1.7	2.4	3.1	2.9	2.5	0.9	0.8	0.8	0.8	0.8
RRIM 600	2.8	3.4	3.9	4.8	3.7	1.1	1.1	1.2	1.0	1.1
GT 1	3.5	4.9	5.8	6.9	5.3	1.4	1.2	1.6	1.6	1.5
CD $P \leq 0.05$	0.13	0.11	0.11	0.12	-	0.09	0.12	0.08	0.09	-

CD $P \leq 0.05$ for clones = 0.13; periods = 0.13; interaction = 0.16

Table 3.9 The level of activities (µmole nitrite formed / min / mg protein) of nitrate reductase in diseased and healthy leaves (mean of three replications) from resistant and susceptible *H. brasiliensis* clones.

Clone	Inoculated plants					Healthy controls				
	Sampling time (h)				Mean	Sampling time (h)				Mean
	24	48	72	96		24	48	72	96	
RRII 105	1.20	0.98	0.98	0.90	1.00	2.71	2.80	2.77	2.70	2.74
PB260	1.26	1.16	1.18	0.98	1.15	2.68	2.93	2.99	2.71	2.87
RRIM 600	1.42	1.81	1.33	1.42	1.50	2.66	2.78	2.92	2.88	2.81
GT 1	1.56	1.46	1.40	1.36	1.45	2.71	2.77	2.79	2.86	2.78
CD $P \leq 0.05$	0.11	0.12	0.10	0.09	-	0.09	0.08	0.07	0.10	-

CD $P \leq 0.05$ for clones = 0.12; periods = 0.12; interaction = 0.20

3.4.2.1.8 Malate dehydrogenase

In susceptible clones, a reduction in malate dehydrogenase activity was noticed after the initial increase (Table 3.10). In RRII 105 the reduction started after 48h of infection but it occurred after 72 h of infection in PB 260. Enhanced malate dehydrogenase activity was noted in both the resistant clones. The initial expression of malate dehydrogenase was greater for GT 1 and RRIM 600. However, its increased production continued up to 72 h after infection and then it declined. At 48 h of infection the activity of malate dehydrogenase was more or less similar for all the four *Hevea* clones studied. Healthy controls of different clones showed lower malate dehydrogenase activity compared to the infected plants.

3.4.3.2 Hydrolase Enzymes

3.4.3.2.1 Chitinase

Chitinase activity increased on *C. cassiicola* infection in all the clones studied and the increase was continuous up to 96 h after infection (Table 3.11). The resistant clones RRIM 600 and GT 1 showed enhanced chitinase activity even after 24 h from infection and the increase was considerably higher than that of the susceptible clones. During the later stages, GT 1 exhibited a two-fold increase than that of RRII 105 and PB 260. The control plants did not show significant variation in enzyme activity.

3.4.3.2.2 Lipase

Increased lipase activity was observed in the initial stages of pathogenesis with all the four *Hevea* clones (Table 3.12). The increase was prominent in susceptible clones and the activity declined from 72 to 96 hrs after infection. In GT 1, there was an increase in lipase activity with respect to their control plants. However, the activity did not increase with the increase in period of infection. In RRIM 600, maximum activity occurred at 48 hrs of infection and then it declined. Controls of the four *Hevea* clones showed more or less similar lipase activity throughout the experimental period.

Table 3.10 The level of activities of malate dehydrogenase in diseased and healthy leaves from resistant and susceptible *H. brasiliensis* clones.

Clone	Inoculated plants					Healthy controls				
	Sampling time (h)				Mean	Sampling time (h)				Mean
	24	48	72	96		24	48	72	96	
RRII 105	2.43	4.85	3.32	2.29	3.22	1.56	1.62	1.84	2.20	1.80
PB260	2.37	4.88	4.92	2.81	3.74	1.82	1.82	1.98	2.16	1.94
RRIM 600	3.11	4.93	5.55	5.46	4.76	1.86	1.92	2.11	2.12	2.00
GT 1	3.33	4.64	5.96	4.76	4.67	1.76	2.10	2.21	2.18	2.06
CD $P \leq 0.05$	0.22	0.24	0.24	0.28	-	0.11	0.19	0.18	0.16	-

CD $P \leq 0.05$ for clones = 0.30; periods = 0.30; interaction = 0.44

* Mean of three replications

* Unit: μmole NADH oxidized / min / 0.2 ml enzyme extract.

Table 3.11 The level of activities of chitinase in diseased and healthy leaves from resistant and susceptible *H. brasiliensis* clones

Clone	Inoculated plants					Healthy controls				
	Sampling time (h)				Mean	Sampling time (h)				Mean
	24	48	72	96		24	48	72	96	
RRII 105	14.13	19.3	22	26.33	20.44	12	12.8	12.6	12.8	12.55
PB260	16.28	21	23.8	27.1	22.05	13.8	15.6	15.8	15.8	15.25
RRIM 600	20.47	26.38	32.6	38.9	29.59	13.9	13.9	13.8	13.9	13.88
GT 1	21.38	30.6	32.16	43.9	32.26	14	13.6	13.9	14.3	13.95
CD $P \leq 0.05$	1.89	1.97	2.66	2.08	-	1.0	1.79	1.88	1.96	-

CD $P \leq 0.05$ for clones = 2.01; periods = 2.01; interaction = 4.32

* Mean of three replications

* Unit: Unit/ min / mg protein.

Table 3.12 The level of aetivities of lipase in diseased and healthy leaves from resistant and susceptible *H. brasiliensis* clones

Clone	Inoculated plants					Healthy controls				
	Sampling time (h)				Mean	Sampling time (h)				Mean
	24	48	72	96		24	48	72	96	
RRII 105	2.49	2.81	1.99	1.99	2.32	1.09	1.12	1.24	1.13	1.14
PB260	2.03	2.63	2.14	1.95	2.18	1.12	1.10	1.16	1.16	1.13
RRIM 600	1.75	1.86	1.67	1.70	1.74	1.13	1.18	1.11	1.10	1.13
GT 1	1.65	1.66	1.68	1.61	1.65	1.16	1.16	1.22	1.17	1.17
CD $P \leq 0.05$	0.22	0.28	0.27	0.19	-	0.09	0.08	0.79	0.10	-

CD $P \leq 0.05$ for clones = 0.23; periods = 0.23; interaction = 0.34

* Mean of three replications

* Unit: meq / min / g sample

3.4.3.2.3 Phosphatase

The tolerant and susceptible clones showed variation in phosphatase activity. In susceptible clones, the activity reduced with the increase in pathogenesity, but on the contrary, the tolerant clones showed increased phosphatase activity in all the stages of infection. Among the clones GT 1 showed remarkable increase during the initial stages of infection. Uninoculated controls did not show much variation of phosphatase activity and it remained lower than the inoculated leaves.

Table 3.13 The level of activities (unit/ min / mg protein) of catalase in diseased and healthy leaves (mean of three replications) from resistant and susceptible *H. brasiliensis* clones

Clone	Inoculated plants					Healthy controls				
	Sampling time (h)				Mean	Sampling time (h)				Mean
	24	48	72	96		24	48	72	96	
RRII 105	5.78	2.98	2.26	2.76	3.45	2.71	2.80	2.37	2.70	2.65
PB 260	4.21	2.36	2.98	2.77	3.08	2.99	2.93	3.95	3.45	3.34
RRIM 600	6.67	7.09	7.56	9.56	7.72	2.78	2.75	2.88	2.88	2.82
GT 1	6.94	8.90	8.96	9.99	8.70	2.41	2.77	2.49	2.86	2.63
CD P ≤0.05	0.77	0.65	0.70	0.83	-	0.23	0.36	0.56	0.38	-

CD P ≤ 0.05 for clones = 0.67; periods = 0.67; interaction = 0.78

* Mean of three replications

3.4.3.3 Lyase enzymes

3.4.3.3.1 Phenylalanine ammonia lyase

The activity of PAL increased on infection in all the *H. brasiliensis* clones tested (Table 3.13). The increase was slow but steady in RRII 105, PB 260 and RRIM 600. The activity reached its peak at 96h after infection. The resistant clones showed greater induction at the later stages of infection. A steep rise in activity of PAL was noticed in GT 1 at the initial stages of disease development and a two-fold increase was noticed after 48h when compared with susceptible clones. The control plants showed similar activity at all the time periods studied.

3.4.3.3.2 Tyrosine ammonia lyase activity

There was an increase in TAL activity in the leaves after inoculation with *C. cassiicola* (Table 3.14). The increase was more prominent in the resistant clones RRIM 600 and GT1. In the susceptible clones the activity increased from 24h to 96h after infection but remained lower than that of the resistant clones. In RRIM 600 the activity increased up to 48h after infection and thereafter showed a reduction at 72 h after infection. A two-fold increase in activity of TAL was noticed in RRIM 600 and GT1 at 48h from infection.

3.4.3.3.3 PEP carboxylase

PEP carboxylase activity got increased after the infection in all the *H. brasiliensis* clones studied (Table 3.15). RRII 105 and PB 260 showed an initial increase up to 48 h after infection and a final reduction thereafter. In RRIM 600, maximum activity was observed after 72 h of infection and later the activity decreased, but was statistically significant. A linear rise in PEP carboxylase activity was noticed in GT 1 and the increase was significant in all the periods studied.

Table 3.13 The level of activities of PAL in diseased and healthy leaves from resistant and susceptible *H. brasiliensis* clones

Clone	Inoculated plants					Healthy controls				
	Sampling time (h)				Mean	Sampling time (h)				Mean
	24	48	72	96		24	48	72	96	
RRII 105	7.6	8.2	8.9	10	8.68	6.2	6.2	7	6.9	6.57
PB260	8.2	8.8	9.6	9.9	9.13	8	8	8	8.2	8.5
RRIM 600	12.6	12.9	14.7	15.8	14	8.8	8.7	8.9	8.9	8.83
GT 1	11.5	17.8	20.1	22.4	17.95	8.9	9.6	9.6	9.7	9.45
CD $P \leq 0.05$	2.66	2.19	2.00	2.79	-	1.0	1.1	0.98	0.96	-

CD $P \leq 0.05$ for clones = 0.27; periods = 0.27; interaction = 0.38

* Mean of three replications

Table 3.14 The level of activities of TAL in diseased and healthy leaves from resistant and susceptible *H. brasiliensis* clones

Clone	Inoculated plants					Healthy controls				
	Sampling time (h)				Mean	Sampling time (h)				Mean
	24	48	72	96		24	48	72	96	
RRII 105	4.4	4.9	4.9	5.3	4.88	4	4	4	4	4
PB260	5.2	5.8	6.3	6.9	6.05	4.2	4.3	4.2	4.2	4.23
RRIM 600	5.6	8.7	7.6	10.3	8.05	4.5	4.5	4.5	4.5	4.5
GT 1	8.6	10.11	12.1	12.8	10.9	5.2	5.6	5.9	5.3	5.5
CD $P \leq 0.05$	0.81	0.16	0.32	0.22	-	0.10	0.10	0.09	0.11	-

CD $P \leq 0.05$ for clones = 0.33; periods = 0.33; interaction = 0.42

* Mean of three replications

Table 3.15 The level of activities of PEP carboxylase in diseased and healthy leaves from resistant and susceptible *H. brasiliensis* clones

Clone	Inoculated plants					Healthy controls				
	Sampling time (h)				Mean	Sampling time (h)				Mean
	24	48	72	96		24	48	72	96	
RRII 105	2.33	4.92	3.4	3.3	3.49	1.88	1.89	1.89	1.81	1.87
PB260	2.61	5.96	5.31	4.38	4.57	2	2	2	2	2
RRIM 600	5.6	5.66	7	6.98	6.31	2.25	2.41	2.48	2.6	2.44
GT 1	5.88	5.92	7.62	8	6.8	2.30	2.3	2.46	2.41	2.37
CD $P \leq 0.05$	0.58	0.66	0.71	0.81	-	0.21	0.18	0.11	0.9	-

CD $P \leq 0.05$ for clones = 0.54; periods = 0.54; interaction = 0.65

* Mean of three replications

* Unit: μmole NADH oxidized / min / 0.2 ml enzyme extract.

3.4.3.4 Synthetase

3.4.3.4.1 Glutamate synthase

Glutamate synthase activity increased in all the *H. brasiliensis* clones studied after infection (Table 3.16). The increase was maximum after 96 h of infection in RRII 105, PB 260 and GT 1. RRIM 600 showed optimum activity at 72 h after infection. A linear increase in activity was observed both in RRII 105 and GT 1 but the activity was almost two fold in GT 1 than in RRII 105. A reduction in activity was noticed at 72 h of infection for PB 260. RRIM 600 showed increase in activity up to 72 h and then a final reduction at 96 h. The activity was lower in healthy controls of both susceptible and resistant clones but resistant clones showed a slight increase over the susceptible clones.

Table 3.16 The level of activities of glutamate synthase in diseased and healthy leaves from resistant and susceptible *H. brasiliensis* clones

Clone	Inoculated plants					Healthy controls				
	Sampling time (h)				Mean	Sampling time (h)				Mean
	24	48	72	96		24	48	72	96	
RRII 105	6.11	6.18	7.20	8.00	6.87	5.2	5.2	5.28	5.2	5.22
PB260	7.12	8.99	8.66	9.80	8.64	5.28	5.16	5.2	5.26	5.23
RRIM 600	10.00	12.19	13.00	9.80	11.25	6.66	6.26	6.28	6.11	6.33
GT 1	10.96	12.97	14.74	15.98	13.66	6.19	6.27	6.66	6.62	6.44
CD $P \leq 0.05$	0.98	0.84	1.23	1.06	-	0.69	0.87	1.05	0.99	-

CD $P \leq 0.05$ for clones = 1.00; periods = 1.00; interaction = 1.90

* Mean of three replications

* Unit: Nanomole of NAD(P)H oxidized / min / mg protein.

3.5 Discussion

3.5.1 Fungal enzymes in offence

3.5.1.1 Cellulase

Cellulolytic enzymes are extensively studied in a wide variety of microorganisms, complexed or noncomplexed (Lynd *et al.*, 2002). Cell wall degrading enzymes released by pathogens are known to be responsible for the pathogenesis in fungal diseases (Mandavia *et al.*, 1999). Cellulase enzyme have been shown to be produced by plant pathogens (Pointing *el al.*, 1998; Jayasinghe *et al.*, 1999; Espino *et al.*, 2005) which are known to facilitate cell wall penetration and tissue maceration in host plants (Sasaki, 1996). In the present study, increased cellulose activity was noticed continuously with the increase in period of incubation. The virulence of a pathogen has been correlated with the increased production of cellulolytic enzymes in many pathosystems studied (Ramana *et al.*, 1997; Zhou *et al.*, 1996).

3.5.1.2 Protease

Extracellular enzymes are important to fungi not only for digestion but also in many instances for the pathogenic process: the enzymes may function in overcoming the natural resistance of the host as well as in providing soluble products that can be absorbed and used as food (Knogge, 1996). The production of extracellular proteases by plant pathogenic fungi is also well documented, and it has been proposed that in some fungus-plant interactions these enzymes may function as pathogenic factors (Dobnson *et al.*, 1996; North, 1982; Pekkarinen *et al.*, 2000). Virulence of the pathogen and its proteolytic activity has been positively correlated by many (Leger and Roberts, 1997; Riou *et al.*, 1991).

In the present study showed increased protease activity with the increase in incubation period in all the *C. cassiicola* isolates studied. It has been suggested that the proteases may facilitate located penetration of the plant cell wall by breaking down the fibrous glycoproteins that contribute to cell wall stability (Carpita and Gibeaut, 1993). Some phytopathogenic fungi such as *Fusarium, Alternaria*, and *Rhizoctonia* produced

serine alkaline proteases, which are indispensable for their growth (Pekkarinen *et al.*, 2000). They are probably nutrient-mobilizing enzymes whose primary function is the support of fungal growth after host cell death has occurred.

The results showed the capability of *C. cassiicola* to produce the hydrolytic enzyme such proteases and Cellulases. These enzymes may be involved in the capability of the fungus to invade vegetal tissues.

3.5.2 Host enzymes in offence

3.5.2.1 Peroxidase

Peroxidase activity of tissues is reported to be well correlated with their resistance by many investigators (Uritani and Stahmann, 1961, Kawashima and Uritani, 1965, Smith and Hammerschmidt, 1988, Angelini *et al.*, 1993, Chandra and Tyagi, 1993, Jite and Tressa, 1999). Breton *et al.* (1996) observed a significantly higher peroxidase activity for the clone GT 1 resistant to *C. cassiicola*. Present study indicated that the resistant clones produced greater quantity of peroxidase on infection, prior to the establishment of the pathogen. Their production may act as a physical and/or chemical barrier for the pathogen. Several authors reported the increase in peroxidase activity in plant tissue response to infection and presumed the participation of the enzyme in forming defence barriers (Hammerschmidt *et al.*, 1982, Arora and Wagle,1985, Purkayastha, 1998). It was suggested that the role of peroxidase in resistance may result in oxidation of phenols to more fungi-toxic compounds around the infected areas (Lyr, 1966, Hammerschmidt and Kuc, 1982). Peroxidase catalyse the fungal polymerization step of lignin synthesis and may therefore be directly associated with the increased ability of systemically protected tissue to lignify (Gross, 1979, Angelini *et al.*, 1900, Luhova *et al.*, 2003.) and the rapid lignin deposition may provide a barrier to the invading pathogen (Vance *et al.*, 1980). In susceptible clones, increase in peroxidase activity was noticed, but the quantity was much lower than that of resistant clones in early stages of pathogenesis. According to Irving and Kuc (1990), in a susceptible interaction, although the defence related products are formed, the pathogen over grows

their production. Peroxidase alone cannot account for resistance. Probably peroxidase is, but one attribute of host tissue, contributing to a physiological status that is inhospitable for the fungus.

3.5.2.2 Polyphenol oxidase

A sharp increase in PPO activities following infection was observed in all the *H. brasiliensis* clones studied, however, the increase was much higher in susceptible clones than resistant clones. Similar increase was noticed in many other host parasite combinations (Jennings *et al.*, 1969, Gangopadhyay and lal, 1986, Luthra *et al.*, 1988b, Shamina and Sarma, 2000). It is generally assumed that PPO in plant cells is mainly compartmentalized in vesicles or plastids (Butt, 1980) and in the cell walls. Disruption of the host membranes is known to occur during invasion by pathogens and the loss of compartmentalization that this entails could account for the rapid increase in PPO activity as reported here with susceptible clones (Lazarovits and ward, 1982). The increased activity of PPO has been reported to be due to the activation of latent polyphenol oxidase (Robb *et al.*, 1962). Tomiyama (1963) suggested that increase in PPO activity may be directly or indirectly involved in resistance. But on the contrary, the present study indicates lower activity of PPO in resistant clones studied.

3.5.2.3 Catalase

Changes in catalase activity as a result of fungal infection have been reported in various host pathogen combinations (Maxwell and Batemann, 1967, Fric and Fuchs, 1970, Vir and Grewal, 1974, Ronald, 2001). In the present study, increased catalase activity was observed continuously with increasing period of infection in resistant clones of *H. brasiliensis* as described by Rudolph and Stahmann (1964) for halo blight of bean. It is often believed that change in the activity of catalase after infection is related to disease resistance (Vir and Grewal, 1975, Lebeda *et al.*, 2001). Fric and fuchs (1970) observed marked increase in catalase activity of resistant wheat leaves infected with *Puccinia graminis tritici*. On the contrary, Gupta *et al.* (1995) reported lower activity of catalase in *Alternaria blight* infected leaves of *Brassica* sp. as compared to healthy. The activity almost disappeared at later stages, which accounted for maximum disease severity suggesting that this enzyme may not have any significant role in disease

resistance. The steady increase in catalase activity in the resistant *H. brasiliensis* clone is indication of its role in defence. Slight initial increase of catalase and final decrease in its activity was observed in susceptible clones in the present study. Many investigators reported such an initial induction and final reduction of catalase activity with different susceptible plants (Montalbini and Mate, 1972, Vir and Grewal, 1975).

3.5.2.4 Ascorbic Acid Oxidase

The innate resistance of plants depends largely upon the organic reducing agent, ascorbic acid which is found to play a significant role in several host parasite combination (Sharma *et al.*, 1975). Since ascorbic acid level is largely influenced by ascorbic acid oxidase, this enzyme plays a role in disease resistance (Aulakh and Grover, 1970). In the present study, increased ascorbic acid oxidase was noted in both resistant and susceptible clones, however, the activity in susceptible clones was lower compared to resistant clones. A toxin produced by *Pyricularia oryzae* was reported to inhibit ascorbic acid oxidase activity (Tamari *et al.*, 1963). Toxins produced by *C. cassiicola* are reported to play a significant role in the pathogenesis (Breton *et al.*, 2000). These toxins may be responsible for the inhibition of ascorbic acid oxidase.

3.5.2.5 IAA Oxidase

Varietal resistance to several diseases of certain crop plants has been related to the higher activities of IAA oxidase (Stebbins, 1992, Pfanz, 1993). In the present study, enzyme activity increased with the period of incubation of infected leaves both for resistant and susceptible clones. Hashim *et al.* (1980) reported such an increase in *H. brasiliensis* clones infected with *Microcyclus ulei*. Resistant clones had greater IAA oxidase activity. This indicates some associationship between resistance of *Hevea* leaves to leaf disease pathogens, with the levels of activity of IAA oxidase. Zmrhal *et al.* (1987) and Mc Dougall (1993) supported the concept that IAA oxidase plays a definite role in the processes of lignification and there is evidence that IAA oxidase may be involved in generating H_2O_2 necessary for lignification to proceed (Mader *et al.*, 1986). The present data support the concept that a local increase of IAA oxidase of the cell walls in invaded area may contribute to the mechanical barrier formation against the pathogens.

3.5.2.6 CAD

The formation of cinnamyl alcohol, the precursor of lignin from their corresponding cinnamyl CoA esters requires two enzymatic steps, which are catalyzed by Cinnamyl-CoA reductase and CAD. The synthesis of cinnamyl alcohol from cinnamaldehyde is catalysed by CAD and is considered as a highly specific step in lignification. Hence enhancement of CAD activity may be a specific biochemical marker for resistance. In the present study, the resistant *H. brasiliensis* clones showed enhancement of CAD activity from 24h to 96h after infection. Such increased activity of CAD during the expression of resistance was observed in *Populus euramiricana* (Grand *et al.*, 1985). Mitchell *et al.* (1994) observed a substrate-specific induction of wheat leaf CAD in relation to its role in regulating the composition of defensive lignin induced at wound margins. Therefore, the higher CAD activity may hasten the lignification process in resistant clones.

3.5.2.7 Nitrate reductase

The assimilatory reduction of nitrates by plants is a fundamental biological process in which a highly oxidized form of inorganic nitrogen is reduced to nitrogen and then to ammonia. The nitrate reducing system consists of nitrate reductase and nitrite reductase, which catalyze the stepwise reduction of nitrate to nitrite and then to ammonia.

Nitrate reductase activity decreased in both susceptible and resistant clones of *Hevea brasiliensis* after *C. cassiicola* infection as indicated by the present study. Post-infectional reduction in the activity of nitrate reductase was investigated by many workers (Robert and Walters, 1988; Murray and Ayres, 1986).

Singh (2004) reported that the activity of nitrate reductase was decreased in all the *Brassica* species infected plant parts, but on the contrary Sadles and Scott (1974) observed an increased enzyme activity in barley leaves infected with powdery mildew fungus during the late stage of infection.

3.5.2.8 Malate Dehydrogenase

Dehydrogenase catalyse the transfer of hydrogen atom from a substratum to a hydrogen acceptor. Qualitative and quantitative changes in the activity of certain dehydrogenases in the diseased plant tissue during pathogenesis have been reported by Stahmann and Demorest (1972) and Reddy and Stahmann (1972). The present study indicated a reduction in malate dehydrogenase activity after two and three days in susceptible and resistant clones respectively. Such an initial increase and final decrease was reported by Madhukar and Reddy (1990) with infected guava fruits. However, Held *et al.* (1965) and Chile and Vyas (1983) observed increased activity in potato and *Piper betel* infected by *Phytophthora infestans* and *Phytophthora parasitica* var *piperina* respectively.

3.5.2.9 Chitinase

The role of chitinases as part of the inducible plant defence response is well documented (Boller, 1987; Collinge *et al.* 1993). In the present study, increased chitinase activity was observed in all the clones after *C. cassiicola* infection, but the activity was higher in tolerant clones. Broglie *et al.* (1991) and Vierheilig *et al.* (1993) reported that tobacco plants constitutively expressing class I chitinase showed increased resistance to *Rhizoctonia solani.* Generally, chitinase activity is at a low level in plants but is induced in response to various stimuli like salicylic acid (Jung *et al.* 1993; Margis-Pinbeiro *et al.* 1994; Chacko *et al.* 2005), virus infections (Lawton *et al.* 1992; Payne *et al.* 1990; Ohme-Takagi *et al.* 1998), and various pathogenic microorganisms (Kastner *et al.* 1998; Mohr *et al.* 1998; Munch-Garthonn *et al.* 1997; Vad *et al.* 1992). Deborah *et al.* (2001) investigated the differential induction of chitinase in rice as response to inoculation with *Rhizoctonia solani* and revealed increasing chitinase activity in rice plants and the level was higher for incompactable than for compactable interaction.

3.5.2.10 Lipase

Changes in phospholipid content in cell organells have been observed in several plants due to infection (Hoppe and Heitefuss, 1974, Sednina *et al.*, 1981). The present study indicated an increase in lipase activity during the early stages of infection in susceptible clones. Lee *et al.* (1992) reported such an increased phospholipase activity in

Alternaria alternata susceptible strawberry cultivars. Since phospholipids are among the primary constituents of higher plant cell membranes, the production of lipase by phytopathogenic organisms during pathogenesis may be related to the changes in host cell permeability that have often been associated with certain plant diseases (Lai *et al.*, 1968). Several investigators suggested that phospholipase enzymes are involved in signal transduction in plant cells in response to various stimuli (Scherer, *et al*, 1988, Martiny-Baron and Scherer, 1989, Palmgren and Sommarin, 1989, Scherer and Andre, 1989, Scherer, *et al*, 1990). Phospholipases activate protein kinases by forming diacylglycerol and inositol 1,4,5-triphosphates (IP3), which function as second messengers in signal transduction system (Vidhyasekaran, 1998). Lipases may be of pathogen as well as of host origin. Observations of the present study indicated that the control plants of *H. brasiliensis* clones showed similar lipase activity (lower than the infected leaves). This proves that lipase originated from the pathogen and not from host in *Hevea-Corynespora* interaction. The lipid degrading enzymes, especially lipase has been detected in extracts of many phytopathogenic fungi like *Erysiphe pisi* (Faull and Gay, 1983), *Thielaviopsis basicola* (Lumsden and Bateman, 1968), *Sclerotium rolfsii* (Tseng and Bateman, 1969) and *Sclerotium sclerotiorum* (Lumsden, 1970).

3.5.2.11 Phosphatase

Phosphatases play a central role in cellular signaling. Recent studies have clearly demonstrated that protein phosphatases function not only by counterbalancing the protein kinases but also by taking a leading role in many signaling events (Luan, 1998). Tomato defense mechanism against the fungal pathogen *Cladosporium fulvum* were activated through phosphatase involved signal transduction (Vera-Estrella, *et al.*, 1994).

C. cassiicola inoculation significantly induced the production of phosphatase in resistant clones revealed the possibility of this enzyme in resistance to Corynespora leaf fall disease of *H. brasiliensis.* Jakobek and Lindgren (2002) studied the expression of a bean acid phosphatase cDNA and correlated it with disease resistance. Beber *et al.* (2000) activated a putative barley acid phosphatase by the chemical inducers of systemic resistance.

3.5.2.12 & 13 PAL & TAL

Changes in PAL and TAL activity as a result of fungal infection have been reported in various host-pathogen interactions (Satyavir, 2003; Chacko *et al.*, 2005). In the present study, enhanced activity of PAL and TAL following infection was noticed in all the *H. brasiliensis* clones studied. But the clones showing resistant reaction had greater activity than the susceptible ones. PAL and TAL are key enzymes in the phenyl propanoid pathway catalyzing synthesis of secondary metabolites including lignin, flavanoids and phytoalexins from L-phenyl alanine. Sundar and Vidhyasekaran (2003) observed positive correlation of red rot resistance to higher activity of PAL and TAL while comparing susceptible and resistant genotypes of sugarcane against *Colletotrichum falcatum*. The enhanced activity of these enzymes may have triggered hypersensitive reaction in the resistant clones.

3.5.2.14 PEP carboxylase

Changes in phosphoenol pyruvate carboxylase activity as a result of fungal infection has been reported in very few host-pathogen combinations (Walters and Ayres, 1983). An increase in phosphoenol pyruvate carboxylase activity has been noticed in *C. cassiicola* infected *H. brasiliensis* clones. Muller *et al.* (2009) reported three times higher phosphoenol pyruvate carboxylase activity in virus infected plant compared to healthy ones. They hypothesize the possibility of an infection-related phosphorylation, which could be part of the plants response to pathogen attack.

3.5.2.15 Glutamate synthase

The present study showed an increase in glutamate synthase activity on C. cassiicola infection. Roberts and Walters (1988) reported enhanced glutamate synthase activity with respect to infection by *Alternaria porri*. But on the contrary, Walters and Ayres (1980) reported reduced activity of glutamate synthase in powdery mildew disease of barley.

The relationship between enzymes and other biochemicals activities can be very interesting for the explanation of reciprocal physiological and biochemical effects of

these enzymes in plants resistance. Biochemical studies indicate that the infected plants had higher post infection total phenols, OD phenols, and proteins levels and showed higher oxidase activity compared to healthy plants. It is believed that the increased biosynthesis of phenolics and stimulated polyphenol oxidase activity are responses to infection leading to greater accumulation of toxic quinones at the site of infection. Similarly increased peroxidase activity might have resulted in enhanced lignification and thus reduced the infection frequency by the pathogen (Angelini *et al.*, 1993; Rea *et al.*, 1998).

The participation of peroxidase and IAA oxidase in the phytoalexin synthesis is evident from many studies (Mahadevan, 1991). Phytoalexins are not normally present in healthy plant tissues; they are synthesized following perception of the potential pathogen by the host in a process that requires increases in the activities of enzymes responsible (Dixon and Harrison, 1990). Breton *et al.* (1997) reported biosynthesis of scopoletin (phytoalexin) in susceptible and resistant clones of *H. brasiliensis* infected with *C. cassiicola*. Scopoletin acts as a stimulator of IAA oxidase (Imbert and Wilson, 1970) and peroxidase activities (Schafter *et al.*, 1971). The fact that scopoletin can alter the host enzyme systems may be of significance in the plants defence system.

It is now established that signals are required to activate defence genes which are commonly present in both susceptible and resistant plants (Vidhyasekaran, 1988a, b., Pontier *et al.*, 1994). Signal molecules have been detected in fungi (Schaffrath, *et al.*, 1995; Vidhyasekaran *et al.*, 1996, 1997), bacteria (He *et al.*, 1993) and viruses (Culver and Dawson, 1991), irrespective of whether they are pathogens or not and are released by the host enzymes when these pathogens come into contact with plants (Ham *et al.*, 1991). The disease resistance can be induced in genetically susceptible varieties by over expressing some of these signals at specified periods.

3.6 Bibliography

Abbas, C. A. , Groves, S. and Gander, J. E. (1989). Isolation, purification and properties of *Penicillium charlesii* alkaline protease. *Journal of Bacteriology*, **171**: 5630-5637.

Adams, D. J. (2004). Fungal cell wall chitinases and glucanases. *Microbiology*, **150**: 2029-2035.

Adel Rio, L., Sandalio, L. M., Corpas, F. J., Palma, J. M. and Barroso, J. B. (2006). Reactive oxygen species and reactive nitrogen species in peroxisomes. Production, scavenging and role in cell signaling. *Plant Physiology*, **141**: 330-335.

Ahmed, S., Bashir, A., Saleem, H., Saadia, M. and Jamil, A. (2009). Production and purification of cellulose degrading enzymes from a filamentous fungus *Trichoderma harzianum*. *Pakistan Journal of Botany*, **41**: 1411-1419.

Aleksieva, P. and Peeva, L. (2000). Investigation of acid proteinase biosynthesis by the fungus *Humicola lutea* 120-5 in an airlift bioreactor. *Enzyme Microbiology and Technology*, **26**: 402-405.

Andrade, V. S., Sarubbo, L. A., Fukushima, K., Miyaji, M., Nishimura, K. and de Campos-Takaki, G. M. (2002). Production of extracellualr proteases by *Mucor circinelloides* using D-glucose as carbon sources/substrate. *Brazilian Journal of Microbiology*, **33**: 106-110.

Angelini, R., Bragaloni, M., Federico, R., Infantino, A. and Portapuglia, A. (1993). Involvement of polyamines, diamine oxidase and peroxidase in resistance of chickpea to *Ascochyta rabiei*. *Journal of Plant Physiology*, **142**: 704-709.

Angelini, R., Manes, F. and Federico, R. (1990). Spatial and functional correlation between diamine-oxidase and peroxidase activities and their dependence upon de-etiolation and wounding in chick-pea stems. *Planta*, **182**: 89-96.

Arora, Y. K. and Bajaj, K. L. (1985). Peroxidase and polyphenol oxidase associated with, induced resistance of mung bean to *Rhizoctonia solani* Kuhn. *Journal of Phytopathology*, **114**: 325-331.

Arora, Y. K., Mehta, N., Thakur, D. P. and Wagle, D. S. (1986). Enzyme changes associated with host-parasite interactions between pearl millet and downy mildew fungus. *Journal of Phytopathology*, **116**: 97-105.

Arora, Y.K. and Wagle, D.S. (1985). Inter-relationship between peroxidase, polyphenol oxidase activities and phenolic content of wheat for resistance to loose smut. *Biochemie und physiologie der pflanzen,* **180**: 75-78.

Aulakh,K.S. and Grower, R.K. (1970). Changes in free amino acids, carbohydrates and ascorbic acid contents of tomato fruits after infection with pathogenic fungi. *Indian Journal of Microbiology,* **10**: 61-64.

Avdiushko, S. A., Ye, X. S. and Kuc, J. (1993). Detection of several enzymatic activities in leaf prints of cucumber plant. *Physiological and Molecular Plant Pathology,* **42**: 441–454.

Ayora, S., Lindgren, P. E. and Gotz, E. (1994). Biochemical properties of a novel metalloprotease from *Staphylococcus hyicus subsp. Hycus* involved in extracellular lipase processing. *Journal of Bacteriology*, **176**: 3218-3223.

Bahkali, A. H., Al-Khaliel, A. S. and Ellchider, K. A. (1997). *In vitro* and *In vivo* production of pectolytic enzymes by some phytopathogenic fungi isolated from Southwest Saudi Arabia. *Journal of King Saudi University*, **9**: 125-137.

Bailey, B. A. (1995) Purification of a protein from culture filtrates of *Fusarium oxysporum* that induces ethylene and necrosis in leaves of *Erythroxylum coca. Phytopathology*, **85**, 1250–1255.

Ball, A. M, Ingram, D. S. and Johnstone, K. (1991). A detached cotyledon test for the isolation of mutants of *Pyrenopeziza brassicae* defective in pathogenicity determinants. *Journal of Phytopathology*, **136**: 204-210.

Bartoli, C. G., Simontacchi, M., Tambussi, E., Montaldi, E. and Puntarulo, S. (1999). Drought and watering dependent oxidative stress: Effect on antioxidant content in *Triticum aestivum* L. leaves. *Journal of Experimental Botany*, **50**: 375-383.

Bau, L. and Feng Yun, L. (1998). Changes in activities and electrophoretic patterns of peroxidase and polyphenol oxidase in cucumerinum. *Scientia Agricultura Sinica*, **31**: 86-88.

Bayliss, K., Sivasithamparam, K., Barbetti, M. and Lagudah, E. (2001). Variability of phenylalanine ammonia lyase and peroxidase activities in leaves of subterranean clover is determined by their susceptibility to *Kabatiella caulivora*. *Phytopathologia Mediterranea*, **40**: 217-223.

Baysal, O., Soylu E. M. and Soylu, S. (2003). Induction of defence-related enzyme and resistance by the plant activator acibenzolar-S-methyl in tomato seedlings against bacterial canker caused by *Clavibacter michiganensis* ssp. *michiganensis*. *Plant Pathology*, **52**: 747-753.

Beber, K., Jarosch, B. Langen, G. and Kogel, K. H. (2000). Expression analysis of genes induced in barley after chemical activation reveals distinct disease resistance pathways. *Molecular Plant Pathology*, **1**: 277–286.

Bernards M. A. and Ellis, B. E. (2001). Phenylalanine ammonia-lyase from tomato cell cultures inoculated with *Verticillium albo-atrum*. *Plant Physiology*, **97**: 1494-1500.

Bestwick, C. S., Adam, A.L., Puri,N. and Mansfield, J. W. (2001). Characterization of and changes to pro- and anti-oxidant enzyme activities during the hypersensitive reaction in lettuce (*Lectuca sativa* L.) *Plant Science*, **161**: 497-506.

Blanco-Portales, R., Medina-Escobar, N., Lopez-Raez, J. A., Gonzalez-Reyes, J. A., Villalba, J. M., Moyano, E., Caballero, J. L. and Munoz-Blanco, J. (2002). Cloning, expression and immunolocalization pattern of a cinnamyl alcohol dehydrogenase gene from strawberry (*Fragaria* x *Ananassa* cv. Chandler). *Journal of Experimental Botany*, **53**: 1723-1734.

Bokma, E., Spiering, M., Chow, K. S., Mulder-Patty, P. M. F., Subroto, T., and Beintema, J. J. (2001). Determination of cDNA and genomic DNA sequences of hevamine, a chitinase from the rubber tree *Hevea brasiliensis*. *Plant Physiology and Biochem*istry, **39:** 367-376.

Boller, T. (1987). Hydrolytic enzymes in plant disease resistance. In: *Plant Microbe Interactions* (Eds. T. Kosuge and W. Nester), Macmillan, New York, **2**: 385-413.

Boller, T. and Mauch, F. (1988). Colourimetric assay for chitinase. *Methods in Enzymology.*, **161**: 430 -435 .

Bolwell, G. P., Cramer, C. L., Lamb, C. J., Schuch, W. and Dixon, R. A. (1986). L-Phenylalanine ammonia-lyase from Phaseolus *vul*garis: Modulation of the levels of active enzyme by trans-cinnamic acid. *Planta*, **169**: 97-107.

Booker, F. L. and Miller, J. E. (1998). Phenylpropanoid metabolism and phenolic composition of soybean [*Glycine max* (L.) Merr.] leaves following exposure to ozone. *Journal of Experimental Botany*, **49**: 1191-1202.

Breton, F, Sanier, C. and Auzac, J. (2000). Role of cassiicolin, a host-selective toxin, in pathogenicity of *Corynespora cassiicola*, causal agent of a leaf fall disease of *Hevea*. *Journal of Rubber Research*, **3** (2): 115-128.

Breton, F., Auzac, J., Garcia, D., Sanier, C. and Eschback, J.M. (1996). Recent researches on *Corynespora cassiicola/Hevea brasiliensis* interaction. *Proceedings of the Workshop on Corynespora Leaf Fall Diseases of Hevea Rubber*, 16-17 December 1996, Medan, pp. 49-78.

Breton, F., Sanier, C. and Auzac, J. (1997). Scopoletin production and degradation in relation to resistance of *Hevea brasiliensis* to *Corynespora cassiicola*. *Journal of Plant Physiology*, **151** (5): 595-602.

Broglie, K., Chet, I., Holiday, M., Cressman, R., Biddle, P., Knowlton, S., Mauvais, C. J. and Broglie, R. (1991). Transgenic plants with enhanced resistance to the fungal pathogen *Rhizoctonia solani*. *Science*, **254**: 1194-1197.

Butt, V. S. (1980). Direct oxidases and related enzymes. In: *Comprehensive Treatise*, Vol.2. (Ed. D.D. Davies). Academic Press, New York. pp: 81-123.

Butt, V. S. (1980). Direct oxidases and related enzymes. In: *The Biochemistry of Plants: A comprehensive Treatise* (Ed. D. D. Davies), **2**: 81-123.

Campbell, M. M. and Ellis, B. E. (1992) Fungal elicitor-mediated responses in pine cell cultures III. Purification and characterization of phenylalanine ammonia-lyase. *Plant Physiology*, **98**: 62-70.

Carpita, N. C. and Gibeaut, D. M. (1993).Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant Journal*, **3**: 1-10.

Caruso, C., Chilosi, G., Caporale, C., Leonardi, L., Bertini, L., Magro, P. and Buonocore V. (1999). Induction of pathogenesis-related proteins in germinating wheat seeds infected with *Fusarium culmorum*. *Plant Science*, **140**: 87-97.

Chacko, N., Philip, S., Zacharia, C.A. and Jacob, C. K. (2005). Artificial induction of chitinase and phyenyl alanine ammonia lyase activity in *Hevea brasiliensis*. In: Preprints of papers. International Natural Rubber Conference, India 2005. (Eds. N.M. Mathew *et al.*) Rubber Research Institute of India, Kottayam, Kerala, India, pp 523-526.

Chandra, J. and Tyagi (1993). Peroxidase activity associated with leaf blight of mung bean (*Vigna radiata* (Linn.) Wilczek). *Indian Journal of Mycology and Plant Pathology*, **23** (2): 184-186.

Chandra, J. and Tyagi (1993). Peroxidase activity associated with leaf blight of mung bean (*Vigna radiate* (Linn) Witezek). *Indian Journal of Mycology and Plant Pathology*, **23**: 184-186.

Chang, M., Horovitz, D., Culley, D. and Hadwiger, L. A. (1995). Molecular cloning and characterization of a pea chitinase gene expressed in response to wounding, fungal infection and elicitor chitosan. *Plant Molecular Biology*, **28**: 105-111.

Chen, C. Q., Belanger, R. R., Benhamou, N. and Paulitz, T. C. (2000). Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. *Physiological and Molecular Plant Pathology*, **56**: 13–23.

Chen, Z., Iyer, S., Caplan, A., Klessig, D. F. and Fan, B. (1997). Differential accumulation of salicylic acid and salicylic acid-sensitive catalase in different rice tissues. *Plant Physiology*, **114**: 193-201.

Chen, Z., Ricigliano, J. W. and Klessig, D. F. (1993a). purification and characterization of a soluble salicylic acid-binding protein from tobacco. *Proceedings of National Academy of Science*, USA. **90**: 9533-9537.

Chen, Z., Silva, H. and Klessig, D. F. (1993b). Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. *Science*, **262**: 1883-1886.

Chile, S.K. and Vyas, K.M. (1983). Free amino acids in relation to Phytophthora leaf rot pathogenesis of *Piper betle*. *Indian Phytopathology*, **36**: 721-722.

Christopher, D. J. Suthinraj, T. and Udhayakumar, R. (2007). Induction of defense enzymes in *Trichoderma viride* treated blackgram plants in response to *Macrophomina phaseolina* infection. *Indian Journal of Plant Protection*. **35**: 299-303.

Chrzanowska, J., Kolaczkowska, M. and Polanowski, A. (1993). Production of exocellular proteolytic enzymes by various species of *Penicillium*. *Enzyme Microbiology and Technol*ogy, **15**: 140-143.

Collinge, D. B., Kragh, K. M., Mikkelsen, J. D., Nielsen, K. K., Rasmussen, U. and Vad, K. (1993). Plant Chitinases. *Plant Journal*, **3**: 31-40.

Conrath, U., Chen, Z., Ricigliano, J. R. and Klessig, D. F. (1995). Two inducers of plant defense responses, 2,6-dichloroisonicotinic acid and salicylic acid, inhibit catalase activity in tobacco. *Proceedings of National Academy of Science*, USA. **92**: 7143-7147.

Culver, J. N. and Dawson, W. O. (1991). Tobacco mosaic virus elicitor coat protein genes produce a hypersensitive phenotype in transgenic *Nicotiana sylvestris* plants. *Molecular Plant Microbe Interactions*, **4**: 458-463.

Dangl, S. L. and Jones, J. D. G. (2001). Plant Pathogens and Integrated defence responses to infection. *Nature*, **411**: 826-833.

Dariot, D. J., Simonetti, A., Plinho, F. and Brandeli, A. (2008). Purification and characterization ofextracellular β-glucosidase from *Monascus ouroureus*. *Journal of Microbiology and Biotechnology*, **18**: 933-941.

Das, S., Agarwal, R. and Singh, D. V. (2003). Differential induction of defense related enzymes involved in lignin biosynthesis in wheat response to spot blotch infection. *Indian Phytopathology*, **56**: 129-133.

De Sa, M. M., Subramaniam, R., Williams, F. E., Douglas, C. J. (1992). Rapid activation of phenylpropanoid metabolism in elicitor-treated hybrid poplar *(Populus*

trichocarpa tom and gray X *Populus deltoids* marsh) suspension-cultured cells. *Plant Physiology*, **98**: 728-737

De Tullio, M. C., Liso, R. and Arrigoni, O. (2004). Ascorbic Acid Oxidase: An Enzyme in Search of a Role. *Biologia Plantarum*, **48**: 161-166.

De Vries, R. P. and Visser, J. (2001). *Aspergillus* enzymes involved in degradation of plant cell wall polysaccharides. *Microbiology and Molecular Biology Reviews*, **65**: 497-522.

Deborah, S. D., Palaniswami, A. and Velazhahan, R. (2001). Differential induction of chitinase and β-1,3-glucanase in rice in response to inoculation with a pathogen (*Rhizoctonia solani*) and a non-pathogen (*Pestalotia palmarum*). *Acta Phytopathologica et Entomologica Hungarica,* **36**: 67-74.

Dharmadhikari, M. S. and Jite, P. J. (1996). Studies on changes in total sugars and ascorbic acid in *Acacia leucophloea* infected with *Haplophragmiopsis ponderosa*. *Indian Journal of Mycology and Plant Pathology*, **26**: 199-201.

Dixon, R.A. and Harrison, M.J. (1990). Activation, structure and organization of genes involved in microbial defense in plants. Advance Genetics, **28**: 165-234.

Dobinson, K. F., Lecomte, N. and Lazarovits, G. (1996). Production of an extracelular trypsin-like protease by the fungal plant pathogen *Verticillium dahliae*. *Canadian Journal of Microbiolology*, **43**: 227-233.

Duff, S. M. G., Sarath, G., Plaxton, W. C. (1994). The role of acid phosphatase in plant phosphorus metabolism. *Physiologia Plantarum*, **90**: 791–800.

Durner, J. and Klessig, D. F. (1996). Salicylic acid is a modulator of tobacco and mammalian catalase. *Journal of Biol. Chem.*, **271**: 28492-28501.

Egea, C., Ahmed, A. S., Candela, M. and Candela, M. E. (2001). Elicitation of proxidase activity and lignin biosynthesis in pepper suspension cells by *Phytophthora capsici*. *Journal of Plant Physiology*, **158**: 151-158.

Ellingboe, A. H. (2001). Plant-pathogen Interactions: Genetic and Comparative Analyses. *European Journal of Plant Pathology*, **107**: 79-84.

Elstner, E. F. (1982) Oxygen activation and oxygen toxicity. *Annual Review of Plant Physiology*, **33**: 73-96.

Espino, J. J., Brito, N., Noda, J. and Gonzalez, C. (2005). *Botrytis cinera* endo-β-1,4-glucanase Cel5A is expressed during infection but is not required for pathogenesis. *Physiology and Molecular Plant Pathology*, **66**: 213-228.

Eventoff, W., Tanaka, N. and Rossmann, M. G. (1976). Crystalline bovine liver catalase. *Journal of Molecular Biology*, **103**: 799-801.

Fan-Ching, Y. and Lin, I. H. (1998). Production of acid protease using thin stillage from a rice-spirit distillery by *Aspergillus niger*. *Enzyme Microbiology Technology*, **23**: 397-402.

Farkas, G. L. and Lovrekovich, L. (1965). Enzyme levels in tobacco leaf tissues affected by the wildfire toxin. *Phytopathology*, **55**: 519-524.

Farley, P. C. and Ikasari, L. (1992). Regulation of the secretion of *Rhizopus Oligosporus* extracellular carboxyl proteinase. *Journal of General Microbiology*, **138**: 2539-2544.

Farrell, A. M., Foster, T. J. and Holland, K. T. (1993). Molecular analysis and expression of the lipase of *Staphylococcus epidermidis*. *Journal of General Microbiology*, **139**: 267-277.

Faull, J.L. and Gay, J.L. (1983). Phospholipase activity in *Erysiphe pisi*, *Physiological Plant Pathology*, **22**: 55-63.

Feierabend, J. and Engel, S. (1986) Photoinactivation of catalase *in vitro* and in leaves. *Arch Biochem Biophys*, **251**: 567-576.

Feierabend, J. and Kemmerich, P. (1983). Mode of interference of chlorosis- inducing herbicides and peroxisomal enzyme activities. *Physiologia Plantarum*, **57**: 346-351.

Fengming, S., Xiuchun, G. and Zhong, Z. (1997). Changes in activities of superoxide dismutase and catalase in cotton seedling after infection with *Fusarium oxysporum* f. sp.*vasinfectum* and their relations to the resistance. *Journal of Zhejiang Agricultural University*, **25**: 373-377.

Fengming, S., Zhong, Z. and Xiuchun, G. (1997). Role of peroxidase in the resistance of cotton seedlings to *Fusarium oxysporum* f. sp. *vasinfectum*. *Journal of Zhejiang Agricultural University*, **23**: 143-148.

Fita, I. and Rossmann, M. G. (1985). The active center of catalase. *Journal of Molecular Biology*, **185**: 21-37.

Fradin, E. F. and Thomma, B. P. H. J. (2006). Physiology and molecular aspects of Verticillium wilt diseases caused by *V. dahliae* and *V. albo-atrum. Molecular Plant Pathology*, **7**: 71-86.

Fric, F. and Fuchs, W.H. (1970). Veranderungen der Aktivitat einiger enzyme im weizenblatt in Abhangigikeit von der temperature labilen vertraglichkeit fur *Puccinia graminis* tritici. *Phytopathologische Zeitschrift*, **67**: 161-174.

Gangopadhyay, S. and Lal, S. (1986). Changes in certain biochemical constituents in maize (*Zea mays* L.) leaf sheath infected with *Rhizoctonia solani* Kuhn. *Indian Journal of Plant Pathology*. **4**: 9-16.

Gao, J., Weng, H., Zhu, D., Yuan, M. and Yu Xi, F. (2008). Production and characterization of cellulolytic enzymes from the thermoacidophilic fungal *Aspergillus terreus* M11 under solid state cultivation of corn stover. *Bioresource and Technology,* **99**: 7623-7629.

Gentile, A., Ferraris, L. and Matta, A. (1988). Variations of Phenoloxidase Activities as a Consequence of Stresses that Induce Resistance to *Fusarium* Wilt of Tomato. *Journal of Phytopathology*, **122**: 45-53.

Gibb, G. D. and Strohl, W. R. (1987). Physiological regulation of protease activity in *Streptomyces peucetius. Canadian Journal of Microbiol*ogy, **34**: 187-190.

Gogoi, R., Singh, D. V. and Srivastava, K. D. (2000). Changing behaviour of defence related enzymes in wheat during infection of *Neovossia indica. Indian Phytopathology*, **53**: 153-156.

Grand C., Sarni F. and Boudet A.M. (1985). Inhibition of cinnamyl- alcohol debydrogenases activity and lignin synthesis in poplar *(Populus euramericana)* tissue by two organic compounds. *Planta,* **163**: 232-237.

Grand, C., Sarni, F. and Lamb, C. J. (1987) Rapid induction by fungal elicitor of the synthesis of cinnamyl alcohol dehydrogenase a specific enzyme of lignin synthesis. *European Journal of Biochemistry,* **169:** 73-77.

Green, N. E., Hadwiger, L. A. and Graham, S. O. (1975). phenylalanine ammonia-lyase and tyrosine ammonia-lyase and lignin in wheat inoculated with *Erysiphe graminis* f. sp. *tritici*. *Phytopathology*, **65**: 1071-1074.

Gross, G.G. (1979). Recent advances in the chemistry and biochemistry of lignin. *Recent Advances in Phytochemistry*, **12**: 177-220.

Gupta, S.K., Gupta, P.P. and Kaushik, C.D. (1995). Changes in leaf peroxidase, polyphenol oxidase, catalase and total phenols due to Alternaria leaf blight in *Brassica* species. *Indian Journal of Mycology and Plant Pathology*, **25** (3): 175-180.

Guzman, S., Ramos, I., Moreno, E., Ruiz, B., Rodriguez-Sanoja, R.,escalante, L., Langley, E. and Sanchez, S. (2005). Sugar uptake and sensitivity to carbon catabollite regulation in *Streptomyces peucetius var. caesius*. *Applied Microbiology and Biotechnology*, **69**: 200-206.

Ham, K.S., Kauffmann, S., Albersheim, P. and Darvill, A.G. (1991). Host – pathogen interactions. XXXIX. A soybean pathogenesis – related protein with β-1,3-Glucanase activity release phytoalexin elicitor - active heat – stable fragments from fungal walls. *Molecular Plant Microbe Interactions*, **4**: 545-552.

Hammerschmidt, R. (1999). Induced disease resistance: How do induced plants stop pathogens. *Physiological and Molecular Plant Pathology*, **55**: 77–84.

Hammerschmidt, R., Nuckles, E.M. and Kue, J. (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiological Plant Pathology*, **20**:73-82.

Hashim, I., Chee, K.H. and Wilson, L.A. (1980). The relationship of phenols and oxidative enzymes with the resistance of *Hevea* to South American Leaf Blight. *Phytopathologische Zeitschrift*, **97**: 332-345.

Hashim, I., Wilson, L. A. and Chee, K. H. (1978). Regulation of indole acetic acid oxidase activities in *Hevea* leaves by natuarally occurring phenolics. *Journal of Rubber Research Institute of Malasia*, **26**: 105.

He, S. Y., Huang, H.C. and Collmer, A. (1993). *Pseudomonas syringae* pv. *Syringae* Harpin: A protein that is secreated via the Hrp pathway and elicits the hypersensitive response in plants. *Cell*, **73**: 1255-1266.

Heidi, J., Mitchell, J., Hall, L. and Barber, M. S. (1994). Elicitor induced cinnamyl alcohol dehydrogenase activity in lignifying wheat leaves. *Plant Physiology*, **104**: 551-556.

Held, A., Kedar, N. and Birk. Y. (1965). Dehydrogenase activity of potato tubers tissue infected with *Phytophthora infestans*. *Phytopathology*, **55**: 970-976.

Herrera, M. D. V., Toro, M. E., Figueroa, L. I. C. and Vazquez, F. (2004). Extracellular hydrolytic enzymes produced by phytopathogenic fungi. *Environmental Microbiology*, **16**: 299-321.

Hoppe, H. H. and Heitefuss, R. (1974). Permeability and membrane lipid metabolism of *Phaseolus vulgaris* infected with *Uromyces phaseoli*. II. Changes in lipid concentration and ^{32}P incorporation into phospholipids. *Physiological Plant Pathology*, **4**: 11-23.

Huckelhoven, R. (2007). Cell-wall associated mechanisms of disease resistance and susceptibility. *Annual Review of Phytopathology*, **45**: 101-127.

Ikotun, T. and Balogun, O. (1987). *In vitro* and *In vivo* production of pectolytic enzymes by some phytopathogenic fungi. *Journal of Basic Microbiology*, **27**: 347-354.

Imbert, M.P. and Wilson, L.A. (1970). Stimulatory and inhibitory effects of scopoletin in IAA oxidase preparations from sweet potato. *Phytopathology*, **9**: 1787-1794.

Irving, H.R. and Kuc, J.A. (1990). Local and systemic induction of peroxidase, chitinase and resistance in cucumber plants by K_2HPO_4. *Physiological and Molecular Plant Pathology*, **37**: 355-366.

Jacobs, A. K., Dry, I. B. and Robinson, S. P. (1999). Induction of different pathogenesis-related cDNAs in grapevine infected with powdery mildew and treated with ethephon. *Plant pathology*, **48**: 325-336.

Jakobek, J. L. and Lindgren, P. B. (2002). Expression of a bean acid phosphatase cDNA is correlated with disease resistance. *Journal of Experimental Botany*, **53**: 387-389.

Jayaraj, J., Wan, A., Rahman, M. and Punja, Z. K. (2008). Seaweed extract reduces foliar fungal diseases on carrot. *Crop Protection*, **27**: 1360-1366.

Jayasinghe, C. K. and Wijesundera, R. L. C. (1999). Cell wall degrading enzyme secretion by *Cylindrocladium quinqueseptatum*. *Journal of Rubber Reaserch Institute of Srilanka*, **82**: 47-60.

Jennings, P.H., Brannman, B.L. and Zscheile, F.P. (1969). Peroxidase and polyphenol oxidase activity associated with leaf spot of maize. *Phytopathology*, **59**: 963-967.

Jite, P.K. and Tressa, J. (1999). Biochemical changes in *Jasminum grandiflorum* infected by *Uromyces hobsoni*. *Indian Phytopathology*, **52** (1): 77-78.

Johri, J. K. and Devi, S. (1998). Ultrastructural studies on *Phytophthora palmivora* infection on betelvine. *Archives of Phytopathology and Plant Protection*, **31**: 233-240.

Jongedijk, E., Tigelaar, H., van Roekel, J. S. C., Bres-Vloemans, S. A., Dekker, I., van den Elzen, P. J. M., Cornelissen, B. J. C. and Melchers, L. S. (1995) Synergetic activity of chitinases and β-1,3-glucananses enhances fungal resistance in transgenic tomato plants. *Euphytica*. **85**: 173-180.

Joosten, M. H. A. J. and De Wit, P. J. G. M. (1989). Identification of several pathogenesis-related proteins in tomato leaves inoculated with *Cladosporium fulvum* (syn. *Fulvia fulva*) as β-1,3-glucanases and chitinases. *Plant Physiology*, **89**: 945-951.

Joseph, A. (1998). Investigation on certain biochemical changes and phyllosphere microflora of *Hevea brasiliensis* as influenced by nitrogenous fertilizer application and *Corynespora cassiicola* inoculation. Ph.D. Thesis, Mahatma Gandhi University, Kottayam, India.

Jung, J. L., Fritig, B. and Hahne, G. (1993). Sunflower (*Helianthus annuus* L.) pathogenesis-related proteins. Induction by aspirin (acetyl-salicylic acid) and characterization. *Plant Physiology*, **101**: 873-880.

Kale, M. C. and Chaudhary, A. D. (2001). Induction of phenylalanine ammonia lyase in groundnut cultivars in response to biotic and abiotic stress. *Indian Phytopathology*, **54**: 288-292.

Kalia, P. (1998). Enzymic association of powdery mildew resistance in garden pea. *Vegetable Science*, **25**: 166-168.

Kang, Z. and Buchenauer, H. (2002). Immunochemical localization of β-1,3-glucanase and chitinase in *Fusarium culmorum* infected wheat spikes. *Physiology and Molecular Plant Pathology*, **60**: 141-153.

Kannaiyan, J., Vidhyasekaran, P. and Kandaswamy, T. K. (1975). Proteolytic enzyme activity of *Claviceps microcephala* causing Ergot disease of Bajra. *Indian Phytopathology*, **28**: 111.

Kanwar, S. S., Kaushal, R. K., Jawed, A., Chimni, S. S. and Punj, V. (2004). Pretreatment of lipase from *Bacillus coagulans* BTS-1 with proteases enhances its activity, *Asian Journal of Microbiology, Biotechnology and Environmental Science*, **6**: 215-219.

Karthikeyan, A. and Bhaskaran, R. (1992) Peroxidase, polyphenol oxidase and nitrate reductase activities in Thanjavur wilt affected coconut palms. *International Journal of Tropical Plant Diseases,* **10**(1): 85-89.

Kastner, B., Tenhaken, R. and Kauss, H. (1998). Chitinase in cucumber hypocotyls is induced by germinating fungal spores and by fungal elicitor in synergism with inducers of acquired resistance. *Plant Journal*, **13**: 447-454.

Kavitha, R. and Umesha, S. (2008). Regulation of defense-related enzymes associated with bacterial spot resistance in Tomato. *Phytoparasitica*, **36**: 144-159.

Kawashima, N. and Uritani, I. (1965). Some properties of peroxidases produced in sweet potato infected by the black rot fungus. *Plant cell Physiology*, **6**: 247-265.

Kenton, P., Mur, L. and Draper, J. (1999). A requirement for calcium and protein phosphatase in the jasmonate-induced increase in tobacco leaf acid phosphatase specific activity. *Journal of Experimental Botany*, **50:** 1331-1341

Khan, W., Prithiviraj, B. and Smith, D. L. (2003). Chitosan and chitin oligomers increase phenylalanine ammonia-lyase and tyrosine ammonia-lyase activities in soybean leaves. *Journal of Plant Physiology*, **160**: 859-863.

Khirbat, S. K. and Jalali, B. L. (1998). Polyphenol oxidase and bound phenol content in the leaves of chick pea (*Cicer arietinum* L.) after inoculation with *Ascochyta rabiei*. *Legume Research*, **21**: 198-200.

Kim, K. K., Song, H. K., Shin, D. H., Hwang, K. Y. and Suh, S. W. (1997). Features of bacterial and pancreatic lipase. *Structure*, **5**: 173-185.

Kok, R. G., van Thor, J. J., Nugteren-Roodzant, I. M., Brouwer, M. B. W. Egmond, M. R., Nudel, C. B., Vosman, B. and Hellingwerf, K. J. (1995). Characterization of the extracellular lipase, LipA, of *Acinetobacter calcoaceticus* BD413: fatty acid repression of LipA expression and degradation of LipA. *Journal of Bacteriology*, **178**: 6025-6035.

Kosuge, T. (1969). The role of phenolics in host response to infection. *Annual Review of Phytopathology*, **7**: 195-222.

Kumar, V., Kumar, A., Verma, V. C., Gond, S. K. and Kharwar, R. N. (2007). Induction of defense enzymes in *Psuedomonas fluorescens* treated chick pea root against *Macrophomina phaseolina*. *Indian Phytopathology*, **60**: 289-295.

Kuzniak, E. and Sklodowska, M. (2005). Fungal pathogen-induced changes in the antioxidant systems of leaf peroxisomes from infected tomato plants. *Planta,* **222**: 192-200.

Lawton, K., Ward, E., Payne, G., Moyer, M. and Ryals, J. (1992). Acidic and basic class III chitinase mRNA accumulation in response to TMV infection of tobacco. *Plant Molecular biology*, **19**: 735-743.

Lazarovits, G. and Ward, E.W.B. (1982). Polyphenol oxidase activity in soybean hypocotyls at sites inoculated with *Phytophthora megasperma* f. sp. *Glycinea*. *Physiological Plant Pathology*, **12**: 227-236.

Lea, P. J. and Ireland, R. J. (1999). Plant amino acids. In: Nitrogen metabolism in higher plants (ed. Singh B. K.) New York: Marcel Dekker, Inc., pp 1–47.

Lea, P. J. and Miflin, B. J. (1974). Alternative route for nitrogen assimilation in higher plants. *Nature*, **251:** 614–616.

Lebeda, A., Luhova, L., Sedlarova, M. and Jancova, D. (2001). The role of enzymes in plant-fungal pathogens interactions. *Journal of Plant Disease Protection*, 108: 89-111.

Lee, S.S., Kawakita, K., Tsuge, T. and Doke, N. (1992). Stimulation of phospholipase A_2 activity in strawberry cells treated with AF- toxin I produced by *Alternaria alternata* strawberry pathotype, *Physiological and Molecular Plant Pathology*, **41**: 283-294.

Leger, R. J. and Roberts, D. W. (1997). Adaptation of proteases and carbohydrases of saprophytic, phytopathogenic and dentomopathogenic fungi to the requirements of their ecological niches. *Microbiology*, **143**: 1983-1992.

Levin, A. Tenhaken, R., Dixon, R. and Lamb, C. (1994). H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell,* **79**: 583-593.

Li, L. and Steffens, J. C. (2002). Overexpression of polyphenol oxidase in transgenic tomato plants results in enhanced bacterial disease resistance. *Planta*, **215**: 239-247.

Li, Y. H., Ding, H., Wang, J., Xu, G. J. and Zhao, F. (2006). A novel thermoacidophilic endoglucanase, Ba-EGA, from a new cellulose degrading bacterium, *Bacillus* sp. AC-1. *Applied Microbiology and Biotechnol*ogy, **70**: 430-436.

Lin, L. S. and varner, J. E. (1999). Expression of ascorbic acid oxidase in Zucchini squash (*Cucurbita pepo* L.). *Plant Physiology*, **96**: 159-165.

Lin, W., Anuratha, C. S., Datta, K., Potrykus, I., Muthukrishnan, S. and Datta, S. K. (1995). Genetic engineering of rice for resistance to sheath blight. *Biotechnology*, **13**: 686-691.

Luan, S. (1998). Protein phosphatases and signaling cascades in higher plants. Trends in *Plant Science*, **3**: 271-275.

Luhova, L., Lebeda, A., Hedererova,D. and Pec, P. (2003). Activities of amine oxidase, peroxidase and catalase in seedlings of *Pisum sativum* L. under different light conditions. *Plant Soil Environment*, **49** (4): 151-157.

Lumsden, R. D. (1970). Phosphatidase of *Sclerotiorum sclerotiorum* produced in culture and infected bean. *Phytopathology*, **60**: 1106-1110.

Lumsden, R. D. and Bateman, D. F. (1968). Phosphatid degrading enzymes associated with pathogenesis in *Phaseolus vulgaris* infected with *Thielaviopsis basicola. Phytopathology*, **58**: 219-227.

Luthra, Y.P., Joshi, U.N., Gandhi, S.K. and Arora, S.K. (1988). Biochemical alterations in downy mildew infected lucern leaves. *Indian Phytopathology*, **41**:100-106.

Lynd, L. K., Weimer, P. J., vanZyl, W. H. and Pretorius, I. S. (2002). Microbial cellulose utilization: fundamentals and biotechnology. *Microbiology and Molecular Biology Review*, **66**: 506-577.

Lyr, H. (1966). On the toxicity of oxidized polyphenol. *Phytopathologische Zeitschrift*, **52**: 229-240.

Mader, M., Nessel,A. and Schloss,P. (1986). Cell compartmentation and specific roles of isozymes. In: *Molecular and Physiological aspectsof Plant Peroxidases.* (Eds. H. Greppin, C. Penel, T. Gaspar). University of Geneva, pp: 247-260.

Madhukar, J. and Reddy, S.M. (1990). Dehydrogenase enzyme in the fruit-rot of *Guava. Indian journal of Mycology and Plant Pathology*, **20** (2): 189-191.

Mahadevan, A. (1991). Phytoalexin Production. In: *Post Infectional Defence Mechanisms*. Today & Tomorrow's Printers and Publishers, New Delhi-110005 (India), **2**: 232.

Malca, I. and Zscheile, F. P. (1964). Dehydrogenase activity in relation to development of the Helminthosporium leaf spot disease of maize. *Phytopathology*, **54**: 1281-1282.

Malik, S., Kumar, P., Panwar, J. D. S., Anjali, and Rathi, Y. P. S. (2002). Physiological and biochemical alterations induced by urdbean leaf crinkle virus in *Vigna mungo* (L.) Hepper. *Annals of Plant Protection Sciences*, **10**: 91-94.

Mandavia, M. K., Gajera, H. P., Andharia, J. H. and Parameswaran, M. (1999). Cell wall degrading enzymes in host-pathogen interaction of Fusarium wilt of chickpea: Inhibitory effects of phenolic compounds. *Indian Phytopathology*, **52**: 285-288.

Marchant, R., Davey, M. R., Lucas, J. A., Lamb, C. J., Dixon, R. A. and Power, J. B. (1998). Expression of a chitinase transgene in rose (*Rosa hybrida* L.) reduces development of blackspot disease (Diplocarpon rosae Wolf). *Mol. Breed.* **4**: 187-194.

Margis-Pinheiro, M., Marivet, J. and Burkard, G. (1994). Bean class IV chitinase gene: Structure, developmental expression and induction by heat stress. *Plant Science*, **98**: 163-173.

Martiny-Baron,G, Scherer,G.F.E. (1989). Phospholipid- stimulated protein kinase in plants. *Journal of Biological Chemistry*, **264**: 18052-18059.

Maxwell, D.P. and Bateman, D.F. (1967). Changes in the activity of some oxidases in extracts of *Rhizoctonia* infected bean hypocotyls in relation to lesion maturation. *Phytopathology*, **57**: 132-136.

Maxwell, D.P. and Bateman, D.F. (1967). Changes in the activity of some oxidases in extracts of *Rhizoctonia* infected bean hypocotyls in relation to lesion maturation. *Phytopathology*, **57**: 132-136.

Mayer, A. M. and Harel, E. (1979). Phenol oxidases in plants. *Phytochemistry*, **18**: 193-215.

McDougall, G. J. (1993). Covalently bound peroxidases and lignification. In: *Plant Peroxidases: Biochemistry and Physiology*. (Eds: K.G. Welinder, S.K. Rasmussen, C. Penel and H. Greppin). University of Geneva, pp: 277-282.

Mehta, A. and Mehta, P. (1993). Production of pectolytic and cellulolytic enzymes by *Fusarium oxysporum* and *Fusarium moniliforme* under different cultivation conditions. *Folia Microbiologica*, **30**: 42-50.

Mehta, A., Chopra, S. and Mehta, P. (1993) Production of cell wall-degrading enzymes by three isolates of *Aspergillus niger* under different cultivation conditions. *Journal of Basic Microbiology,* **33**: 193-199.

Mendgen K. and Deising H. (1993). Infection structures of fungal plant pathogens-a cytological and physiological evaluation. *New Phytol.* **124**:193-213.

Mendgen, K., Hahn, M. and Deising, H. (1996). Morphogenesis and mechanisms of penetration by plant pathogenic fungi. *Annual Review of Phytopathology*, **34**: 367-386.

Metraux, J. P. and Boller, T. (1986). Local and systemic induction of chitinase in cucumber plants in response to viral, bacterial and fungal infections. *Physiological and Molecular Plant Pathology,* **28,** 161–169.

Mishra, N. P., Mishra, R. K. and Singhal, G. S. (1993). Changes in the activities of anti-oxidant enzymes during exposure of intact wheat leaves to strong visible light at different temperatures in the presence of protein synthesis inhibitors. *Plant Physiology*, **102**: 903-910.

Mitchell, H. J., Hall, J. L. and Barber M. S. (1994). Elicitor induced cinnamyl alcohol dehydrogenase activity in lignifying wheat (*Triticum aestivum* L.) leaves. *Plant Physiology*, **104**: 551-556.

Moerschbacher, B. M., Noll, U., Ocampo, C. A., Flott, B. E., Gotthardt, U., Wüslefeld, A. and Reisener, H. (1990). Hypersensitive lignification response as the mechanism of non-host resistance of wheat against oat crown rust *Physiologia Plantarum*, **78**: 609-615.

Moerschbacher, B. M., Kogel, K. H., Noll, U. M. and Reisener, H. J. (1986). An elicitor of the hypersensitive lignification response in wheat leaves isolated from the rust fungus *Puccinia graminis f. sp.. tritici.* I. Partial purification and characterization. *Zeitschrift fur Naturforschung*, **41c**: 830-838.

Moerschbacher, B. M., Noll, U. M. Flott, B. E. and Reisener, H. J. (1986). Lignin biosynthetic enzymes in stem rust infected, resistant ans susceptible near-isogenic wheat lines. *Physiological and Molecular Plant Pathology*, **33**: 33-46.

Mohammadi, M. and Kazemi, H. (2002) Changes in peroxidase and polyphenol activity in susceptible and resistant wheat heads inoculated with *Fusarium graminearum* and induced resistance. *Plant Science,* **162**: 491–498.

Mohammadi, M. and Kazemi, H. (2002) Changes in peroxidase and polyphenol activity in susceptible and resistant wheat heads inoculated with *Fusarium graminearum* and induced resistance. *Plant Science,* **162**: 491–498.

Mohr, U., Lange, J., Boller, T., Wiemken, A. Vogeli-Lange, R. (1998). Plant defense genes are induced in the pathogenic interaction between bean roots and *Fusarium solani*, but not in the symbiotic interaction with the arbuscular mycorrhizal fungus *Glomus mosseae*. *New Phytol*, **138**: 589-598.

Muller, K., Doubnerova, V., Synkova, H., Cerovska, N. and Ryslava, H. (2009). Regulation of phosphoenol pyruvate carboxylase in PVY (NTN) infected tobacco plants. *Biology and Chemistry*, **390**: 245-251.

Munch-Garthoff, S., Neuhaus, J. M., Boller, T., Kemmerling, B. and Kogel, K. H. (1997). Expression of β-1,3-glucanase and chitinase in healthy, stem-rust-affected and elicitor-treated near-isogenic wheat lines showing Sr5-or Sr24-specified race-specific rust resistance. *Planta*, **201**: 235-244.

Murray, A. J. S. and Ayres, P. G. (1986). Uptake and translocation of nitrogen by mildew barley seedlings. *New Pathologist*, **104**: 355-365.

Nicholson, R. L. and Hammerschmidt, R. (1992). Phenolic compounds and their role in disease resistance. *Annual Review of Phytopathology*, **30**: 369-389.

North, M. J. (1982). Comparative biochemistry of the proteinases of eucaryotic microorganisms. *Microbiology Reveiw*, **46**: 308-340.

Oh, Y. S., Shih, I. L., Tzeng, Y. M. and Wang, S. L. (2000). Protease produced by *Pseudomonas aeruginosa* K-187 and its application in the deproteinization of shrimp and crab shell wastes. *Enzyme Microb. Technol.*, **27**: 3-10.

Ohme-Takagi, M., Meins, F. and Shinshi, H. (1998). A tobacco gene encoding a novel basic class II chitinase: A putative ancestor of basic class I and acidic class II chitinase genes. *Mol. Gen. Genet,* **259**: 511-515.

Olmos, E. and Hellin, E. (1997). Cytochemical localization of ATPase plasma membrane and acid phosphatase by cerium based in a salt-adapted cell line of *Pisum sativum*. *Journal of Experimental Botany*, **48**: 1529-1535.

Ormaetxe, I. I., Escuredo, P. R., Arress-Igor, C. and Bacana, M. (1998). Oxidative damage in pea plants exposed to water defecit or paraquat. *Plant Physiology*, **116**: 173-181.

Palmgren, M.G. and Sommarin, M. (1989). Lysophosphatidylcholine stimulates ATP-dependent protein accumulation in isolated oat root plasma membrane vesicles. *Plant Physiology*, **90**: 1009-1014.

Pardo A. (1996). Effect of surfactants on cellulase production by *Nectria catalinensis*. *Current Microbiology*, **33**: 275-278.

Payne, G., Ahl, P., Moyer, M., Harper, A., Beck, J., Meins, F. and Ryals, J. (1990). Isolation of complementary DNA clones encoding pathogenesis related proteins P and Q, two acidic chitinases from tobacco. *Proceedings of national Academy of Science*, USA. **87**: 98-102.

Pegg, G. S., Webb, R. I., Carnegie, A. J., Wingfield, M. J. and Drenth, A. (2009). Infection and disease development of Quambalaria spp on Corymbia and Eucalyptus. *Annual Review of Phytopathology,* **58**: 642-654.

Pekkarinen, A., Mannonen, L., Jones, B. L. and Niku-Paavola, M. L. (2000). Production of proteases by *Fusarium* species grown on barley grains and in media containing cereal proteins. *Journal of Cereal Science*, **31**: 253-261.

Perl-Treves, R. and Perl, A. (2002). Oxidative stresses: An introduction. In: *Oxidative Stresses in Plants* (Eds. Dierk Inze and Marc Van Montagu) Taylor and Francis, London and New York. Pp. 8.

Pfanz, H. (1993). Oxidation of IAA by extracellular peroxidases. In: *Plant peroxidases: Biochemistry and Physiology*.(Eds. K.G. Welinder, S.K. Rasmussen, C. Penel and H. Greppin). University of Geneva, pp. 169-174.

Pillonel C, Hunziker P, Binder, A. (1992) Multiple forms of the constitutive wheat cinnamyl alcohol dehydrogenase. *Journal of Experimental Botany*, **43**: 299-305.

Pointing, S. B., Buswell, J. A., Jones, E. B. G. and Vrijmoed, L. L. P. (1999). Extracellular cellulolytic enzyme profiles of five lignicolous mangrove fungi. *Mycological Research*, **103**: 696-700.

Politycka, B. (1998). Phenolics and the activities of phenylalanine ammonia-lyase, phenol-β-glucosyltransferase and β-glucosidase in cucumber roots as affected by phenolic allelochemicals. *Acta Physiologiae Plantarum*, **20**: 405-410.

Ponteir, D., Godiard, L., Marco, Y. and Roby, D. (1994). Hsr 203 J, a tobacco gene whose activation is rapid, highly localized and specific for incompatible plant/pathogen interactions. *Plant Journal*, **5**: 507-521.

Prats, E., Martinez, F., Rojas-Molina, M. M. and Rubiales, D. (2007). Differential effects of phenylalanine ammonia lyase, cinnamyl alcohol dehydrogenase, and energetic metabolism inhibition on resistance of appropriate host and nonhost cereal-rust interactions. *Phytopathology*, **97**: 1578-1583.

Purkayastha, R.P. (1998). Disease resistance and induced immunity in plants. *Indian Phytopathology*, **51** (3): 211-221.

Pushalkar, S. P., Rao, K. K. and Menon, K. (1995). Production of ß-Glucosidase by *Aspergillus terreus. Current Microbiology*, **30**: 255-258.

Radiacommare, R., Kandan, A., Nandakumar, R. and Samiyappun, R. (2004). Association of hydrolytic enzyme chitinase against *Rhizoctonia solani* in *Rhizobacteria* treated rice plants. *Journal of Phytopathology,* **152**, 365–370.

Raman, V. V., Reddy, V. K., Reddy, S. M. and Reddy, K. J. (1997). Production of cellulases, hemicellulases, pectinases, proteinases and lipases by *Cephalosporium maydis* isolated from *Zea mays* stalks. *Microbial Biotechnology*, **1**: 187-192.

Rameshsundar, A. and Vidhyasekaran, P. (2003). Induction of defense-related biochemical changes by elicitors of red rot pathogen and a non-pathogen in sugarcane cell culture. *Inadian Phytopathology,* **56**: 255-261.

Rao, G. P., Singh, M. and Singh, H. N. (1992). Nitrate reductase activity in smut t infected leaf tissues of sugarcane. *Indian Phytopathology*, **45**: 123-124.

Rauscher, M., Mendgen, K., and Deising, H. 1995. Extracellular proteases of the rust fungus *Uromyces viciae-fabae. Experimental Mycolog,y* **19**: 26-34.

Ray, H., Douches, D. S. and Hammerschmidt, R. (1998). Transformation of potato with cucumber peroxidase: Expression and disease response. *Physiological and Molecular Plant Pathology*, **53**: 93–103.

Riou, C., Freyssinet, G. and Fevre, M. (1991). Production of cell wall-degrading enzymes by the phytopathogenic fungus *Sclerotinia sclerotiorum. Applied Environmental Microbiology*, **57**: 1478-1485.

Robb, A.D., Mapson, L.W. and Swain, T. (1964). Activation of the latent lyrosinase of broad bean. *Nature*, **201**: 503-504.

Roberts, A. M. and Walters, D. R. (1988). Nitrogen assimilation and metabolism in rusted leek leaves. *Physiological and Molecular Plant Pathology*, **32**: 229-235.

Roby, D., Broglie, K., Cressman, R., Biddle, P., Chet, I. and Broglie, R. (1990). Activation of bean chitinase promoter in transgenic tobacco plants by phytopathogenic fungi. *Plant Cell*, **2**: 999-1007.

Rodriguez, I. A., Escobedo, C. P., Paramo, M. G. Z., Romero, E. L. and Camacho, H. C. (2005). Degradation of cellulose by the bean-pathogenic fungus *Colletotrichum lindemuthianum*.Produciton of extracellular cellulolytic enzymes by cellouse induction. *Antonie van Leeuwenhock.*, **87**: 301-310.

Ronald, P. (2001). Signaling in rice disease resistance. In: *Delivery and perception of pathogen signals in plants.* (Eds. Noel, T. Keen, Shigeyuki Mayama, Jan E. Leach and Shinji Tsuyumu). APS Press, The American Phytopathological Society. St. Paul, Minnesota, pp. 137-144.

Rubin, B. A. and Artsikhovskaya, E. V. (1964). Biochemistry of pathological darkening of plant tissues. *Annual Review of Phytopathology*,**2**: 157-178.

Rudolph, K. and Stahmann, M.A. (1964). Interactions of peroxidase and catalases between *Phaseolus vulgaris* and *Pueudomonas phaseolicola* (Halo blight of bean). *Nature*, **204**: 474-475.

Sadler, R. and Scott, K. J. (1974). Nitrogen assimilation and metabolism in barley leaves infected with the powdery mildew fungus. *Physiological Plant Pathology*, **4**: 235-247.

Saharan, G. S., Joshi, U. N. and Saharan, M. S. (2000). Phenolic compounds and oxidative enzymes in healthy and Alternaria blight infected leaves of clusterbean. *Acta Phytopathologica et Entomologica Hungarica*, **34**: 299-306.

Saharan, M. S., and Saharan, G. S., Gupta, P. P. and Joshi, U. N. (2001). Phenolic compounds and oxidative enzymes in clusterbean leaves in relation to Alternaria blight severity. *Acta Phytopathologica et Entomologica Hungarica*, **36**: 237-242.

Samia M. and El- Khallal (2007). Induction and modulation of resistance in tomato plants against *Fusarium* wilt disease by bioagent fungi (Arbuscular Mycorrhiza) and/or hormonal elicitors (Jasmonic Acid & Salicylic Acid): 2-changes in the antioxidant enzymes, phenolic compounds and pathogen related-proteins. *Australian Journal of Basic and Applied Sciences*, **1**(4): 717-732.

Saravanakumar, D., Vijayakumar, C., Kumar, N. and Samiyappan, R. (2007). PGPR-induced defense responses in the tea plant against blister blight disease. *Crop Protection*, **26**: 556-565.

Sarkar, K. R. and Joshi, R. D. (1977). Peroxidase and catalase activity in MLO infected Brinjal. *Inadian Phytopathology*, **30**: 518-521.

Sarwar, N., Jamil, F. F. and Praveen, R. (2001). Accumulation of phytoalexins and phenylalanine ammonia lyase in chickpea after inoculation with *Ascochyta rabiei* and their role in defence mechanism. *Pakistan journal of Botany*, **33**: 373-382.

Sasaki, I and Nagayama, H. (1996). β-Glucosidase of *Botrytis cinera*: its involvement in the pathogenicity of the fungus. *Bioscience, Biotechnology and Biochemistry*, **60**: 54-56.

Satyavir (2003). Red rot of sugarcane–current scenario. *Indian Phytopathology*, **56**: 245-254.

Scandalios, T. G. (1993). Oxygen stress and superoxide dismutase. *Plant Physiology*, **101**: 7-12.

Schafer, W. (1994). Molecular mechanisms of fungal pathogenicity to plants. *Annual Review of Phytopathology,* **46**: 461-477.

Schaffrath, U., Scheinpflug, H. and Reisner, H. (1995). An elicitor from *Pyricularia oryzae* induces resistance responses in rice: isolation, characterization and physiological properties. *Physiological and Molecular Plant Pathology*, **46**: 293-307.

Scherer, G. F. E. and Andre, B. (1989). A rapid response to a plant hormone: auxin stimulates phospholipase A_2 *in vivo* and *in vitro*. *Biochemical and Biophysical Research Communications*, **163**: 111-117.

Scherer, G. F. E., Andre, B. and Martiny-Baron, G. (1990). Hormone-activated phospholipase A2 and lysophospholipid-activated protein kinase: a new signal transduction chain and a new second messenger system in plants? *Curr. Top. Plant Biochemistry and Physiology*, **9**: 190-218.

Scherer, G. F. E., Martiny- Baron, G. and Stoffel, B. (1988). A new set of regulatory molecules in plants: a plant phospholipid similar to platelet- activating factor stimulates protein kinase and prototranslocating ATPase in membrane vesicles. *Planta*, **175**: 241-253.

Schwanz, P. and Polle, A. (2000). Differential stress responses of antioxidative systems to drought in pedunculate oat (*Quercus rober*) and maritime pine (*Pinus*

pinaster) grown under high CO_2 concentrations. *Journal of Experimental Botany*, **52**: 133-143.

Scott, K. J. (1965). Respiratory enzymic activities in the host and pathogen of barley leaves infected with *Erysiphe graminis*. *Phytopathology*, **55**: 438-441.

Scott, K. J. and Smillie, R. M. (1963). Possible relationship between photosynthesis and the rise in respiration in diseased leaves. *Nature*, **197**: 1319-1320.

Sednina, G.V.,Aksenova, V.A. and Khartina, G.A. (1981). Changes in lipid composition of mitochondrial membranes in kidney bean infected with *Rhizoctonia solani*, *Faziologiya Rastenii*, **28**: 1030-1036.

Shallom, D. and Shoham, Y. (2003). Microbial Hemicellulases. *Current Opinion of Microbiology*, **6**: 219-228.

Shamina, A. and Sarma,Y.R. (2000). β-1,3 Glucanase, Polyphenol oxidase and peroxidase activities in relation to tolerance of black pepper to *Phytophthora capsici*. *Proceedings of International Conference on Integrated Plant Disease Management for Sustainable Agriculture*. Indian Phytopathological Society, Division of Plant Pathology, IARI, New Delhi. pp.1143-1144.

Sharma, J.N. and Kaul, J.L. (1999). Biochemical nature of resistance in apple to *Venturia inaequalis* causing scab III. Proteins and amino acids. *Journal of Mycology and Plant Pathology*, **29** (2): 227-231.

Sharma, S.L., Chowfla, S.C., Sohi, H.S. and Sharma, M.M. (1975). Factors affecting resistance of tomato varieties to buckene rot (*Phytophthora parasitica*). *Indian Journal of Experimental Biology*. **13**: 323-325.

Shiraishi, T., Yamaoka, N. and Kunoh, H. (1989). Association between increased phenylalanine ammonia-lyase activity and cinnamic acid synthesis and the induction of temporary onaccessibility caused by *Erysiphe graminis* germ tube penetration of the barley leaf. *Physilogy and Molecular plant pathology*, **34**: 75-83.

Shiraishi, T., Yamaoka, N., Nicholson, R. L. and Kunoh, H. (1995). Phenyl alanine ammonia-lyase in barley: activity enhancement in response to *Erysiphe*

graminis f.sp. *hordei* (race 1), a pathogen and *Erysiphe pisi*, a nonpathogen. *Physiological and Molecular Plant Pathology*, **46**: 153-162.

Singh, H. N. P. (1993). Changes in sugar and vitamin C in banana fruits during pathogenesis. *Advanced Plant Science*, **6**: 33-36.

Singh, H. V. (2004). Biochemical transformation in *Brassica* spp due to *Peronospora parasitica* infection. *Annals of Plant Protection Sciences*, **12**: 301-304.

Smith, J. A. and Hammerschmidt, R. (1988). Comparative study of acidic peroxidases associated with induced resistance in cucumber, muskmelon and watermelon. *Physiological and Molecular Plant Pathology*, **33**: 255-261.

Smith, S. H., McCall, S. R. and Harris, J. H. (1968). Alterations in the auxin levels of resistant and susceptible hosts induced by curly top virus. *Phytopathology*, **58**: 575-577.

Srivastava, H. S. (1980). Regulation of nitrate reductase activity in higher plants. *Phytochemistry*, **19**: 725-733.

Srivastava, O. P. and Van Huystee, R. B. (1973). Evidence for close association of peroxidase, polyphenol oxidase and IAA oxidase isoenzymes of peanut suspension culture medium. *Canadian Journal of Botany*, **51**: 2207-2215.

Staples, R. C. and Stahmann, M. A. (1964). Changes in proteins and several enzymes in susceptible bean leaves after infection by the bean rust fungus. *Phytopathology*, **54**: 760-764.

Stebbins, G.L. (1992). Comparative aspects of plant morphogenesis: A cellular, molecular and evolutionary approach. *American Journal of Botany*, 7: 589-598.

Strodtkotter, I., Padmasree, K., Dinakar, C., Speth, B., Niazi, P. S., Wojtera, J., Voss, I., Do, P. T., Nunes-Nesi, A., Fernie, A. R., Linke, V., Raghavendra, A. s. AND Scheibe, R. (2009). Induction of the AOX1D isoform of alternative oxidase in *A. thaliana* T-DNA insertion lines lacking isoform AOX1A is insufficient to optimize photosynthesis when treated with antimycin A. *Molecular Plant*, **2**: 284-297.

Sukhwal, R. and Purohit, S. D. (2003). Accumulation of phenolics and changes in activity of oxidative enzymes in maize infected with *Helminthosporium species*. *Journal of Mycology and Plant Pathology*, **33**: 236-239.

Sunder, A. R. and Vidhyasekaran, P. (2003). Differential induction of phenylpropanoid metabolites in suspension cultured cells of sugarcane by fungal elicitors. *Acta Phytopathologica et Entomologica Hungarica*, **38**: 29-42.

Takahashi, M. and Asada, K. (1983) Superoxide anion permeability of phospholipid membranes and chloroplast thylakoids. *Arch Biochem Biophys*, **226:** 558-566.

Tamari, K., Ogasawara, N. and Kaji, J.(1963). Biochemical products of the metabolism of *Pyricularia oryzae*. In: The Rice blast disease. The Johns Hopkins Press, Baltimore, pp: 35-68.

Temple, S. J., Vance, C. P. and Gantt, J. S. (1998). Glutamate synthase and nitrogen assimilation. *Trends in Plant Sciences*, **3**: 51–56.

Tomiyama, K. (1963). Physiology and Biochemistry of disease resistance of plants. *Annual Review of Phytopathology*, **1**: 295-324.

Tseng, T. C. and Bateman, D. F. (1969). A phosphatidase produced by *Sclerotium rolfsii*. *Phytopathology*, **59**: 359-363.

Tucker, S. L. and Talbot, N. J. (2001). Surface attachment and pre-penetration stage development by plant pathogenic fungi. *Annual Review of Phytopathology*, **39**: 385-417.

Uritani, I. And Stahmann, M.A. (1961). Changes in nitrogen metabolismin sweet potato with black rot. *Plant Physiology*, **36**: 770-782.

Vad, K., de Neergaard, E., Madriz-ordenena, K., Mikkelsen, J. D. and Collings, D. B. (1993). Accumulation of defense-related transcripts and cloning of a chitinase mRNA from pea leaves (*Pisum sativum* L.) inoculated with *Ascochyta pisi* Lib. *Plant Science*, **92**: 69-79.

Van Loon, L. C. (1998). Occurrence and properties of plant pathogenesis-related proteins.In: *Pathogenesis-related proteins in plants* (eds. Datta, S. K. and Mathukrishnan, S.). CRC Press, Boca Raton, pp. 1-19.

Vance, C. P., Sherwood, R. T. and Kirk, T. K. (1980). Lignification as a mechanism of disease resistance. *Annual Review of Phytopathology*, **81**: 259-288.

Velazhahan, R., Samiyappan, R. and Vidhyasekaran, P. (2000) Purification of an elicitor-inducible antifungal chitinase from suspension-cultured rice cells. *Phytoparasitica,* **28:** 131-139.

Vera-Estrella, R. Higgins, V. J. and Blumwold, E. (1994). Plant defense response to fungal pathogens. *Plant Physiology*, **106**: 97-102.

Vidhyasekaran, P. (1988a). Physiology of Desease Resistance in Plants. Volume I. CRC Press, Florida, pp. 149.

Vidhyasekaran, P. (1988b). Physiology of Desease Resistance in Plants. Volume I. CRC Press,

Vidhyasekaran, P. (1997). Fungal pathogenesis in plants and crops : molecular biology and host defense mechanisms. Marcel Dekker, New York. pp. 553.

Vidhyasekaran, P. (1998). Molecular biology of pathogenesis and induced systemic resistance. *Indian Phytopathology*, **51** (2): 111-120.

Vidhyasekaran, P., Velazhahan, R., Samiyappan, R., Ponmalar, R.T. and Muthukrishnan, S. (1996). Isolation of a 35 kDa chitinase from suspension cultured rice cells and its potential in the development of sheath blight – resistant transgenic rice plant. In : *Rice Genetics III* (Ed. G. Khush). International Rice Research Institute, Philippines. pp. 868-872.

Vierheilig, H., Alt, M., Neuhaus, J. M., Boller, T. and Wiemken, A. (1993). Colonization of transgenic *Nicotiana sylvestris* plants, expressing different forms of *Nicotiana tabacum* chitinase, by the root pathogen *Rhizoctonia salani* and by the mycorrhizal symbiont *Glomus mosseae*. *Molecular Plant-Microbe Interaction*, **6**: 261-264.

Vincent, J. B., Crowder, M. W. and Averill, B. A. (1992). Hydrolysis of phosphate monoesters: a biological problem with multiple chemical solusions. *Trends Biochem. Sci*, **17**: 105-110.

Vir, S. and Grewal, J.S. (1974). Physiologic specilization in Asochyta rabiei (pass.) Rab. The causal organism of gram blight. Indian Phytopathology, **27**: 355-360.

Vir, S. and Grewal, J.S. (1975). Changes in catalase activity of gram plant induced by *Ascochyta rabiei* infection. *Indian Phytopathology*, **28**: 223-225.

Von Lelyveld, L. J. and Brodrick, H. T. (1975a). Enzymic responses of avocado leaves to Phytophthora root rot. *Agroplantae*, 7: 13-16.

Walter M. H. (1992). Regulation of lignification in defence. In: *Plant gene research: genes involved in plant defence* (eds. Boller, T. and Meins, F.) Wien: Springer, 327–352.

Walter, M. H., Grima-Pettenati, J., Grand, C., Boudet, A.M., Lamb, C.J. (1988) Cinnamyl-alcohol dehydrogenase, a molecular marker specific for lignin synthesis: cDNA cloning and mRNA induction by fungal elicitor. Proceedings of National Academy of Science, USA **85**, 5546–5550.

Walters, D. R. and ayres, P. G. (1980). Effects of powdery mildew disease on uptake and metabolism of nitrogen by roots of infected barley. *Physiological Plant pathology*, **17**: 369-379.

Walters, D. R. and ayres, P. G. (1983). Changes in nitrogen utilization and enzyme activities associated with CO_2 exchanges in healthy leaves of powdery mildew-infected barley. *Physiological Plant Pathology*, **23**: 447-459.

Weber, D. J., Clare, B. and Stahmann, M. A. (1967). Enzymic changes associated with induced and natural resistance of sweet potato to *Ceratocystis fimbriata*. *Phytopathology*, **57**: 421-424.

Wei, D. L., Kohtaro, K., Shoji, U. and Hui, L. T. (1996). Purification and characterization of an extracellular β-glucosidase from the wood-grown fungus *Xylaria regalis*. *Current Microbiology*, **33**: 297- 301.

Weststeijn, E. A. (1976). Peroxidase activity in leaves of *Nicotiana tabacum* var. *Xanthi* nc. before and after infection with tobacco mosaic virus. *Physiological Plant Pathology*, **8**: 63-71.

Wojtaszek, P. (1997). Oxidative burst: an early plant response to pathogen infection. *Biochem. Journal*, **322**: 681- 692.

Yazdi T., Woodward, J. R. and Radford, A. (1990). The cellulase complex *of Neurospora crassa*: activity, stability and release. *Journal of General Microbiology*, **136**: 1313-1319.

Zhao, X., She, X., Du, Y. and Liang, X. (2007). Induction of antiviral resistance and stimulary effect by oligochitosan in tobacco. *Pesticide Biochemistry and Physiology*, **87**: 78-84.

Zhou, E., Kerong, W. and Jiayun, L. (1996). Comparisons of extra cellular enzyme activities and oxalate production among *Cryphonectric parasitica* isolates with different virulence levels. *Mycologia Sinica*, **15**: 182-187.

Zmrhal, Z., Scidlova, F. and Machackova, I. (1987). Histochemical IAA Oxidase localization in the shoot of wheat. *Biologia Plantarum*, **29**: 94-97.

Chapter 4

Secondary Metabolites in *H. brasiliensis* – *C. cassiicola* interaction

1. Introduction

The fungal pathogens regulate various metabolic pathways of the host during pathogenesis. Atleast in part this regulation is carried out through the action of fungal metabolites (enzymes and other low molecular weight compounds). Each metabolite affect specific metabolic reactions in the host. Physical barricades develop soon after the infection and prevent the subsequent advance of the parasite. The metabolic changes in plant tissues infected by parasites have been reported by many researchers (Malhotra, 1993; Egea *et al.*, 2001; De Ascensao and Dubery, 2003; Strange and Scott, 2005). The visible host reaction to an invading microorganism may not only be due to pathogen toxins but also due to a sequence of reactions in which host metabolites play a role (Mandal and Mitra, 2007). Metabolites like lignin, sugars, phenols, amino acids and proteins play a pivotal role in disease resistance.

2. Reviews

2.1 Carbohydrates

Sugars are inevitable for the growth of the fungi; hence the quantity and quality of sugars play an important role in disease resistance. Sugars, being the precursors for the synthesis of phenolics, phytoalexins and lignin, play a vital role in disease resistance. Based on the sugar requirements for success or failure in pathogenesis, plant diseases are classified as high or low sugar diseases (Horsfall and Diamond, 1957). Many investigations were carried out on carbohydrate metabolism in response to biotic stress and the results shere are wide variation in the host-pathogen interaction in relation to the sugar content in the host (Veermohan *et al.*, 1994; Kumar and Singh, 1996; Dagade, 2003).

Post infectional increase in total and non-reducing sugar was noticed in all pea cultivars except Bonneville on infection by *Erysiphe polygoni* in which there was a

decrease in total sugar content (Guleria *et al.*, 1997). They also found reduction in reducing sugar content in the leaves of both resistant and susceptible clones. Dagade (2003) reported significant reduction in quantity of sugars in *Piper colubrinum* and *P. nigrum* on inoculation with *Phytophthora capsici*. Significant depletion of non-reducing sugars was also observed in inoculated plant parts.

Decreased reducing, non-reducing and total sugars and starch content in *Capsicum* leaves inoculated with *Alternaria solani* was reported by Veermohan *et al.* (1994).

Kaur and Dhillon (1989) observed decrease in sugar content in all the groundnut varieties infected with *Cercosporidium personatum*. Aulakh and Sandhu (1970) also reported quantitative and qualitative decrease in carbohydrates due to tikka disease incidence. Oke (1988) observed that the sugar contents were greatly reduced in *Corynespora* infected leaves of tobacco. Biochemical changes in groundnut leaves due to infection by early and late leaf spot pathogens were studied by Sindhan and Parashar (1996) who reported reduction in quantity of reducing, non-reducing and total sugars in healthy and infected leaves of resistant cultivars. Prasad *et al.* (1997) observed a gradual decrease in total starch and total reducing sugar due to downy mildew disease on *Lathyrus sativus*. Sharma *et al.* (1993) reported a progressive decrease in the amount of reducing, non-reducing and total sugars in brinjal following inoculation with *Phomopsis vexans*.

Vidhyasekaran (1974) studied the possible role of sugars in restriction of lesion development in finger millet infected by the weak pathogen *Helminthosporium tetramera*. Lesions did not enlarge beyond the third day after inoculation, and infected leaves were found to contain a higher concentration of soluble sugars indicating that these sugars may restrict the development of the disease. Sugar concentrations of leaves were highest in plants exposed to continuous light and infection in such plants was very mild. The most severe infection was found in plants kept in continuous darkness.

Attempts have been made to control diseases by altering sugar content of leaves. Naidu *et al.* (1979) showed that calcium ammonium nitrate application increased the sugar content and controlled bacterial leaf blight in rice. Horton and Keen (1966) reported that when glucose or maleic hydrazide was sprayed on onion, *Pyrenochaeta terrestris* induced onion pink root symptom development was greatly decreased.

2.2 Phenols

Alterations of phenols metabolism following infection have been observed in many plant diseases (Parr *et al.*, 1996; Saharan *et al.*, 1999; De Ascensao and Dubery, 2003). The accumulations of oxidized phenolic compounds in plants, which are toxic to certain pathogens, are observed to play a key role in defence reactions (Spanos *et al.*, 1997; Nandagopal and Kulkarni, 1998; Ejechi *et al.*, 1999; Borua and Das, 2000). Chopra *et al.* (1974) found higher polyphenolic constituents in the resistant variety of watermelon against *Alternaria*. They observed nine phenolic compounds in the resistant and six in the susceptible variety and concluded that phenolic compounds alone or in combination with amino acids are involved in the resistance mechanism.

Hammerschmidt and Nicholson (1977) studied the resistance of corn to *Colletotrichum graminicola* with respect to phenol metabolism and lesion development in susceptible, resistant and hypersensitive-resistant inbred varieties. They found that at 42 hours after inoculation the soluble phenols in the resistant and hypersensitive-resistant varieties had increased by approximately 74 and 110 per cent respectively, as compared to no detectable increase in the susceptible inbred varieties. Chattopadhyay and Bera (1980) examined the quantitative changes in phenol content of rice leaves infected with *Helminhosporium oryzae* and reported an initial increase up to 72 hours and a decrease thereafter in resistant cultivars, but in susceptible plants phenol content increased up to 120 hours after infection.

Harms and Terbea (1984) noticed an accumulation of phenolic compounds both in resistant and susceptible isogenic lines of barley and wheat seedlings infected with *Puccinia graminis*. *Fusarium* infected sugarcanes exhibited a marked increase in phenol

content (Kumar *et al.*, 2000). However, the quantum of their accumulation varied according to the level of virulence of isolates and the degree of resistance of sugarcane varieties. Singh *et al.* (2002) studied the total phenolic content in seven tomato accessions consisting of five resistant cultivars and two susceptible ones and observed high phenol contents in *Fusarium* resistant cultivars than the susceptible. According to Prats-Perez (2000) coumarin synthesis of sunflower plant induced in response to adverse environment condition, both biotic and abiotic. It was considered to be part of the defence strategy against microorganisms, insects and parasitic plants.

Saharan *et al.* (1999) suggested the role of phenols and oxidative enzymes particularly peroxidase in defence against Alternaria blight disease of cluster bean. Total phenols increased in all the varieties with the advancement of crop age while ortho-dihydric phenols and flavanols decreased. Saharan, *et al.* (2001) reported higher total phenolics in susceptible cluster bean cultivars with the increase in Alternaria blight severity. They also observed that ortho-dihydroxy phenols increased with the increase in disease severity up to 25 per cent and with further increase in disease severity, these phenols started to decline.

Mahajan (1999) evaluated total phenols in four susceptible and 11 resistant lines of cauliflower at five growth stages in relation to downy mildew and reported a negative correlation of disease incidence with total phenol. Nandagopal and Kulkarni (1998) conducted studies on biochemical changes in resistant and susceptible varieties of durum wheat against *Exserohilum hawaiiensis* and reported increased levels of phenolics in resistant varieties.

In a number of plant-pathogen interaction, a reduction in phenolic content was noticed. Oke (1988) reported that the phenol content showed a decreasing trend in tobacco leaves infected with *C. cassiicola*. Gogoi *et al.* (2001) studied the effect of the highly aggressive isolate KB-2 of the Karnal bunt pathogen (*Neovossia indica*) on phenol metabolism and reported that in the resistant durum wheat line, total phenols did not change for six days, but declined significantly at 10 days after infection.

2.3 Total free amino acids

Amino acids are important in plant resistance development and various changes in their levels have been shown in diseased plants (Paul, 1998; Sharma and Kaul, 1999; Dagade, 2003). Resistance to soybean stem canker was observed to increase with an increase in amino acid content. Relative quantity of amino acids was suggested to be partially responsible in determining relative resistance of tissues, particularly since balance of amino acids was important for growth of the pathogen *in vitro* (Chand and Walker, 1969).

Kanchaveli and Tsereteli (1972) reported the association of amino acid content with resistance of apple to *Venturia inaequalis*. Mitter *et al.* (1997) investigated the biochemical changes in chickpea genotypes resistant and susceptible to grey mould (*Botrytis cinerea*) and found that the sulphur containing amino acid content doubled in the resistant genotypes compared to the susceptible ones. Lupashku and Enaki (1998) observed an increase in free amino acid levels after the infection of triticale genotypes by *Fusarium* spp causing wilt.

Total free amino acid content was maximum in healthy matured fruits and it decreased about 50 per cent in *Aspergillus* infected bael fruits (Verma *et al.,* 1991). Sharma *et al.* (1992) found that the healthy leaves of resistant maize cultivars had significantly higher quantity of free amino acid, but it reduced in leaves infected with *Exserohilum turcicum* in all the cultivars and the reduction rate was more for susceptible than for resistant ones. Biochemical studies on susceptible, tolerant and immune genotypes of blackpepper to *Phytophthora capsici* revealed increased total free amino acid content in Panniyur 1 which is tolerant (Dagade, 2003).

Sharma and Kaul (1999) extensively studied the biochemical nature of resistance in apple to *Venturia inaequalis* causing scab and reported higher amino acids content in the young expanding leaves of resistant cultivars than in the susceptible ones. Amino acid content declined after inoculation and the decrease was more prominent in the

incompatible combinations. Kaul and Munjal (1980) reported a decrease in the free amino acids in apples on account of rotting caused by fungal pathogens.

2.4 Total protein

Increase in total protein content of plants on infection is observed to be associated with their resistance. Behrozin and Tehrani (1997) studied the total protein changes in the first leaves of two wheat cultivars infected with *Puccinia striiformis* and observed maximum increase in the total protein content in the resistant cultivars 72 hours after inoculation. Such an increase was noticed after 100 hours after inoculation in susceptible cultivars. A final reduction was also observed in the total protein content after 144 hours after infection in both the resistant and susceptible cultivars. A progressive increase in protein content of healthy and inoculated tomato plants was noticed with the increase in plant age (Sutha *et al.*, 1998).

Hoj *et al.* (1989) noticed that after infection of *Erysiphe graminis*, total proteins accumulated to high concentrations in resistant plants but not in the susceptible barley.

Ushamalini *et al.* (1998) noticed a reduction in protein content in cowpea infected with seed borne fungi. (Amadioha (1999) described a reduction in total protein contents in potato tubers during pathogenesis by *Rhizoctonia bataticola*. Hosagoudar *et al.* (1997) investigated the biochemical changes in the leaves of ebony tree affected with black mildew caused by *Meliola diospyri* and pointed out a reduction in total protein content in the infected leaves. Potpour *et al.* (2000) showed that the total protein content increased in resistant and susceptible barley seedlings inoculated with *Erysiphe graminis* f. sp. *hordei* 12 hours after inoculation and reached maximum level 72 hours after inoculation. Biswas *et al.* (2002) studied the effect of foliar spray of *Chaetomium globosum* for biocontrol in wheat and reported high total protein content in the plant after the treatment.

3. Materials and methods

3.1 Total sugars

One hundred milligrams of sample were hydrolyzed by keeping it in a boiling water bath for three hours with 5 ml of 2.5 N-HCl and cooled to room temperature. It was neutralized with sodium carbonate, the volume made up to 100 ml and centrifuged. Aliquots of 0.5 and 1ml were taken for analysis from the supernatant collected. The volume was adjusted to 1 ml by adding distilled water and then 4 ml of anthrone reagent was added. The mixture was heated for eight minutes, cooled rapidly and the green to dark green colour read at 630 nm. Total sugars were calculated form the standard graph plotted with glucose standards (Hodge and Hofreiter, 1962).

3.2 Total reducing sugar

The sugars were extracted from 100mg of the sample with 80% hot ethanol twice (5ml at each time). The supernatant collected was evaporated by keeping it on a water bath at 80^0C. 10 ml of water was added to dissolve the sugars. 0.1 and 0.2 ml of aliquots were pipetted out to separate test tubes and the volume was adjusted to 2ml with distilled water. 1ml of alkaline copper tartrate reagent was added to each test tube. The tubes were placed in a boiling water bath for 10 min. 1ml of arsenomolybdate reagent was added to it after cooling the tubes. The final reaction mixtures volume was raised to 10 ml with water and the absorbance of blue colour was read at 620 nm after 10 min. The quantity of reducing sugars was calculated form the graph drawn from glucose standards (Nelson, 1944).

3.3 Total non-reducing sugars

The content of the non-reducing sugar fraction was calculated by subtracting the quantity of the reducing sugars from that of the total soluble carbohydrate of the corresponding tissues.

4.3.4 Total phenols

The leaf samples (1g) were ground with a pestle and mortar in 10ml of 80% ethanol. The homogenate was centrifuged at 10,000rpm for 20 min and the supernatant was saved. The extraction was done twice with the residue, pooled all the supernatant and evaporated to dryness. The residue obtained was dissolved in 5ml of distilled water.

Different aliquots (0.2 to 2ml) were pipetted out into test tubes and the volume was made up to 3ml with water. Folin-ciocalteau reagent (0.5ml) was added and kept for 3 min. Two ml of 20% Na_2CO_3 solution was added to each tube and the contents mixed thoroughly. The tubes were placed in boiling water bath for exactly 1 min, cooled and the absorbance measured at 650nm against the reagent blank. The standard curve was prepared by using graded concentrations of catechol and the total phenol content calculated based on it.

4.3.5 Total protein

Total proteins were estimated by employing Lowry's methods (Lowry *et al.*, 1951) Leaf extract was prepared by grinding 500 mg of sample with pestle and mortar in 5 to 10 ml of phosphate buffer. Supernatant collected after centrifugation was used for protein estimation. The extract sample (0.1 and 0.2 ml) were pipetted out in separate test tubes. Sterile distilled water was added to make the volume to 1ml. A tube with 1ml of sterile distilled water served as control. Alkaline copper solution (5ml) was added to each test tube including blank. The sample was mixed well and allowed to stand for 10 min and 0.5 ml of folin-ciocalteau reagent was added, mixed well and incubated at room temperature in the dark for 30 min. A blue colour was developed. The absorbance readings were taken at 660 nm. Total protein was calculated from a standard graph plotted from graded concentrations of bovine serum albumin.

4.3.6 Total free amino acids

Plant samples (500mg) were ground in a pestle and mortar. 10ml of 80% ethanol was added to the homogenate and centrifuged. The supernatant was collected and the extraction was done twice with the residue. All the supernatant was pooled and used for the quantitative estimation of total free amino acids.

Ninhydrin solution (1ml) was added to 1ml of the extract and the total volume was made up to 2ml with distilled water. The tubes were heated in a boiling water bath for 20 min, 5 ml of the diluent was added and mixed well with the contents. The purple colour developed was read after 15 min against a reagent blank, in a spectrophotometer, at 570nm.

4.4. Results

4.4.1 Metabolites

4.4.1.1. Total sugars

C. cassiicola infection led to the decrease in total sugar content in all the *H. brasiliensis* clones studied except PB 260, which showed a slight increase in sugar content during the early stages of pathogenesis (Table 4.1). In inoculated leaves of resistant clones, the alterations in total sugar content were almost negligible when compared with uninoculated leaves during the early stages of disease development. However, the level of total sugars was high in resistant clones and it increased with the increase in period of infection. For PB 260, there was not much variation in the level of total sugars between infected and healthy plants.

4.4.1.2. Reducing sugars

Increased reducing sugar content was observed in the leaves inoculated with the pathogen (Table 4.2). The quantity of reducing sugar was higher in resistant clones than the susceptible and the increase in level of reducing sugar attained peak at 96 hrs of infection. For RRII 105 a reduction in reducing sugar content was noticed after 72 hrs of infection. Lower levels of reducing sugars were noticed with healthy control plants.

infection. For RRII 105 a reduction in reducing sugar content was noticed after 72 hrs of infection. Lower levels of reducing sugars were noticed with healthy control plants.

4.1.3. Non-reducing sugars

A reduction in non-reducing sugar content was observed in all the *H. brasiliensis* clones inoculated with *C. cassiicola* (Table 4.3). On the contrary, higher non-reducing sugar accumulation was noticed in all the healthy control plants. In infected leaves, there was an increase in non-reducing sugar content as the period of infection increased. GT 1 and RRIM 600 had more non-reducing sugar content than RRII 105 and PB 260, however, the quantity was lower than that of the healthy controls.

Table 4.1 The level of activities of total sugar in diseased and healthy leaves from resistant and susceptible *H. brasiliensis* clones

Clone	Inoculated plants					Healthy controls				
	Sampling time (h)				Mean	Sampling time (h)				Mean
	24	48	72	96		24	48	72	96	
RRII 105	12.62	13.97	16.38	17.13	15.02	15.33	18.06	18.40	20.59	18.10
PB260	11.69	14.06	15.27	16.68	14.42	11.24	13.40	14.21	17.68	14.13
RRIM 600	14.33	15.22	18.25	21.86	17.41	16.74	18.53	22.49	26.20	20.99
GT 1	17.13	19.78	21.95	25.22	21.02	18.62	20.77	24.91	29.09	23.34
CD $P \leq 0.05$	0.89	0.93	0.99	1.24	-	0.87	0.97	0.89	0.92	-

CD $P \leq 0.05$ for clones = 0.98; periods = 0.98; interaction = 1.13

* Mean of three replications

Table 4.2 The level of activities of reducing sugar in diseased and healthy leaves from resistant and susceptible *H. brasiliensis* clones

Clone	Inoculated plants					Healthy controls				
	Sampling time (h)				Mean	Sampling time (h)				Mean
	24	48	72	96		24	48	72	96	
RRII 105	1.52	1.89	2.10	0.96	1.86	1.00	1.00	0.08	1.09	1. 04
PB260	1.99	2.18	2.66	2.68	2.37	1.24	1.26	1.45	1.40	1.33
RRIM 600	2.22	2.36	2.59	2.67	2.46	1.47	1.61	1.67	1.76	1.62
GT 1	2.37	2.48	2.99	3.12	2.74	1.46	1.56	1.58	1.66	1.56
CD $P \leq 0.05$	0.44	0.46	0.42	0.42	-	0.34	0.41	0.44	0.36	-

CD $P \leq 0.05$ for clones = 0.43; periods = 0.43; interaction = 0.49

* Mean of three replications

Table 4.3 The level of activities of non-reducing sugar in diseased and healthy leaves from resistant and susceptible *H. brasiliensis* clones

Clone	Inoculated plants					Healthy controls				
	Sampling time (h)				Mean	Sampling time (h)				Mean
	24	48	72	96		24	48	72	96	
RRII 105	1.10	2.08	14.28	15.21	13.16	14.33	17.06	17.32	19.50	17.05
PB260	9.70	11.88	12.61	14.00	12.04	10.00	12.14	12.76	16.28	12.79
RRIM 600	12.11	12.86	15.66	19.19	14.95	15.17	16.92	20.82	24.44	19.33
GT 1	14.76	17.30	18.96	22.10	18.28	17.16	19.21	23.33	27.43	21.78
CD $P \leq 0.05$	0.98	1.36	1.76	1.39	-	1.67	1.54	1.65	1.39	-

CD $P \leq 0.05$ for clones = 1.56; periods = 1.56; interaction = 1.85

* Mean of three replications

4.1.4. Total phenols

In all the *H. brasiliensis* clones, the quantity of total phenols increased continuously from 24 to 96 hours after infection (Table 4.4). Maximum increase was observed after 96 hours of infection in all the clones. The resistant clones showed more total phenols than the susceptible ones. Healthy controls of resistant clones had significantly high quantity of total phenols than the susceptible clones studied.

4.1.5. Total free amino acid

The total free amino acid content was more in susceptible healthy clones and it reduced after the infection with *C. cassiicola* (Table 4.5). Total free amino acid content was much lower in resistant clones and it remained so after infection. An initial decrease in quantity of total free amino acid was noticed in all the *H. brasiliensis* clones studied which marginally increased at later stages of infection, but remained lower than that of the healthy controls.

4.1.6. Total protein

During initial stages of disease, a rise in protein level was observed in the inoculated leaves of GT 1 and RRIM 600, which was followed by gradual decrease up to 96 hours after infection (Table 4.6). In susceptible *H. brasiliensis* clones, the protein content increased slowly from 24 to 96 hours after inoculation. Total protein quantity was higher at 96 hours of infection in susceptible clones. Maximum protein accumulation was noticed after 48 hrs of infection in resistant varieties which declined thereafter.

Table 4.4 The level of activities of total phenol in diseased and healthy leaves from resistant and susceptible *H. brasiliensis* clones

Clone	Inoculated plants					Healthy controls				
	Sampling time (h)				Mean	Sampling time (h)				Mean
	24	48	72	96		24	48	72	96	
RRII 105	7.96	8.17	8.96	10.12	8.80	4.28	4.16	4. 22	4.24	4.23
PB260	6.23	8.91	9.23	10.99	8.56	4.33	4.33	4.33	4.33	4.33
RRIM 600	8.91	12.36	13.77	15.16	12.55	5.40	5.06	5.08	5.20	5.19
GT 1	10.49	14.18	16.18	18.3	14.79	5.91	5.96	5.96	5.90	5.93
CD $P \leq 0.05$	0.97	0.99	1.45	1.67	-	0.93	0.89	0.93	0.93	-

CD $P \leq 0.05$ for clones = 1.00; periods = 1.00; interaction = 1.98

* Mean of three replications

Table 4.5 The level of activities of total free amino acid in diseased and healthy leaves from resistant and susceptible *H. brasiliensis* clones

Clone	Inoculated plants					Healthy controls				
	Sampling time (h)				Mean	Sampling time (h)				Mean
	24	48	72	96		24	48	72	96	
RRII 105	0.682	0.366	0.444	0.516	0.502	0.911	0.986	0.922	0.929	0.937
PB 260	0.491	0.334	0.363	0.319	0.377	0.928	0.922	0.962	0.962	0.944
RRIM 600	0.220	0.218	0.211	0.289	0.235	0.663	0.660	0.638	0.631	0.648
GT 1	0.320	0.182	0.162	0.210	0.219	0.443	0.412	0.410	0.416	0.42
CD $P \leq 0.05$	0.95	0.99	0.96	0.99	-	0.99	0.99	0.93	0.98	-

CD $P \leq 0.05$ for clones = 0.11; periods = 0.11; interaction = 0.18

* Mean of three replications

Table 4.6 The level of activities of total protein in diseased and healthy leaves from resistant and susceptible *H. brasiliensis* clones

Clone	Inoculated plants					Healthy controls				
	Sampling time (h)				Mean	Sampling time (h)				Mean
	24	48	72	96		24	48	72	96	
RRII 105	26.63	37.10	40.00	43.16	36.72	22.16	22.19	22.39	22.41	22.28
PB260	24.22	28.18	36.99	41.11	32.62	21.90	22.16	22.18	22.37	22.15
RRIM 600	34.47	37.16	37.20	28.18	34.25	20.11	21.24	21.11	20.98	20.86
GT 1	39.33	39.87	31.12	28.18	34.62	17.26	17.34	17.30	17.18	17.27
CD $P \leq 0.05$	2. 22	2.08	2.28	2.11	-	2.00	2.18	2.09	2.21	-

CD $P \leq 0.05$ for clones = 2.13; Periods = 2.13; Interaction = 2.44

* Mean of three replications

5. Discussion

5.1 Metabolites

5.1.1 Total sugars

The infection of *H. brasiliensis* leaves with *C. cassiicola* caused a decline in total sugars content after pathogenesis. Comparatively more total sugars present in resistant clones but lower than that of the healthy controls. The decrease in carbohydrate content in infected leaves indicated its utilization by the pathogen for its growth and reproduction as reported earlier by Padmanaban (1973). Oke (1988) also reported qualitative and quantitative decrease in total sugars due to the infection of tobacco leaves with *C. cassiicola.* According to Goodman *et al.* (1967), the carbohydrate in infected plants may be utilized for meeting the energy requirements of host plants due to increased respiration. The reduction of sugar content in infected leaves of resistant varieties can be because a major part of these sugars is shifted to polyphenol synthesis (Neish, 1964; Dagade, 2003).

5.1.2 Reducing sugars

C. cassiicola inoculation altered the level of reducing sugar content in all the *H. brasiliensis* clones studied. The present study revealed higher quantities of reducing sugars in both resistant and susceptible clones. Severe incidence of disease in plants having higher reducing sugars indicate that corynespora disease of rubber is a high sugar disease as suggested by Horsfall and Dimond (1957). In general, infection increased the reducing sugar content (Sankpal and Nimbalkar, 1979, Dhumal and Nimbalkar, 1982) of plants. Sindhan *et al.* (1996) reported reduction in reducing sugar content in flag smut resistant wheat varieties.

5.1.3 Non-reducing sugars

The level of non-reducing sugars decreased in all the inoculated leaves of *H. brasiliensis* clones studied. This post infectional reduction in non-reducing sugars might be due to the rapid hydrolysis of sugars during pathogenesis through the enzymes secreted by the pathogen (Jaypal and Mahadevan, 1968). A reduction in non-reducing sugars due to pathogenic invasion was observed by many workers in various crops like brinjal and wheat (Sharma *et al.*, 1993, Sindhan and Parashar, 1996). Veermohan *et al.*

(1994) reported lower levels of non-reducing sugars in capsicum leaves inoculated with *Alternaria solani*. Joseph (1998) reported a reduction in non-reducing sugar of fertilizer treated *C. cassiicola* infected *H. brasiliensis* plants. The present study conforms these findings.

5.1.4 Total protein

Considerable increase in protein content was noticed with the *C. cassiicola* infected *H. brasiliensis* clones. Vidhyasekaran *et al.* (1973) reported increase in protein contents in six varieties of rice due to *Helminthosporiose* infection and suggested that the increase might be due to co-polymerization of host protein and fungal protein (Vanderplank, 1983, Sharma and Kaul, 1999) or due to enhanced enzymatic activity. In resistant *H. brasiliensis* clones, an initial increase in protein content was noticed which may be due to their role in cell protection mechanisms as indicated by Von-Broembsonand Hadwiger (1972). The alterations in protein content in resistant *H. brasiliensis* are not as much as observed in susceptible clones at 96 hrs after infection. The present study is in conformity with the findings of Grzlinska (1969), Chattopadhyaya and Bera (1978, Chowdhury (1995) and Setty *et al.* (2001) indicating high post-infectional increase in protein level of susceptible varieties of crop plants in comparison to the resistant varieties.

5.1.5 Total free amino acid

Total free amino acid content was maximum in healthy leaves, which decreased on infection by *C. cassiicola*. This depletion in amino acid content may either be due to its utilization by the pathogen (Nema, 1991) or due to the synthesis of defence-related enzymes at the time of signal transduction (Sharma and Kaul, 1999). Kanchaveli and Tsereteli (1972) reported the association of higher amino acid contents with the resistance of apple to *Venturia inaequalis*. There are indications that amino acids might induce host resistance to fungal pathogens (Dekker, 1969; Mitter *et al.*, 1997; Sharma and Kaul, 1999).

Sharma *et al.* (1992) also reported a reduction in total free amino acid content in resistant and susceptible maize varieties infected with *Exserohilum turcicum*. Amino

acids are believed to be important in the plant resistance mechanisms (Kumar and Yadav, 2002). Chand and Walker (1969) reported increase in resistance to soybean stem canker with the increase in amino acid content.

The results of the present study indicate the involvement of free aminoacid present in the susceptible clones for the pathogenesis in Corynespora leaf disease of *H. brasiliensis.*

5.1.6 Total phenol

Angiosperm plants contain many kinds of phenolic compounds like monophenols, phenolic acids, pterocarpans, isoflavans, isoflavons, isoflavanones, glucosides of isoflavanoides and anthocyanidins (Vidhyasekaran, 1988). Phenolics play an important role in metabolic regulation of an infected plant. The presence of high concentration of phenolic compounds is considered to be one of the major factors of an incompatible host-pathogen interaction (Farkas and Kirlay, 1962).

It has been reported that the resistant cultivars of tomato contained more phenol when infected by *Fusarium* while the susceptible varieties recorded lower phenol content (Singh *et al.*, 2002).

In the present study, total phenolic compounds were observed to accumulate in all the clones after the infection and the quantity was higher in tolerant clones.The post infectional increase in phenolic contents could be due to several factors including enhancement of synthesis, translocation of phenolics to the site of infection and hydrolysis of phenolic glycosides by fungal glycosides to yield free phenols (Sharma *et al.*, 1983). The presence of high phenolic contents prior to inoculation and considerable increase after inoculation in resistant genotypes suggested a positive role of phenolic compounds in resistance of *Hevea* genotypes to Corynespora leaf fall disease. High phenolic contents create a toxic atmosphere in resistant clones and hence the fungus fails to establish and colonize on these clones. Phenols by their simple structure penetrate the microorganisms and cause considerable damage to the cell metabolisms (Kalaichelvan and Elangovan, 1995). The structure and function of OH group in the phenols is highly

interrelated and the positional effect of OH or COOH shows the toxicity (Heipieper *et al.*, 1991).

6. Bibliography

Amadioha, A. C. (1999). Changes in the biochemical composition of stored potato tubers during pathogenesis by *Rhizoctonia bataticola*. *Archives of Phytopathology and Plant Protection*, **32**: 151-159.

Aulakh, K. S. and Grower, R. K. (1970). Changes in free amino acids, carbohydrates and ascorbic acid contents of tomato fruits after infection with pathogenic fungi. *Indian Journal of Microbiology*, **10**: 61-64.

Behrozin, M. and Tehrani, A. S. (1997). Study of the total protein changes on the first leaves of two wheat cultivars infected with *Puccinia stiiformis*. *Applied Entomology and Phytopathology*, **66**: 4-5.

Biswas, S. K., Srivastava, K. D., Biswas, D. R. and Aggarwal, R. (2002). Effect of foliar spray of *Chaetomium globosum* on total protein, nitrogen and carbon contents of wheat. *Annals of Plant Protection Sciences*, **10**: 76-79.

Borua, I. and Das, P. (2000). Changes in activities of polyphenol oxidase, acid phosphatase and phenol content in developing chilli varieties susceptible and resistant to *Colletotrichum capsici*. *Crop Research*, **19**: 230-234.

Chand, J. N. and Walker, J. C. (1969). Relation of free amino acids in cucumber leaves to the development of angular leaf spot. *Phytopathologische Zeitschrift*, **64**: 94-97.

Chattopadyay, S. B. and Bera, A. K. (1980). Phenols and polyphenol oxidase activity in rice leaves infected with *Helminthosporium oryzae*. *Phytopathologische Zeitschrift*, **98**: 59-63.

Chopra, B. L., Jhooty, J. S. and Bajaj, K. L. (1974). Biochemical differences between 2 varieties of water melon resistant and susceptible to *Alternaria cucumeriana*. *Zeitschrift*, **79**: 47-52.

Chowdhury, A. R. (1995). Biochemical changes associated with induction of resistance in groundnut plants to *Puccinia arachidis* by seed treatment with non-

conventional chemicals. *Indian Journal of Mycology and Plant Pathology*, **25**: 231-234.

De Ascensao, A. R. C. F. and Dubery, I. A. (2003). Soluble and wall-bound phenolics and phenolic polymers in *Musa acuminata* roots exposed to elicitors from *Fusarium oxysporum* f. sp. *cubense*. *Phytochemistry*, **63**: 679-686.

Degade, S. B. (2003). Biochemical studies on *Phytophthora capsici* inoculated and uninoculated plants of *Piper* spp. *The Madras Agricultural Journal*, **90**: 697-701.

Dekker, J. (1969). L-methionine induced inhibition of powdery mildew and its reversal by folic acid. *Netherlands Journal of Plant Pathology*, **75**: 182-185.

Dhumal, K. N. and Nimbalkar, J. D. (1982). Physiological studies on grassy shoot disease infected sugarcane cultivars. *Indian Phytopathology*, **35**: 341-343.

Egea, C., Ahmed, A. S., Candela, M. and Candela, M. E. (2001). Elicitation of peroxidase activity and lignin biosynthesis in pepper suspension cells by *Phytophthora capsici*. *Journal of Plant Physiology*, **158**: 151-158.

Ejechi, B. O., Nwafor, O. E. and Okoko, F. J. (1999). Growth inhibition of tomato-rot fungi by phenolic acids and essential oil extracts of pepper fruits (*Dennettia tripetala*). *Food Research International*, **32**: 395-399.

Farkas, G. L., Kiraly, Z. and Solymosy, F. (1962). Role of phenolic compounds in the physiology of plant disease and plant disease resistance. *Phytopathologische Zeitschrift*, **44**: 105-150.

Gogoi, R., Singh, D. V. and Srivastava, K. D. (2000). Changing behaviour of defence related enzymes in wheat during infection of *Neovossia indica*. *Indian Phytopathology*, **53**: 153-156.

Goodman, R.N., Kiraly, Z. and Zaitlin, M. (1967). The biochemistry and physiology of infectious plant diseases. D. Van Nostrand Co., Princeton, New jersey, pp: 354.

Grzelinska, A. (1969). Changes in protein level and activities of several enzymes in susceptible and resistant tomato plants after infection by *Fusarium oxysporum* f. *lycopersici* (Sacc.) Synder et Hanson. *Phytopathologische Zeitschrift*, **66**: 374-380.

Guleria, S., Paul, B. and Bajaj, K. L. (1997). Biochemical changes in powdery mildew (*Erysiphe polygoni* DC.) resistant and susceptible cultivars of pea (*Pisum sativum*). *Plant Disease Research*, **12**: 185-188.

Hammerschmidt, R. and Nicholson, R. L. (1977). Resistance of maize to anthracnose: changes in host phenols and pigments. *Phytopathology*, **67**: 251-258.

Harms, H. and Terbea, M. (1984). Metabolism of phenolic compounds in healthy and brown rust-infected barley and wheat varieties. *Phytopathologische Zeitschrift*, **111**: 283-296.

Heipieper, H. J., Keweloh, H. and Rehm, H. J. (1991). Influence of phenols on growth and membrane permeability of free and immobilized *Eschericia coli*. *Applied Environmental Microbiology*, **57**: 1213-1217.

Hodge, J.E. and Hofreiter, B.T. (1962). In: Methods in carbohydrate chemistry (Eds. Whistler, R.L. and Be Miller, J.N.). Academic Press, New York.

Hoj, P. B., Hartman, D. J., Morrice, N. A., Doan, D. N. P. and Fincher, G. B. (1989). Purification of 1,3-β-glucan endohydrolase enzyme II from germinated barley and determination of the primary structure from cDNA clone. *Plant Molecular Biology*, **13**: 31-42.

Horsfall, J.G. and Dimond, A.E. (1957). Interactions of tissue sugar, growth substances and disease susceptibility. *Z. Pflanzenkrankh Pflanzenchute*, **64**: 415-421.

Horton, J. C. and Keen, N. T. (1966). Sugar repression of endopolygalacturonase and cellulose synthesis during pathogenesis by *Pyrenochaeta terrestris* as a resistance mechanism in onion pink root. *Phytopathology*, **56**: 908.

Hosagoudar, V. B., Abraham, T. K., Krishnan, P. N. and Vijayakumar, K. (1997). Biochemical changes in the leaves of ebony tree affected with black mildew. *Indian Phytopathology*, **50**: 439-440.

Jaypal, R. and Mahadevan, A. (1968). Biochemical changes in banana leaves in response to leaf spot pathogenesis. *Indian Phypathology*, **21**: 43-48.

Joseph, A. (1998). Investigation on certain biochemical changes and phyllosphere microflora of *Hevea brasiliensis* as influenced by nitrogenous fertilizer application and *Corynespora cassiicola* inoculation. Ph.D. Thesis, Mahatma Gandhi University, Kottayam, India.

Kalaichelvan, P. T. and Elangovan, N. (1995). Effect of phenolics on *Drechslera oryzae*. *Indian Phytopathology*, **48**: 271-274.

Kanchaveli, L. A. and Tsereteli, G. L. (1972). Investigation on the resistance of apple varieties to *Venturia inaequalis* (Cook) Wint. and the determination of anatomical and biochemical indicators of resistance. *Tr. Inst. Zashch. Rast. Gruz. SSR*, **23**: 304-311.

Kanchaveli, L. A. and Tsereteli, G. L. (1972). Investigation on the resistance of apple varieties to *Venturia inaequalis* (Cook) Wint. and the determination of anatomical and biochemical indicators of resistance. *Tr. Inst. Zashch. Rast. Gruz. SSR*. **23**: 304-311.

Kaul, J. L. and Munjal, R. L. (1980). Post-infection biochemical changes in apple fruits due to rot causing fungal pathogens. *Gartenbauwissenschaft*, **45**: 185-187.

Kaur, J. and Dhillon, M. (1989). Biochemical alterations in groundnut (*Arachis hypogaea.*) leaf induced by *Cercosporidium personatum* (Berk. And Curt.) Deighton. *Indian Journal of Mycology and Plant Pathology*, **19**: 151-156.

Kumar, B. and Yadav, B. P. (2002). Studies on metabolic changes like chlorophyll, sugars and amino acids contents induced by the virus in cucumber leaf. *Annals of Biology*, **18**: 147-151.

Kumar, R. and Singh, S. B. (1996). Changes in biochemical constituents of sunflower leaves in relation to Alternaria blight development. *Indian Journal of Mycology and Plant Pathology*, **26**: 234-236.

Kumar, S., Kumar, B. and Kumar, V. (2000). Changes in phenolic and amino acid contents of sugar cane induced by isolates of red rot pathogen. *Annals of Agricultural and Biological Research,* **5**: 75-78.

Lowry, O.H., rosebrough, N.J., farr, A.C. and Randall, P.J. (1951). Protein measurement with the folin- phenol reagent. *Journal of Biological Chemistry*. **193**: 265-277.

Lupashku, G. A. and Enaki, V. V. (1998). The role of free amino acids in triticale genotypes infected by fusarial wilt. *Rusian Agricultural Science*, **5**: 16-21.

Mahajan, V. (1999). Relationship of phenols and sugars in downy mildew resistance of cauliflower. *Journal of Maharashtra Agricultural Universities*, **24**: 97-99.

Malhotra, S. K. (1993). Biochemical components of tomato genotypes in relation to Fusarium wilt. *Indian Journal of Mycology and Plant Pathology*, **23**: 302-304.

Mandal, S. and Mitra, A. (2007). Reinforcement of cell wall in roots of *Lycopersicon esculentum* through induction of phenolic compounds and lignin by elicitors. *Physiological and Molecular Plant Pathology*, **71**: 201-209.

Mitter, N. Grewal, J. S. and Pal, M. (1997). Biochemical changes in Chickpea genotypes resistant and susceptible to grey mould. *Indian Phytopathology*, **50**: 490-498.

Naidu, D., Rao, S. and Rao, S. C. (1979). Effect of nitrogen nutrition and bacterial leaf blight of rice leaves. *Phytopathologische Zeitschrift*, **96**: 83-86.

Nandagopal, N. and Kulkarni, S. (1998). Studies on biochemical changes in resistant and susceptible varieties of durum wheat against *Exserohilum hawaiiensis* (Bugnicourt) Subram and Jain ex. M. B. Ellis- a causal agent of leaf blight. *Karnataka Journal of Agricultural Sciences*, **11**: 83-89.

Neish, A.C. (1964). Major pathways of biosynthesis of phenols. In: *Biochemistry of phenolic compounds*. (Ed. E.B. Harborne). Academic Press, New York, pp. 245-359.

Nelson, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. *Journal of Biological chemistry*, **153**: 375-380.

Nema, A. G. (1991). Changes in chlorophyll, nitrogen, protein, amino acid and some enzyme contents in betelvine leaves infected with *Xanthomonas campestris* pv *betlicola. Indian Phytopathology*, **44**: 9-14.

Oke, O. A. (1988). Changes in chemical constituents of tobacco leaves infected with *Corynespora cassiicola* and *Colletotrichum nicotianae*. *Phytopathologische Zeitschrift*, **122**: 181-185.

Padmanaban, P. (1973). Studies on Alternaria leaf spot disease of cotton caused by *Alternaria macrospora* Zimm. M.Sc. (Agriculture) thesis, Tamil Nadu Agricultural university, India.

Parr, A. J., Waldron, K. W., Ng, A. and Parker, M. L. (1996). The wall-bound phenolics of Chinese water chestnut (*Eleocharis dulcis*). *Journal of Science and Food Agriculture*, **71**: 501-507.

Paul, S. T. (1998). Biochemical and biological bases of resistance in solanaceous vegetables against bacterial wilt incited by *Ralstonia solanacearum*. *Ph. D. (Agriculture) Thesis*, Kerala Agricultural Univrersity, Thrissur, Kerala, p. 269.

Potpour, M., Mohammadi, M., Torabi, M. and Shariftehrani, A. (2000). Peroxidase specific activity pattern in resistant and susceptible barley seedlings inoculated with *Erysiphe graminis* f.sp. *hordei*, the causal agent of powdery mildew disease. *Iranian Journal of Agricultural Sciences*, **31**: 415-426.

Prasad, B. K., Sinha, N. P., Singh, S. P., Dayal, S., Kumar, S., Jallaludin and Prabladprasad. (1997). Changes in carbohydrate in khesari (*Lathyrus sativus*) due to downy mildew disease. *Indian Phytopathology*, **50**: 362-364.

Prats-Perez, E., Bazzalo, M., Leon, A. and Jorrin-Novo, J. (2000). Accumulation of soluble phenolic compounds in sunflower capitula correlates with tolerance to *Sclerotinia sclerotiorum*. In: *Proceedings of 15th International Sunflower Conference*, June 12-15, Toulouse, France, **2**: 35-41.

Saharan, M. S., Saharan, G. S. and Joshi, U. N. (1999). Phenolic compounds and oxidative enzymes in healthy and Alternaria blight infected leaves of clusterbean. *Acta Phytopathologica et Entomologica Hungarica*, **34**: 299-306.

Saharan, M. S., Saharan, G. S., Gupta, P. P. and Joshi, U. N. (2001). Phenolic compounds and oxidative enzymes in cluster bean leaves in relation to Alternaria blight Severity. *Acta Phytopathologica et Entomologica Hungarica*, **34**: 299-306.

Sankpal, S. D. and Nimbalkar, J. D. (1979). Effect of smut infection on sucrose metabolism in sugar mechanism. *Indian Phytopathology*, **32**: 306-307.

Setty, T. A. S., Kumar, T. B. A., Gowda, K. T. P., Hattappa, S., Ramaswamy, G. R. and Prasad, N. (2001). Biochemical changes due to *Peronosclerospora sorgi* infection in resistant and susceptible maize genotypes. *Environment and Ecology*, **19**: 751-755.

Sharma, J. P., Mishra, B. and Jha, K. (1992). Biochemical relationship in resistant and susceptible cultivars with turcicum leaf blight disease in maize. *Indian Phytopathology*, **45**: 241-243.

Sharma, J.N. and Kaul, J.L. (1999). Biochemical nature of resistance in apple to *Venturia inaequalis* causing scab III. Proteins and amino acids. *Journal of Mycology and Plant Pathology*, **29** (2): 227-231.

Sharma, O. P., Sugha, S. K., Dev, K. and Banyal, D. (1993). Biochemical alterations in brinjal leaves and fruits due to infection by *Phomopsis vexans*. *Indian Journal of Mycology and Plant Pathology*, **23**: 318-319.

Sharma, S. G., Narayan, R., Lal, S. and Chaturvedi, C. (1983). Role of phenolic compounds in resistance of maize to leaf blight caused by *Drechslera* state of *Cochliobolus heterostrophus*. *Indian Phytopathology*,**36**: 43-46.

Sindhan, G. S. and Parashar, R. D. (1996). Biochemical changes in ground nut leaves due to infection by early and late leaf spot pathogen. *Indian Journal of Mycology and Plant Pathology*, **26**:210-212.

Sindhan, G. S., Parashar, R. D. and Hooda, I. (1996). Relationship between biochemical parameters and flag smut resistance in wheat. *Indian Journal of Mycology and Plant Pathology*, **26**: 291-293.

Singh, J., Banerjee, M. K., Chakraborty, S. and Kalloo, G. (2002). Role of phenolics and peroxidase in resistance to Fusarium wilt in tomato (*Lycopersicon esculentum* Mill.). *Annals of Agri Bio Research,* **7**: 41-46.

Spanos, K. A., Pirrie, A. and Woodward, S. (1997). *In vitro* expression of resistance responses to *Secridium* species in micropropagated shoots of *Cupressus sempervirens* and *Chamaecyparis lawsoniana*. *Canadian Journal of Botany*, **75**: 1103-1109.

Strange, R. N. and Scott, P. R. (2005). Plant disease: a threat to global food security. *Annual Review of Phytopathology*, **43**: 83-116.

Sutha, R., Ramiah, M. and Rajappan, K. (1998). Changes in protein and amino acid composition of tomato due to topovirus infection. *Indian Phytopathology*, **51**: 136-139.

Ushamalini, C., Rajappan, K. and Gangadharan, K. (1998). Changes in the biochemical constituents of cowpea due to seed borne fungi, Indian *Phytopathology*, **51**: 258-260.

Vanderplank, J.E. (1983). Genetic and Molecular Basis of Plant Pathogenesis, New York, pp: 349.

Veermohan, C., Govindarajlu, T. and Ramassamy, V. (1994). Biochemical and physiological changes in chilli leaves inoculated with *Alternaria solani*. *Advances in Plant Science*, **7**: 29-34.

Verma, S., Gupta, S., Singh, R. V. and Abidi, A. B. (1991). Changes in biochemical constituents of bael fruits infected with *Aspergillus* species. *Indian Phytopathology*, **50**: 362-364.

Vidhyasekaran, P. (1974). Possible role of sugars in restriction of lesion development in fingermillet leaves infected with *Helminthosporium tetramera*. *Physiological Plant Pathology*, **4**: 555-565.

Vidhyasekaran, P. (1988). Physiology of disease resistance in plants. Vol I & II. CRC Press Inc. Florida.

Von- Broembsen, S. L. and Hadwiger, L. A. (1972). Characterization of disease resistance responses in certain gene- for- gene interaction between flax and *Melampsora* Lini. *Physiological Plant Pathology*, **2**: 207-215.