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BIOCHEMISTRY OF HOST PARASITE INTERACTION – *CUSCUTA CHINENSIS* LAM. ON *CHROMOLAENA ODORATA* (L) KING & H. E ROBINS. A FUNDAMENTAL APPROACH

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Abstract: The genus *Cuscuta* (Dodders, Convolvulaceae) is one of the most significant lineages of parasitic plants from economic, conservation, and anthropogenic perspectives. The present study was undertaken to evaluate the impact of *Cuscuta chinensis* in the invasive species *Chromolaena odorata*. The anatomy of haustoria and biochemical changes in the host by the holoparasitic angiosperm *Cuscuta chinensis* parasitizing different tissues of *C. odoratum* were studied under light microscope. *C. chinensis* invasion in host tissue preferentially enhanced the activities of superoxide dismutase (SOD) and guaiacol peroxidase (POX) whereas marginally the catalase (CAT) activity. The biotic stress in the plant induces the formation of reactive oxygen species such as hydrogen peroxide and was effectively scavenged by the enzyme POX. The significant activity of SOD inturn supports the H_2O_2 formation in the host tissue. The low profile of phenol also supports the profile of PPO. The significant level of PPO activity suggests its functions as an anti-nutritive defense against parasites by producing browning in the infected tissues of the host. Increased concentration of total soluble protein and proline in the infected host suggest its defense against the parasite. The IR spectral analysis also suggests the presence of different functional group in the infected species compared to control revealing their active phase in secondary metabolite synthesis. The data was strongly supported by the activity profile of Phenylalanine ammonia lyase. Chlorophyll content showed a marginal decrease in the infected host compared to control. Free amino acids also showed a significant profile in the host plant. The results of the present study related with the tolerance shown by the host suggest the non compatible relationship between the parasites on the host plant.

Key words: Oxidative stress, Antioxidant machinery, Reactive oxygen species, Protein, Flavonoid, Phenylalanine ammonia lyase, Parasite.

INTRODUCTION

Cuscuta (Dodder) is a genus found throughout the temperate to tropical regions of the world, with the greatest species diversity in subtropical and tropical regions. Dodder infestations reduce crop yield and increase harvesting costs. The damage of dodder to the host plant varies from moderate to severe depending on the growth of the host plant and on the number of haustoria attachments to the host plant. Dodder management is only achieved using combined preventive, cultural, mechanical and chemical methods that aim at control of existing populations prior to seed production and control of subsequent seedlings. Fields with dodder history need to be monitored frequently, and new dodder plants must be removed as soon as possible.

Parasitic plants have direct consequences on their hosts. *Cuscuta chinensis*, for instance, been proved for the holoparasite along open land in Kerala. Here, the parasite prefers the competitively dominant species. In order to place the knowledge gained from these studies into a broader context, there is need for biochemical studies of parasitic plants in the communities in which they naturally occur. The objectives of the present investigation includes study of changes in host (*Chromolaena odorata)* infected by the parasite (*Cuscuta chinensis*) in comparison with the control non infected (*Chromolaena odorata).*

MATERIALS AND METHODS

Plant Material

Cuscuta chinensis is the parasite and *Chromolaena odorata* the host plant (Fig. 1 a & b). The results are compared with the non infected *C. odorat*a as control.

Fig. 1. a - *Chromolaena* - Plant not infected with *Cuscuta* (Control); b - *Chromolaena* - Infected Plant

Relative Water Content

Leaf RWC was determined and calculated using the formula:

RWC = [(FW-DW)/(TW-DW)]x100

Where FW is fresh weight, DW is dry weight and TW is turgid weight.

Morphological analysis

Fertile aerial parts of plants were prepared according to usual techniques for herbarium material conservation (Bridson and Forman, 1992). The measurements were made with a scale and the observations were done under stereoscopic microscopic Olympus SZH, coupled to an Olympus C-35AS-4 camera.

Anatomical analysis

To analyze the trichomes distribution, leaves and petioles were embedded in historesin and transversely sectioned in a rotary microtome with a C steal knife (Leica). The transverse sections were stained in 0.05% toluidin blue O in acetate buffer (pH 4.4) (Feder and Brien, 1968) for 5 min. Trichomes were isolated, clarified with NaOCl2% diluted in water 1:8 (v:v), washed in water and stained in safranin 1% in ethanol 50% (Johansen 1940) and observed at covered glass sheet in an Olympus BH2 microscope coupled to an Olympus C-35AD4 camera.

Analytical

Total phenol content of leaves was estimated by the method of Mayr *et al.* (1995). The soluble proteins were estimated by using the method of Lowry *et al.* (1951). Sugar content of leaves was estimated by the method of Miller (1972). Aluminium chloride colorimetric technique was used for flavonoid estimation (Siddique *et al.*, 2010). Total chlorophyll was estimated by the methods of Vicas *et al.* (2010). Carotenoids also estimated by the method of Vicas *et al.* (2010). Total free amino acids were determined using the method of Moore and Stein (1948)

IR spectroscopy

IR spectroscopy: The leaves of each experimental condition (approximately 3-4 cm) taken from plants were pooled as one sample. The samples were immediately dried in an oven for 2 days at 60°C. Tablets for FTIR spectroscopy were prepared in an agate mortars, by mixing leaves powder (2 mg) with KBr (1:100 p/p). The absorbance spectra were measured between 300 and 4500/cm. At least three spectra were obtained for each sample (Anilkumar *et al.*, 2012).

Biochemical

Isolation of PAL was made following the method of Morrison *et al.* (1994). The activity of PAL was estimated by the method of Whetten and Sederoff (1992). Peroxidase was isolated and assayed following the method of Goliber (1989). Peroxidase activity was assayed using guaiacol as substrate (Ingham *et al.*, 1998). Catalase was isolated and assayed as per the method of Swapna (2003). α-amylase activity was carried out by a modification of procedures described

by Rinderknecht *et al.* (1967) and Wahlefeld (1974). The ascorbate peroxidase activity determination was carried out according to the modified methods of Yanagida *et al.* (1999). The SOD was extracted and assayed following the method of Fridovich (1997). Polyphenol oxidase (PPO) activity was determined as per the procedure given by Mayer *et al.* (1965).

Statistical Analysis

The data was statistically evaluated by one way ANOVA and t-test. The results are average of 6 replications and are represented as mean ± SD.

RESULTS AND DISCUSSION

Parasitic invasion in host leads to an altered metabolism involves a number of complex morphological, biochemical and physiological changes.

Haustorial penetration into host tissue

Haustorial development is initiated from epidermal and cortical parenchyma cells just external to the pericycle. The cells divide anticlinally and periclinally to form a group of cells. These differentiating cells have conspicuous nuclei, densely stained cytoplasm and abundant starch grains. The Protuberance shows polarity (Fig. 2a & b).

Leaf relative water content (RWC)

Leaves of host show high RWC during infection (89.7%) compared to control (78%). The high RWC of the leaf can be explained by the higher rate of absorption of water with low transpi-ration rate (Kramer and Boyer, 1995).

Photosynthetic pigments

The leaves of host infected by *C. chinensis* showed a marginal decreased total chlorophyll content than those of control (Table 1). Chlorophyll-b content was significantly reduced than chlorophyll-a in the tested plants ($P < 0.01$). Meanwhile, the carotenoid content was highest in the infected plants than control. This in turn affects the photosynthetic activity in the infected plant. These changes may be due to the induction induced by the parasite on chlorophyll synthesis, photosystem efficiency, activity of photosynthetic enzymes and on plant water balance (Mobin and Nafees, 2007). Mobin and Nafees, (2007) reported that in most of the stressed plants, the chlorophyll a-content exceed that of chlorophyll-b. Similarly, the interaction also caused an increase in carotenoid content. Further analysis showed that chlorophyll content of leaves was positively correlated with

Table 1. Pigment profile (mg/g) of infected host (*Chromolaena odorata*), control and the parasite *Cuscuta chinensis*

Pigments (mg/g)	Host-Infected	Control	Parasite
Chlorophyll a	1.05	0.779	ND
Chlorophyll b	1.03	1.47	ND
Total chlorophyll	2.08	2.25	ND
Carotenoids	0.54	0.32	ND

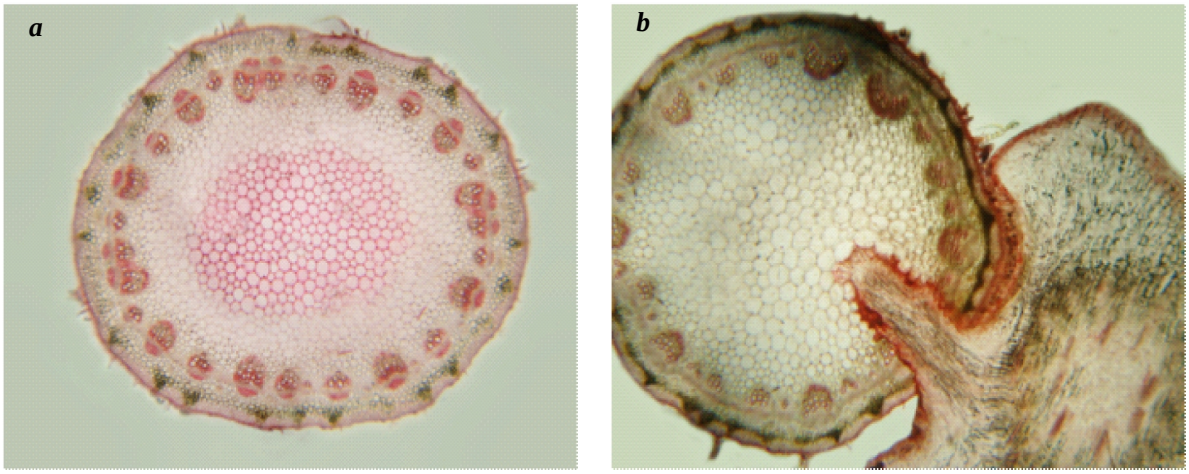

Fig. 2. a- *Chromolaena* - CS of stem (Control); b- *Chromolaena* - CS - Stem of host plant showing haustorial penetration

leaf relative water content (r = 0.75). Thus, the altered total chlorophyll content in the host may be due to the interaction between host and parasites.

Flavonoid content analysis

The flavonoid content in most of the infected plant species were maximum in infected species followed control and the parasite (Fig.3). Flavonoid plays a role in the defense of the plant. It also influences the resistance of plants to adverse environmental conditions, including air pollution and its reducing power is directly propor-tional to its concentration (Raza and Murthy, 1988). The study revealed that the significant flavonoids con-tent of plants may be due to the high secondary metabolism in the plants.

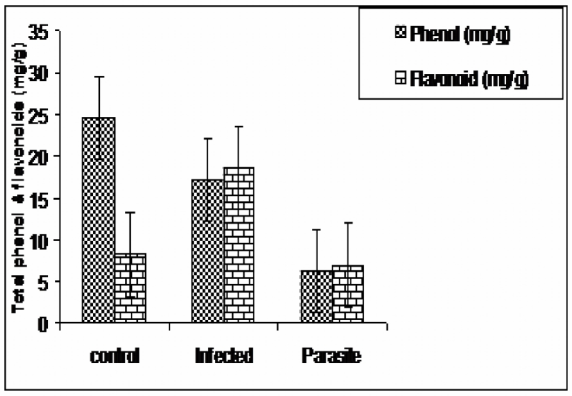

Fig. 3. Total phenol & flavonoid content in *Chromolaena odorata* infected with *Cuscuta chinensis* compared with control and the parasite. The results are mean of triplicates with two independent experiments ± SD

Soluble sugar

Significant differences were observed among the experimentals in terms of soluble sugar content (Fig. 4). In the host *C. odorata*, soluble sugar content decreased but higher than the parasite. The results are different from other environmental stress impacts like drought which resulted in an increased soluble sugar concentration in the cotton tolerant cultivar but no change in the case of sensitive cultivar. Fazeli *et al.* (2006) suggested that increase in soluble sugar under drought may be due to catabolism of polysaccharides.

Soluble protein

Soluble protein content increased marginally in the infected plant (Fig. 4). These findings can be related to some earlier studies in which it has been observed that concentration of soluble protein increased only in stress tolerant genotype with comparison of the cold-sensitive genotype under freezing treatment (Aghaee *et al.*, 2011). Increase in soluble protein, one of the important mechanism induce signal transduction, RNA processing, translation, protein processing, redox homeostasis, photosynthesis, photo-respiration, and metabolisms of carbon, nitrogen, sulfur and energy.

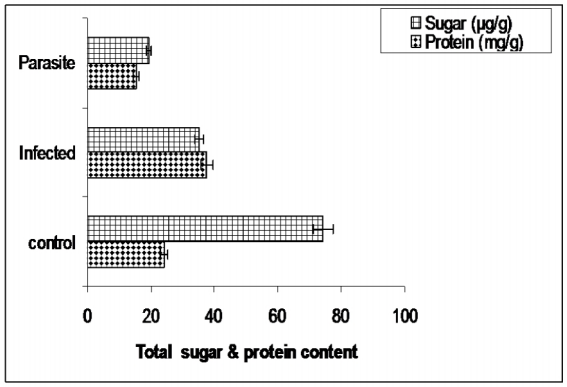

Fig. 4. Total sugar and protein content in *Chromolaena odorata* infected with *Cuscuta chinensis* compared with control and the parasite. The results are mean of triplicates with two independent experiments ± SD

Table 2. Amino acid profile (µg/g) of infected host (*Chromolaena odorata*), control and the parasite *Cuscuta chinensis*

Amino acids (µg/g)	Host	Control	*C. chinensis*
Tyrosine	9704.9	1704.82	721.27
Phenylalanine	666.6	117.1	49.54
Serine	1552.4	272.48	115.28
Glycine	695.92	122.3	51.72
Aspartic acid	4666.6	819.78	346.83
Proline	6607.1	1160.64	491.04
Cysteine	5920	1040	440
Isoleucine	804.34	141.28	59.7

Similarly, the free amino acids such as tyrosine, phenyl alanine, serine, glycine, aspartate, proline, cysteine and isoleucine also showed a higher profile in *C. chinensis* infected plants than control and in the parasite (Table 2). This suggests the active metabolic phase of the host compared to the control plants.

Total phenol content (TPC)

Phenol content showed a marginal decrease in *C. chinensis* invaded *C. odorata*. than in control plants (Fig. 3). The influence of invasion of parasite in the host tissue influences the content of total phenolics. Davies and Hobson (1981), reported more flavonoids (quercetin and kaempferol) synthesize in field-grown tomato than in greenhouse. A similar trend was confirmed in tomatoes were grown under a covering material that allowed the transmission of solar UV radiation up to 400 nm, there was a higher content of TPC of about 10%-16% in the fruits than in those grown under UV-exclusion conditions.

Phenylalanine ammonia lyase (PAL)

Phenylalanine ammonia lyase, the initiator enzyme of Phenyl propanoid pathway was assayed in the plants. The active phase of the enzyme in the parasite invaded host tissue compared with the control suggests its role in secondary (Fig.5). The high level of flavonoids in the infected plant supports the assay data of PAL.

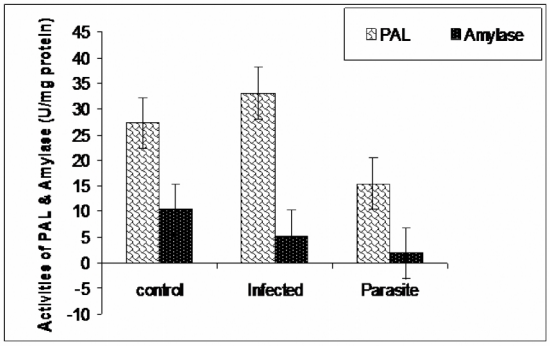

Fig. 5. Phenylalanine ammonialyase (PAL) and amylase enzyme activities in *Chromolaena odorata* infected with *Cuscuta chinensis* compared with control and the parasite. The results are mean of triplicates with two independent experiments ± SD

Antioxidant enzymes

Antioxidant enzymes such as SOD, PPO and POX activities showed an increase, while, CAT and APX decreased marginally in the leaves of *C. odorata* under *C. chinensis* invasion when compared to the control. The interaction effect of host x parasite treatments was highly significant ($P < 0.01$) for SOD, POX, APX, PPO and CAT (Table 3). The maximum increase in the SOD activities was observed in the experimental (2 fold) compared to the control. The maxi-mum POX activity observed was 25.7 U/mg protein. The control and the parasite showed a decrease in POX activity. The analysis of total CAT activity in-dicated a reduction under parasite infection i.e., 6.0 compared to control (6.3 U/mg protein). The increased activity by PPO in infected host reveals its role in the conversion of phenols in to unpalatable quinines.

Biotic stress is inevitably associated with in-creased oxidative stress due to enhanced accu-mulation of ROS, particularly O_2^- and H_2O_2 in chloroplasts, mitochondria, and peroxisomes. As a result, the induction of antioxidant enzyme activities is a general adaptation strategy which plants use to overcome oxidative stresses (Foyer and Noctor, 2003). Our results are consistent with other studies reporting the increased SOD activity in response to drought stress in sunflower (*Gunes et al.*, 2008) and poplar (*Xiao et al.*, 2008).

Table 3. Antioxidant enzymes - Polyphenol oxidase (PPO), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD) activities in *Chromolaena odorata* infected with *Cuscuta chinensis* compared with control and the parasite. The results are mean of triplicates with two independent experiments ±SD.

Antioxidant enzymes (U/mg)	Control	Host-Infected	Parasite
PPO	23.4±0.88	29.5±0.323	12.8±0.27
POX	17.6±0.09	25.7±0.432	6.9±0.086
CAT	6.3±0.23	6.0±0.097	1.9±0.038
APX	9.6±0.17	9.5±0.065	2.8±0.032
SOD	9.2±0.36	18.7±0.367	2.9±0.21

According to the present results, the maximum increase in the SOD activity was observed in the *C. chinensis* invaded *C. odorata*., which might lead to their higher protection against parasite invasion. However, the control showed a significant decrease in SOD activity, this may be related to the poor availability of $O^{\bullet}{}_2$, H_2O_2, which resulted from the low action of SOD. Therefore, it is important that H_2O_2 be scavenged rapidly by the antioxidative defence system to water and oxygen (Guo *et al*., 2006).

The present study indicates a significant in-crease in POX activity in plants under infection stress (Table 3). Some previous studies, as parallel with this results, reported the increased POX activity under drought stress conditions in various plants, like sunflower (*Gunes et al*., 2008), and poplar (*Xiao et al*., 2008). The important point here is a decrease in POX activity in control suggests the active role of the enzyme in detoxification of H_2O_2, which may reflect the low ROS scavenging need in the control species.

In terms of the results, CAT activity decreased in experimental plant (Table 3). The decline in CAT activity is regarded as a general response to many stresses (*Gunes et al*., 2008). The reduction of CAT activity is sup-posedly due to the inhibition of enzyme synthesis or change in the assembly of enzyme subunits under stress conditions. It may also be associated with degradation caused by induced peroxisomal proteases or may be due to the photo-inactivation of the enzyme. All in all, the increase in POX might be consid-ered as a key point for the decom-position of H_2O_2, especially under CAT inactivation.

According to the results, the observed higher constitutive activity of SOD and POX and marginal decreased activity of CAT and APX under biotic stress reveals the differential response of the enzyme in the plant. As a consequence, *C. odorata* may have a better protection mechanism against oxidative damage under parasitic stress by maintaining the higher constitutive and induced activity of antioxidant enzymes, compared with other hosts especially the sensitive ones.

Pathogen-induced PPO activity continues to be reported for a variety of plant taxa, including monocots and dicots (Deborah *et al.,* 2001). Similarly, studies describing correlations of high PPO levels in cultivars or lines with high pathogen resistance continue to provide support for a pathogen defense role of PPO. PPO-over expressing plants showed reduced bacterial growth, whereas PPO antisense-suppressed lines supported greater bacterial numbers. These studies are the only direct demonstrations to date of PPO's importance in pathogen defense.

The APX displayed a low profile compared to SOD, POX and PPO. Increased SOD activity and decreased CAT and APX activities in plants exposed to *C. chinensis* was comparable with the metal toxicity reported by Singh *et al*. (2007). APX is mainly located in chloroplasts (Singh *et al*., 2007), and since infection can cause chloroplast ultra structural damage affecting their integrity and associated metabolism (Li *et al*., 2006), decreases in APX activity would be expected in the leaves. Biotic stress also interferes with enzyme activity and with other proteins by binding to intracellular thiols (-SH) and thus inactivating them (Meharg and Hartley-Whitaker, 2002). The decreased activities of CAT and APX indicated their inactivation/degeneration due to As-induced oxidative stress (Singh *et al*., 2007).

~-amylase Activity

Amylase activity was decreased when compared to the control (Fig.5). The present amount of decreased reducing sugars with biotic stress was against with the report of Thakur and Sharma (2005) with salt stressed sorghum seeds and okra seeds. The accumulation of reducing sugars in the plants is to lower the osmotic potential of the cells and to mitigate the loss of turgidity. The decrease in the activity of amylase followed by the increase in the sugar levels may be attributed to the osmotic adjustment by the sugars as a response to stress induced water deficit (Singh, 2004).

IR Spectral analysis

The IR spectrum of plant samples are shown in Fig. 6 a,b & c. A summary of the most characteristic absorption bands and their tentative assignments are given in Table 4 for

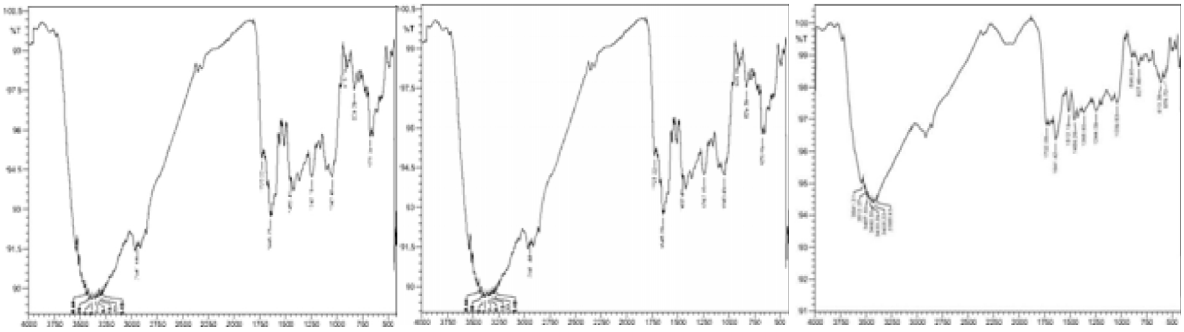

Fig. 6. a, b & c . IR spectrum of infected plant, control plant and the parasite.

Table 4. The characteristic IR absorption bands shown by host plant, control plant and parasite

Control	Host	Parasite *Cuscuta chinensis*
	401.19	
	430.13	
	493.78	
		576.72
		613.36
	621.08	
675.09		
	759.55	
		827.46
829.39		
	833.25	
		896.9
916.19		
		1039.63
1043.49		
	1105.21	
	1240.23	
1242.16		
		1249.09
	1263.37	
	1282.66	
		1365.6
1452.4	1452.4	
		1456.26
		1512.19
		1641.42
1645.28		
1728.22		
		1732.08
2941.44		
	3248.13	
		3388.93
	3404.36	
3408.22		3408.22
3433.29		3433.29
3456.44		
		3460.3
		3487.3
		3512.37
		3541.31

the both infected and for the control sample. The FT-IR spectrum exhibits the characteristic finger print band features. The control plants showed 18 bands whereas infected showed 21.

The complexity of FT-IR spectra in the 1450 to 600 /cm region makes it difficult to assign all the absorption bands, and because of the unique patterns found there, it is often called the fingerprint region. Absorption bands in the 4000 to 1450 /cm region are usually due to stretching vibrations of diatomic units, and this is sometimes called the group frequency region (Cledir Santos *et al.*, 2010)

The absorption bands at 3420 and 3434/cm are representative for C-H, O-H and N-H stretching vibrations, characteristic of the presence of amino acids. In all samples, it is noticed that the bands at 2918, 2921 and 2928/cm are due to the stretching vibration of $-CH_3$ and $-CH_2$ groups (Thenmozhi *et al.*, 2011) indicative of the chlorophyll groups. The 1632, 1640 and 1629/cm bands are due to stretching vibration of carbonyl group characteristic of the secondary amides and other compounds containing C=o group (Elfatih *et al.*, 2010). The bands at 1434/cm and

1411/cm represent the bending vibrations of CH indicative of the lignins. The 1232 and 1216 /cm bands in all samples predict the presence of ester carbonyl (Demetra *et al*., 2011). The C-O-C groups exhibit strong bands at 1095/cm and very strong bands at 1101/cm respectively. The absorption bands at 1100-1000 /cm in the fingerprint region indicate several modes such as C-H deformation or C-O or C-C stretching, pertaining to carbohydrates. Carbohydrates in the leaves were the major constituents of these absorption bands (Huang et al., 2011). The peak at 1032 /cm to 1070/cm in the spectrum also indicates the starch content in the sample. The stronger the relative intensity of the band, the higher the chemical constituents. The secondary peaks at 770-922/cm are assigned as characteristic absorption of the carbohydrate (Sandak *et al*., 2010). The absorbance bands at 837-721/cm represent C-H in plane and out of plane bending for the benzene ring and bands at 553-633/cm represent C-O-O and P-O-C bending of aromatic compounds (phosphates). The infrared spectrum is able to identify not only the major components in organic materials, but also to find some differences among them. These differences may be due to the industrial environment.

From the spectrum, one can notice that the bands 3408, 3433 and 3456/cm are present in the samples only in control and they are absent in infected samples. The bands 2941/cm indicating the chlorophyll groups is strongly present in control but absent in infected which were supported morphologically as paleness suggesting the susceptibility nature. The secondary amides 1631 to 1653/cm are present in the samples in all infected samples and the absorption is also strong. It is also strong in the control sample. Likewise the lignins are present in the sample from strong absorption to weak absorption whereas it has medium absorption in the control sample. The presence of carbohydrate and starch (1100-1000/cm) in the samples is varying from strong to medium absorption, but they have medium absorption in the control sample. The intensive broad absorption band appears in the characteristic carbohydrate region with a maximum at 1058 and 1033/cm. The phosphate groups are present with a medium to weak absorption band whereas in the control sample it possesses medium absorption through 544-633/cm.

CONCLUSIONS

Biotic stress increased the caroteneoid pigments, protein, free amino acid and proline contents in the studied species. Antioxidant enzymes (SOD, POX) showed higher levels of activities. Meanwhile, catalase and ascorbate peroxidase activities reduced. The positive relationships were observed among activities of antioxidant enzymes, and phenol contents. These results suggest that *C. odorata* display higher tolerance to parasite stress due to higher osmotic adjustment and antioxidant protection. Biotic stress was found not to alter the plant physiology while the mobilization of starch was reduced. To overcome the negative influence, starch mobilization was partially restored as a result of amylase activity.

FT-IR technology suggest a disease detection potential accurately and early in plants under stress. The FT-IR technology could be integrated with an autonomous agricultural vehicle for reliable and real-time plant disease detection to achieve superior plant disease control and management. Further studies will focus on explorative multifactor approaches for investigating pathogen injury under various stresses, including DNA microarray, scanning electron microscopy, and vibrational spectroscopy.

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