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Biochemical Changes in Okra Infected with *Cercospora abelmoschi* Ell. and Ev.

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ABSTRACT

Present study was carried out to evaluate the changes occurring in biochemical's (viz., total phenols, total sugars, chlorophyll a, b and total chlorophyll) in Cercospora infected okra leaves from different plots, two weeks after complete execution of fungicide treatments. Results revealed that phenol content in lower leaves(1.58 mg g^{-1}) and upper leaves (1.52 mg g^{-1}), total protein content in lower (9.32 mg g^{-1}) and upper leaves (9.23 mg g^{-1}) were significantly higher in trifloxystrobin + tebuconazole @ 0.1% applied plants. Total sugar content in lower (12.33 mg g^{-1}) and upper leaves (10.08 mg g^{-1}) were low in trifloxystrobin + tebuconazole @ 0.1% treatment. The reduction in Chlorophyll a and chlorophyll b was observed to be lowest in lower leaf (1.15 and 1.06 mg g^{-1} respectively) and upper leaf (1.28 and 1.02 mg g^{-1} respectively) in trifloxystrobin + tebuconazole @ 0.1% sprayed plants. The reduction in the total chlorophyll content was relatively less in trifloxystrobin + tebuconazole @ 0.1% as compared to other treatments due to reduced disease intensity and thus maintaining greenness of the leaves. Significant negative correlation existed between disease severity and total phenols (-0.903), total proteins (-0.903), chlorophyll a (-0.901), chlorophyll b (-0.788), total chlorophyll (-0.856) while significant positive correlation existed between total sugars and disease severity (0.932). Significantly high positive correlation existed between proteins and phenols (0.862), total chlorophyll (0.889), chlorophyll a (0.844), chlorophyll b (0.868). Similarly, significant high positive correlation existed between phenols and total chlorophyll (0.854), chlorophyll a (0.803), chlorophyll b (0.839). Significantly high negative correlation between total sugars and phenols (-0.924), proteins (-0.836), chlorophyll a (-0.919), chlorophyll b (-0.838), total chlorophyll (-0.897) existed.

Key words: *Cercospora leaf spot, okra, trifloxystrobin + tebuconazole, Biochemicals, phenol, proteins, sugars, chlorophyll*

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INTRODUCTION

Okra, *Abelmoschus esculentus* (L.) Moench, is an important warm season vegetable crop grown mainly in the tropical or sub-tropical regions during summer and rainy season (Thomson and Kelly, 1957). Okra is cultivated for a variety of uses but mainly for its edible leaves and immature green seed pods or fruits. Okra dry seeds contain 18-20% oil and 20-23% crude protein (Siemonsma and Kouame, 2004). Fresh okra leaves are used as vegetables while the roots and stems are used for clearing the cane juice from which 'gur' or brown sugar is prepared (Moyin-Jesu, 2007).

Diseases play a vital role in yield losses of the crop. In India, two species of *Cercospora viz., C. malayensis* Stev. and Solh. and *C. abelmoschi* Ell. and Ev. were found to cause leaf spots in okra. These species differ in symptom production. The common biochemical constituents like phenols, proteins, sugars and chlorophyll are important in imparting resistance to the crop plants. But almost all living animals and plant show biochemical changes after infected by infectious agent (in Fishes by Mahananda *et al*., 2010 and in trees by Bora and Joshi, 2013). Sometimes, host plant is induced to synthesize these compounds upon infection. Hence, present investigation was undertaken with the objective to study the different biochemical changes in okra infected with *Cercospora abelmoschi* .

MATERIAL AND METHODS

The experiment was conducted during *kharif* 2016-17 at the Agricultural College Farm, Bapatla, Guntur, Andhra Pradesh. The experiment was laid out in randomized block design (RBD) with four replications.

Susceptible F1 hybrid (Sahiba) was sown with spacing of 60 x 30 cm. Healthy and diseased leaf samples were collected from different plots two weeks after complete execution of treatments. The collected leaf samples were properly labeled and packed in polyethylene bags for biochemical analysis.

Estimation of phenols was carried out by following Folin –Ciocalteau Reagent and by using the standard curve the concentration of phenols in the test sample were expressed as mg phenols per g plant sample on fresh weight basis (Mallick and Singh, 1980). Total Protein estimation was carried out according to the procedure described by the Lowry *et al.* (1951). Total sugars were estimated following anthrone method (Gerhardt *et al*., 1994). Chlorophyll a, chlorophyll b and total chlorophyll were estimated by using the following formulae (Arnon, 1949).

Chlorophyll a (mg g^{-1} tissue) = [12.7 (A663) - 2.69 (A645)] × V/ 1000 × W

Chlorophyll b (mg g^{-1} tissue) = [22.9 (A645) - 4.68 (A663)] × V/ 1000 × W

Total Chlorophyll (mg g^{-1} tissue) = [20.2 (A645) +8.02 (A663)]x V/1000 x W

Where: A = Optical density at respective wave length (nm)
V = Final volume of chlorophyll extract in 80% acetone
W = Fresh weight of the tissue extracted

RESULTS AND DISCUSSIONS

Total Phenol Content (mg g^{-1}): Variation in phenol content was observed to certain extent in infected leaves with respect to their position. In lower leaves, phenol content ranged from 0.98 to 1.58 mg g^{-1}while in upper leaves, it varied between1.25 and 1.52 mg g^{-1}. Phenol content in lower leaves was found significantly high (1.58 mg g^{-1}) over other treatments when infected leaves were sprayed with a combination fungicide trifloxystrobin + tebuconazole @ 0.1%. Combination fungicide treated upper leaves (1.52 mg g^{-1}) and propiconazole sprayed upper leaves were on a par (1.48 mg g^{-1}) with phenol content. However, in upper leaves the amount of phenols were low compared to lower leaves probably due to the low inoculum pressure in trifloxystrobin + tebuconazole sprayed plants (Table 1).

Nearly, three fold high per cent increase over control in phenols was observed in lower leaves (61.23%) over upper leaves (21.60%) in trifloxystrobin + tebuconazole fungicide treated plants. In the same treatment a maximum of 97.50 % increase in phenols was observed over healthy in lower leaves and 87.65% in upper leaves. The increase in phenolics concentration might arise from the release of phenol from their glucosides by the enzyme glucosidase of their host or pathogen (Pridham, 1965). Therefore the early accumulation of phenolic compounds at the infection site will trigger defense responses thereby slowing down the pathogen development through rapid cell death (Fernandez and Heath, 1989). The present study corroborate with the earlier findings and it was found that in addition to phenol accumulation there was an additive impact of fungicides that reduced the amount of inoculum thus reducing the disease severity. A significant negative correlation (-0.903) existed between total phenols and disease severity (Table 5). Similar report was given by Younes and Elyousr (2014) where disease severity was negatively correlated with phenol contents in okra powdery mildew resistant genotypes.

Table 1. Total phenol content (mg g^{-1}) due to Cercospora leaf spot in okra leaves

S. No.	Treatments	PDI (%)		Total Phenol content (mg g^{-1})		Per cent increase over control		Per cent increase over healthy	
		Lower leaf	Upper leaf	Lower leaf	Upper leaf	Lower leaf	Upper leaf	Lower leaf	Upper leaf
1	Mancozeb	85.28 (67.54)	57.72 (49.43)	1.01	1.34	3.06	7.2	26.25	65.43
2	Thiophante methyl	82.23 (65.26)	62.20 (52.05)	1.09	1.29	11.22	3.2	36.25	59.26
3	Trifloxystrobin + Tebuconazole	31.68 (34.11)	16.38 (23.64)	1.58	1.52	61.23	21.6	97.50	87.65
4	Pyraclostrobin	77.78 (61.89)	58.35 (49.79)	1.19	1.32	21.4	5.6	48.75	62.96
5	Hexaconazole	73.90 (59.54)	57.80 (49.47)	1.25	1.42	25.55	13.6	56.25	75.3
6	Propiconazole	57.25 (49.16)	44.07 (41.53)	1.43	1.48	45.91	18.4	78.75	82.72
7	Control (water spray)	95.55 (79.75)	88.62 (70.32)	0.98	1.25				
8	Healthy	0.00	0.00	0.8	0.81				
	SEm±			0.015	0.013				
	CD (P≤0.05)			0.045	0.039				
	CV %			5.40	4.17				
	T cal value			10.37* (df 26), P ≤ 0.05					

* Figures in parenthesis are arcsine transformed values

Total Protein Content (mg g^{-1}): There was no significant difference in total protein content in okra lower and upper leaves on cercospora leaf spot infection and it ranged from 5.98 (control with water spray) to 9.32 mg g^{-1} (trifloxystrobin + tebuconazole) and 6.27 (control with water spray) to 9.23 mg g^{-1} (trifloxystrobin + tebuconazole), respectively. However, significantly low protein content was observed in control which was ranged from 5.98 (lower leaves) to 6.27 mg g^{-1} (upper leaves) (Table 2). A significant negative correlation (-0.903) existed between total proteins and disease severity (Table 5). Significantly high total protein (9.32 and 9.23 mg g^{-1} in lower and upper leaves) was present in fungicide treated plants that expressed low per cent disease severity than other fungicide treated plants with high PDI. Per cent decrease in protein content over healthy was maximum in control (42.77% - lower and 38.04% - upper leaves) while it was minimum in trifloxystrobin + tebuconazole treated plants (9.25% - lower and 8.79% - upper leaves).

Plant pathogens such as viruses, bacteria, fungi and nematodes elicit the synthesis of host proteins which help in restricting the multiplication and spread of pathogens in the healthy tissue (Datta *et al.,* 1999). Present results clearly confirms the earlier statement where the healthy tissue was found with high amount of protein content either in lower (10.27 mg g^{-1}) or upper leaves (10.12 mg g^{-1}). Due to the amount of inoculum that has established there was considerable reduction in the total protein content in diseased leaf samples. Difference in the treated diseased samples might be due to variations in the efficacy of the chemical fungicides. More effective the chemical lesser was the degradation in proteins which could be substantiated by Datta *et al.* (1999) that decrease in protein content in susceptible cultivars might be due to degradation of the host proteins by the proteolytic enzymes secreted by the virulent pathogens or may be due to the changes in the metabolic activity because of plant, pathogen (Mary and Subramanian, 2014) and fungicide interactions.

Table 2. Total protein content (mg g^{-1}) due to Cercospora leaf spot in okra leaves

S. No.	Treatments	PDI (%)		Total Protein content (mg g^{-1})		Per cent increase over control		Per cent decrease over healthy	
		Lower leaf	Upper leaf	Lower leaf	Upper leaf	Lower leaf	Upper leaf	Lower leaf	Upper leaf
1	Mancozeb	85.28 (67.54)	57.72 (49.43)	6.71	6.93	12.2	10.5	34.66	31.52
2	Thiophante methyl	82.23 (65.26)	62.20 (52.05)	6.78	7.01	13.4	11.8	33.98	30.73
3	Trifloxystrobin +Tebuconazole	31.68 (34.11)	16.38 (23.64)	9.32	9.23	55.9	47.2	9.25	8.79
4	Pyraclostrobin	77.78 (61.89)	58.35 (49.79)	7.45	7.54	24.6	20.3	27.51	25.49
5	Propiconazole	73.90 (59.54)	57.80 (49.47)	7.57	7.63	26.6	21.7	26.29	24.6
6	Hexaconazole	57.25 (49.16)	44.07 (41.53)	8.54	8.47	42.8	35.1	16.84	16.3
7	Control (water spray)	95.55 (79.75)	88.62 (70.32)	5.98	6.27			41.77	38.04
8	Healthy	0.00	0.00	10.27	10.12				
	SEm±			0.09	0.16				
	CD (P≤0.05)			0.27	0.47				
	CV %			4.73	8.24				
	T cal value			9.32* (df 26)), P ≤ 0.05					

* Figures in parenthesis are arcsine transformed values.

Total Sugar Content (mg g-1): Lower and upper leaves of okra on infection with Cercospora leaf spot resulted in varied amounts of total sugar content that ranged from 12.33 (trifloxystrobin + tebuconazole) to 18.25 mg g-1 (control with water spray) in lower leaves and from 10.08 (trifloxystrobin + tebuconazole) to 15.94 mg g-1 (control with water spray) in upper leaves. Infection by the pathogen in plant induces changes in biochemical constituents like total sugars, amino acids etc. (Siddaramaiah and Hegde, 1990) resulting in poor nutritive value thus indicating reduction in sugars (Madhavarao et al., 1981) due to increased disease severity (Naik et al., 1988 and Ghosh, 1996). A significant positive correlation (0.932) existed between total sugars and disease severity (Table 5). The results are in

agreement with Younes and Elyousr (2014) where disease severity was positively correlated with total sugars in okra genotypes screened for powdery mildew resistance. Among the infected samples maximum PDI and total sugar was recorded in control ((83.98- lower and 60.04 % upper leaves) (18.25 lower and 15.94 mg g-1 upper leaves) respectively). The amount of decrease in total sugar content over both control and healthy samples was found maximum in trifloxystrobin + tebuconazole treated samples (Table 3).

Reduction in the amount of total sugars in the fungicide treatment with least PDI values could be due to the effect of chemical on the pathogen in managing it. In less effective fungicides infection was high and there will be a greater demand for sugars by the pathogen compared to the treatments with less PDI. Thus, in an successful host pathogen interaction, there will be requirement of sugars for increased respiration or utilization of sugars by the fungi which in turn depends on the capability of fungi to secrete carbohydrate degrading enzyme (Prasad *et al*., 1960).

Table 3. Total sugar content (mg g^{-1}) due to Cercospora leaf spot in okra leaves

S. No.	Treatments	PDI (%)		Total Sugar content (mg g^{-1})		Per cent decrease over control		Per cent decrease over healthy	
		Lower leaf	Upper leaf	Lower leaf	Upper leaf	Lower leaf	Upper leaf	Lower leaf	Upper leaf
1	Mancozeb	85.28 (67.54)	57.72 (49.43)	17.08	14.26	6.41	10.53	9.58	19.3
2	Thiophante methyl	82.23 (65.26)	62.20 (52.05)	16.64	13.89	8.82	12.86	11.91	21.4
3	Trifloxystrobin + Tebuconazole	31.68 (34.11)	16.38 (23.64)	12.33	10.08	32.44	36.76	34.73	42.95
4	Pyraclostrobin	77.78 (61.89)	58.35 (49.79)	15.22	12.94	16.6	18.82	19.43	26.77
5	Hexaconazole	73.90 (59.54)	57.80 (49.47)	14.98	11.92	17.91	25.22	20.6	32.54
6	Propiconazole	57.25 (49.16)	44.07 (41.53)	13.82	11.28	24.27	29.27	26.84	36.16
7	Control (water spray)	95.55 (79.75)	88.62 (70.32)	18.25	15.94			3.39	9.79
8	Healthy	0.00	0.00	18.89	17.67				
	SEm±			0.041	0.04				
	CD (P≤0.05)			0.12	0.13				
	CV %			1.04	1.29				
	T cal value			8.18* (df 26), P ≤ 0.05					

* Figures in parenthesis are arcsine transformed values

Chlorophyll a Content (mg g^{-1}): Significant reduction in Chlorophyll a was noticed in infected leaves (0.73 lower and 0.79 mg g^{-1} upper leaves) compared with healthy (1.21 lower and 1.32 mg g^{-1} upper leaves). In upper leaves, relatively high concentration of chlorophyll a (0.79 to 1.28 mg g^{-1}) was observed indicating lesser damage due to reduced infection than lower leaves (0.73 to 1.15 mg g^{-1}) (Table 4). A significant negative correlation (-0.901) existed between chlorophyll a and disease severity (Table 5).

Chlorophyll b Content (mg g^{-1}): The amount of chlorophyll b content was relatively low over chlorophyll a and Chlorophyll b reduction was comparatively high than the chlorophyll a in both lower and upper leaves. In lower leaves, chlorophyll b content ranged from 0.41 (Control) to 1.06 mg g^{-1} (trifloxystrobin + tebuconazole) and from 0.44(Control) to 1.02 mg g^{-1} (trifloxystrobin + tebuconazole) in upper leaves. In lower leaves, reduction in chlorophyll b in trifloxystrobin + tebuconazole was found on a par with hexaconazole and propiconazole treated samples while in upper leaves in addition to the earlier it was found on par with pyraclostrobin (Table 4). A significant negative correlation (-0.788) existed between chlorophyll b and disease severity (Table 5). The results were in agreement with Muqit *et al.* (2007) who reported the high Chl a/b ratio which indicates more degradation or lower synthesis of chlorophyll 'b' than 'a' due to *okra Yellow Vein Mosaic Virus* infection.

Total Chlorophyll Content (mg g^{-1}): Trifloxystrobin + tebuconazole treated samples were found to be least effected due to infection, with significantly high amount of total chlorophyll (2.22 mg g^{-1} in lower and 2.30 mg g^{-1} in upper leaves) among the treatments (Table 4). A significant negative correlation (-0.856) existed between total chlorophyll and disease severity (Table 5). Bawden (1999) reported that abnormalities in the form and destruction of chloroplasts are common features of disease tissue in plants infected with pathogens, which usually exhibited reduced photosynthetic rate, phosphorylation, hill reaction and carbon dioxide assimilation. Palanisamy *et al.* (2009) reported the effect of okra *Yellow Vein Mosaic Virus* infection on PS II efficiency and thylakoid membrane integrity. The present studies

corroborate with the earlier findings. The disease under study was found to develop in the form of sooty effuse and has strong impact on photosynthetic area and the pathogen during the process of establishment may bring about rapid changes in the cells that may result in accumulation of reactive oxygen species that in turn may affect the integrity of thylakoid membrane. Thylakoids being the centres for chlorophyll synthesis get affected due to the disruption of thylakoid integrity.

Table 4. Total chlorophyll, chlorophyll a, chlorophyll b content (mg g^{-1}) due to Cercospora leaf spot in okra leaves

S. No.	Treatments	Chlorophyll a (mg g^{-1})		Chlorophyll b (mg g^{-1})		Total chlorophyll (mg g^{-1})	
		Lower leaf	Upper leaf	Lower leaf	Upper leaf	Lower leaf	Upper leaf
1	Mancozeb	0.88	0.97	0.44	0.57	1.30	1.54
2	Thiophante methyl	0.96	1.09	0.47	0.75	1.43	1.84
3	Trifloxystrobin+ Tebuconazole	1.15	1.28	1.06	1.02	2.22	2.30
4	Pyraclostrobin	0.99	1.13	0.60	0.96	1.60	2.09
5	Hexaconazole	1.04	1.14	1.00	0.97	2.04	2.11
6	Propiconazole	1.06	1.16	1.05	1.00	2.10	2.16
7	Control (Water spray)	0.73	0.79	0.41	0.44	1.14	1.23
8	Healthy	1.21	1.32	1.15	1.10	2.36	2.42
	SEm±	0.01	0.01	0.02	0.03	0.04	0.03
	CD (P≤0.05)	0.04	0.02	0.06	0.08	0.11	0.09
	CV %	5.27	3.00	12.20	12.91	9.12	6.06
	T cal value	10.42* (df 26), P ≤ 0.05		10.46* (df 26), P ≤ 0.05		10.29* (df 26), P ≤ 0.05	

* Figures in parenthesis are arcsine transformed values

Table 5. Correlation coefficients between biochemical parameters and Per cent disease index (PDI) due to Cercospora infection on okra leaves

Biochemical parameters	PDI (%)
Total Phenols	-0.903
Total Proteins	-0.903
Total Sugars	0.932
Total chlorophyll	-0.856
Chlorophyll a	-0.901
Chlorophyll b	-0.788

CORRELATION BETWEEN BIOCHEMICAL PARAMETERS

Significantly high positive correlation existed between proteins and phenols, total chlorophyll, chlorophyll a and chlorophyll b. Conversion of majority of the proteins in to phenols to overcome the stress at infection site will slow down the pathogen development. Similarly, significantly high positive correlation existed between phenols and total chlorophyll, chlorophyll a and b. As phenols play an important role in resisting the pathogen entry and establishment more the concentration of phenols lesser will be the degradation of chlorophylls.

Between total sugars and phenols, proteins, chlorophyll a, chlorophyll b and total chlorophyll significantly high negative correlation was observed (Table 6). As more sugars are demanded by the invading pathogen for its biomass production and thus the production of protein or phenols gets reduced. As the pathogen establishes chlorophyll pigments will be degraded. Kumar *et al.* (2017) observed that phenol content and total chlorophyll content was positively correlated to each other while total soluble sugar content was negatively correlated with other both the traits in leaves of *YVMV* resistant and susceptible lines of okra.

Table 6. Correlation between biochemical parameters due to Cercospora leaf spot on okra leaves

	Phenols	Proteins	Sugars	Total chlorophyll	Chlorophyll a	Chlorophyll b
Phenols	1					
Proteins	0.862*	1				
Sugars	-0.924*	-0.836*	1			
Total Chlorophyll	0.854*	0.889*	-0.897*	1		
Chlorophyll a	0.803*	0.844*	-0.919*	0.942*	1	
Chlorophyll b	0.839*	0.868*	-0.838*	0.982*	0.861*	1

* Significant at 5 % r = 0.556 No. of observations = 14

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