

# STUDIES ON BIOCHEMICAL BASES OF RESISTANCE AGAINST *COLLETOTRICHUM GLOEOSPORIOIDES* (PENZ)

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## ABSTRACT

Investigation was done to understand the biochemical changes of chlorophyll, OD phenol and sugar content in two moderately resistant pomegranate cultivars Dholka, Yerkund local and two susceptible cultivars Arakta and Ganesh were studied in leaves inoculated with *Colletotrichum gloeosporioides* along with healthy leaves. Dholka contained significantly higher amount of chlorophyll a (2.88mg), Chlorophyll b (2.00mg) and total chlorophyll (4.88mg), OD phenol (3.00mg), total phenol (2.22mg) Reducing sugar (10.46mg), Non reducing sugar (6.69 mg) and total sugar (17.66mg) followed by cultivar Yerkund local while Ganesh recorded least amount of chlorophyll a (2.43mg), chlorophyll b (1.70mg), chlorophyll total chlorophyll (4.14mg) and OD phenol (2.28mg), Total phenol (1.42mg), Reducing sugar (9.58mg) were found in cultivar Ganesh and Non reducing sugar (5.59mg) and total sugar (15.69mg) was found in Arakta. As disease infection increased Chlorophyll a, Chlorophyll b and Total Chlorophyll were reduced. All cultivars showed reduction in Chlorophyll. Maximum reduction of Chlorophyll a (47.19%) and total chlorophyll (44.36%) in Arakta and Ganesh respectively, while Yerkund local showed maximum reduction in chlorophyll b (45.75%). Total phenol content of infected leaves was increased over healthy leaves but at higher rate in Arakta (42.86%). Maximum reduction in reducing sugar (18.56%) non reducing sugar (15.37%) and total sugar (17.28%) was observed in Var. Ganesh.

## INTRODUCTION

*Colletotrichum gloeosporioides* causes anthracnose disease of pomegranate in India. In India anthracnose was first reported by MCRAC (1924) it causes preharvest and post harvest losses in pomegranate the biochemical constituents such as chlorophyll a, b and total chlorophyll, reducing, non reducing total sugar OD phenol and total phenol are important in imparting disease resistance to the plant. Susceptibility and resistance against any disease is depend upon biochemical constituent present in it. To develop ecofriendly management practices biochemical bases of resistance is important. Plant shows biochemical changes after infection by infectious agents (in fishes by Mahananda *et al.*, 2010 and in soybean by Chavan and Suryavanshi, 2014)

The biochemical chlorophyll plays important role in photosynthesis infection of *Colletotrichum* causes death of leaf tissue which result in to reduction in chlorophyll (Chavan and Suryavanshi, 2014) chlorophyll reduction due to infection of *Colletotrichum gloeosporioides* was less in resistant variety of groundnut (Benagi, 1995) OD phenol and total phenol are the precursors of lignin and tanin (Oku 1960) which coat the cell wall and make it an barriers against fungal penetration and hydrolytic enzyme attack (Herrman and Cobb *et al.* 1967)

The resistance in resistant and moderately resistant genotypes of chilli might be due to appreciable amount of total phenol recorded (Rani *et al.*, 2007) however Gupta *et al.*, 2010 in French bean and Prasath and Pannuswami 2008 in chilli recorded that high amount of total and OD phenol was

observed in diseased fruits as compare to healthy one.

Activation of aromatic metabolism is a characteristic feature of plants under stress and it's role in disease resistance has been reviewed time to time but the work on investigation biochemical bases of resistance against *Colletotrichum gloeosporioides* (Penz.) Penz and Sac causing anthracnose is scanty. The study was undertaken to find out the biochemical bases of resistance against *Colletotrichum gloeosporioides* it is useful to find out the internal chemical changes in the plant at the time of infection of pathogens.

## MATERIALS AND METHODS

Present study was carried out at department of SSAC and Plant Pathology, College of Agriculture V.N.M.K.V., Parbhani during year 2013-14. Healthy and diseased pomegranate leaf samples of the moderately resistant and Var. Dholka and Yerkund local and susceptible Var. Arakta and Ganesh were selected on the basis of their reaction to the *C. gloeosporioides* from screening nursery as per scale given by Mayee and Datar 1986. Further biochemical constituents *viz.*, (chlorophyll a, b and total chlorophyll), sugars (reducing, non reducing and total sugar) and phenol (OD phenol, total phenol) were estimated from the selected cultivars applying standard and scientific protocol as mentioned below. Estimation of sugars is carried out by Benedict's method given by Plummer, 1988. Estimation of chlorophyll is carried out according to method given by Hiscox, 1979. Quantitative analysis of chlorophyll was done by using DMSO (Dimethyl sulphide) in the test tube. The optical density of coloured extracted solution was measured

at 645nm, 652nm and 663nm for chlorophyll a, b and total chlorophyll respectively. The chlorophyll a, b and total chlorophyll were estimated using the formulae as given below.

$$\text{mg Chlorophyll a g}^{-1} \text{ tissue} = 12.7(A_{663}) - 2.69(A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{mg Chlorophyll b g}^{-1} \text{ tissue} = 22.9(A_{645}) - 4.68(A_{663}) \times \frac{V}{1000 \times W}$$

$$\text{mg Total Chlorophyll g}^{-1} \text{ tissue} = 20.7(A_{645}) + 8.02(A_{663}) \times \frac{V}{1000 \times W}$$

Where,

A = Absorbance at specific wavelength

V = Final volume of chlorophyll extract in DMSO

W = Fresh weight of tissue extracted.

Phenol was estimated by Folin-Ciocalteu Reagent. The Orthodihydroxy phenols were estimated using Arnow's reagent (Mahadevan, 1966) method. The O.D. phenol were calculated in terms of catechol in  $\mu\text{g/g}$  of tissue from standard curve of Chlorogenic acid.

## RESULTS AND DISCUSSION

The experimental results obtained in present investigation revealed the significant variation in moderately resistant and susceptible varieties of pomegranate Table No.1 for different biochemical constituents as discussed below.

### Chlorophyll (chlorophyll a, b and total)

The results revealed that moderately resistant variety Dholka contained significant high amount of chlorophyll a (2.88mg) chlorophyll b (2.00mg) and total chlorophyll (4.88mg) followed by another moderately resistant variety Yerkud local by recording chlorophyll a (2.84mg), chlorophyll b (1.80mg) and total chlorophyll (4.64mg) on the other hand lowest chlorophyll a (2.43mg), chlorophyll b (1.70mg) and total

chlorophyll (4.14mg) were found in cultivar Ganesh but it was at par with Arakta which recorded (2.45 %) chlorophyll a, (1.75%) chlorophyll b and (4.20%) total chlorophyll.

Interaction between varieties and inoculation for chlorophyll a, chlorophyll b and total chlorophyll was found significant among four varieties assessed for biochemical resistance. All varieties show reduction in chlorophyll a. Maximum (47.19%) reduction chlorophyll a was found in susceptible Var. Arakta while less in Dholka (28.27%). Chlorophyll b also reduced as disease progressed. Yerkud local showed maximum reduction in chlorophyll b (45.73%) the average high reduction in chlorophyll b pattern was seen in susceptible Var. Arakta (39.73%) and Ganesh (40.65%), Total chlorophyll was also reduced with disease progress. The reduction percentage in susceptible variety was high. It was (44.36%) in Ganesh and (43.97%) in Arakta.

Investigations on chlorophyll content revealed that chlorophyll (a, b and total) content in healthy leaves of resistant variety is maximum as compared to diseased leaves. Chavan and suryavanshi, 2014 reported that in soybean cultivars infected with *Colletotrichum truncatum*, the chlorophyll a, b and total content was reduced drastically but at higher rate in susceptible cultivar than resistant cultivar.

As disease progressed decline in chlorophyll was observed it might be due to obstacles created by pathogen in chlorophyll synthesis these findings are in agreement with Gupta *et al.* (2010).

### Phenols content

The moderately resistant cultivars were found to be superior with respect to phenol content. The moderately resistant cultivar Dholka was content significantly high amount of OD Phenol (3.00mg) and total phenol (2.22mg). This was followed by another moderately resistant variety Yerkud local which recorded OD Phenol (2.97mg) and total phenol (2.18mg), while least phenol content was observed in susceptible variety Ganesh which recorded OD phenol (2.58mg) and total phenol

**Table 1: Variation in biochemical alterations in susceptible and moderately resistant pomegranate varieties infected by *Colletotrichum gloeosporioides***

Treat. No.	Treatment	Biochemical Parameters (mg/ 100 g)							
		Chlorophyll-a	Chlorophyll-b	Total Chlorophyll	OD Phenol	Total Phenol	Reducing Sugar	Non Reducing Sugar	Total Sugar
Varieties (V)									
V <sub>1</sub>	Dholka	2.88	2.00	4.88	3.00	2.22	10.46	6.69	17.16
V <sub>2</sub>	Yerkud local	2.84	1.80	4.64	2.97	2.18	10.24	6.58	16.82
V <sub>3</sub>	Arakta	2.45	1.75	4.20	2.78	1.70	9.99	5.59	15.69
V <sub>4</sub>	Ganesh	2.43	1.70	4.14	2.58	1.42	9.58	6.54	16.12
S.E. +	0.01	0.08	0.13	0.08	0.01	0.08	0.08	0.04	
C.D. at 1%	0.03	0.25	0.39	0.25	0.02	0.25	0.24	0.12	
Disease Reaction (D)									
D <sub>0</sub>	Healthy	3.27	2.26	5.53	2.42	1.58	10.66	6.84	17.50
D <sub>1</sub>	Diseased	2.04	1.37	3.41	3.25	2.18	9.47	5.91	15.39
S.E. +		0.008	0.05	0.09	0.05	0.01	0.06	0.05	0.02
C.D. at 1%		0.025	0.17	0.28	0.17	0.01	0.17	0.17	0.08
Interaction (V X D)									
S.E. +		0.01	0.11	0.18	0.11	0.01	0.12	0.11	0.05
C.D. at 1%		0.05	0.35	0.56	0.35	0.03	0.35	0.34	0.17
C.V. %		1.12	1.05	7.13	7.28	1.05	2.06	3.35	0.61
		S	S	S	NS	S	S	S	S

**Table 2: Interaction V x D for Chlorophyll a**

Treat. No.	Variety (V)	Disease Reaction (D)		% Decrease over control
		D0	D1	
V <sub>1</sub>	Dholka	3.36	2.41	28.27
V <sub>2</sub>	Yerkud local	3.32	2.37	28.61
V <sub>3</sub>	Arakta	3.2	1.69	47.19
V <sub>4</sub>	Ganesh	3.1	1.68	45.81
Mean		3.25	2.04	37.21
S.E. +		0.01		
C.D. at 1%		0.05		
C.V. %		1.12		

**Table 3: Interaction V x D for Chlorophyll b**

Treat. No.	Variety (V)	Disease Reaction (D)		% Decrease over control
		D0	D1	
V <sub>1</sub>	Dholka	2.37	1.62	31.65
V <sub>2</sub>	Yerkud local	2.34	1.27	45.73
V <sub>3</sub>	Arakta	2.19	1.32	39.73
V <sub>4</sub>	Ganesh	2.14	1.27	40.65
Mean		2.26	1.37	39.38
S.E. +		0.11		
C.D. at 1%		0.35		
C.V. %		1.05		

**Table 4: Interaction V x D for Total Chlorophyll**

Treat. No.	Variety (V)	Disease Reaction (D)		% Decrease over control
		D0	D1	
V <sub>1</sub>	Dholka	5.7	4.03	29.30
V <sub>2</sub>	Yerkud local	5.66	3.63	35.87
V <sub>3</sub>	Arakta	5.39	3.02	43.97
V <sub>4</sub>	Ganesh	5.32	2.96	44.36
Mean		5.52	3.41	38.20
S.E. +		0.18		
C.D. at 1%		0.56		
C.V. %		7.13		

**Table 5: Interaction V X D for Total Phenol**

Treat. No.	Variety (V)	Disease Reaction (D)		% Increase over control
		D0	D1	
V <sub>1</sub>	Dholka	1.90	2.55	34.21
V <sub>2</sub>	Yerkud local	1.86	2.50	34.41
V <sub>3</sub>	Arakta	1.40	2.00	42.86
V <sub>4</sub>	Ganesh	1.17	1.67	42.74
Mean		1.58	2.18	37.76
S.E. +		0.01		
C.D. at 1%		0.03		
C.V. %		1.05		

**Table 6: Interaction V X D for Reducing Sugar**

Treat. No.	Variety (V)	Disease Reaction (D)		% Decrease over control
		D0	D1	
V <sub>1</sub>	Dholka	10.78	10.14	5.94
V <sub>2</sub>	Yerkud local	10.79	9.68	10.29
V <sub>3</sub>	Arakta	10.50	9.48	9.71
V <sub>4</sub>	Ganesh	10.56	8.6	18.56
Mean		10.66	9.48	11.10
S.E. +		0.12		
C.D. at 1%		0.35		
C.V. %		2.06		

**Table 7: Interaction V X D for Non Reducing Sugar**

Treat. No.	Variety (V)	Disease Reaction (D)		% Decrease over control
		D0	D1	
V <sub>1</sub>	Dholka	7.03	6.35	9.67
V <sub>2</sub>	Yerkud local	7.11	6.05	14.91
V <sub>3</sub>	Arakta	6.14	5.25	14.50
V <sub>4</sub>	Ganesh	7.09	6.00	15.37
Mean	6.84	5.91	13.59	
S.E. +		0.11		
C.D. at 1%		0.34		
C.V. %		3.35		

**Table 8: Interaction V X D for Total Sugar**

Treat. No.	Variety (V)	Disease Reaction (D)		% Decrease over control
		D0	D1	
V <sub>1</sub>	Dholka	17.82	16.5	7.41
V <sub>2</sub>	Yerkud local	17.91	15.73	12.17
V <sub>3</sub>	Arakta	16.64	14.74	11.42
V <sub>4</sub>	Ganesh	17.65	14.6	17.28
Mean		17.51	15.39	12.07
S.E. ±		.05		
C.D. at 1%		0.17		
C.V. %		0.61		

(1.42mg). The value of OD phenol (2.78mg) in Arakta was at par with Ganesh.

Interaction between varieties and inoculation was non significant for OD phenol but significant for total phenol. There was a significant increase in total phenol after inoculation the increase in total phenol was high in susceptible var. Arakta (42.86%) while less in Dholka (34.21%).

As regards with disease reaction as disease infection increased chlorophyll a, chlorophyll b and total chlorophyll were reduced. OD phenol and total phenol were increased after infection while reducing sugar, non reducing sugar and total sugar were decreased as disease infection increased. High amount of total phenol (2.55mg) was observed in diseased leaves of moderately resistant variety Dholka than healthy leaves (1.90mg). Agrawal and Agrawal (1980) reported that infected fruits contain high amount of polyphenol oxidase and peroxidase this findings supports the present study.

OD Phenol is the precursors of lignin and tannin (Oku, 1960) which coat the cell wall and make it an effective against fungal penetration and hydrolytic enzyme attack Herrmann (1962) and Cobb *et al.* (1967). Total phenol and OD phenol were higher in amounts in healthy leaves of resistant genotypes than susceptible genotype of soyabean (Chavan and Suryavanshi, 2014).

The resistance in resistant and moderately resistant plants might be due to the presence of appreciable amount of total phenol Rani *et al.* (2007) these findings were in agreement with present findings. Gupta *et al.* (2010) reported the similar findings. The work of previous worker Anand *et al.* (2009), Prasath and Ponnouswami (2008), Pushpa Agrawal (1982) confirmed the present findings. It indicate that presence of maximum amount of phenol and OD phenol at the time of infection may give resistance against infection.

### Sugar content

The moderately resistant cultivar Dholka was found to be superior with respect to sugar content. The values of reducing sugar (10.46mg), Non reducing sugar (6.69mg) and total sugar (17.66mg) in Dholka variety was significantly superior over other varieties. This was followed by Yerkud local by recording reducing sugar (10.24mg), Non reducing sugar (6.58mg) and total sugar (16.82mg). In case of reducing and non reducing sugar Var. Dholka and Yerkud local were at par with each other. Least reducing sugar (9.58mg) was found in Ganesh while non reducing sugar (5.59mg) and total sugar (15.69mg) was found in susceptible Var. Arakta.

Interaction between varieties and inoculation for reducing sugar, non reducing sugar and total sugar was found significant. Among four varieties maximum reduction in reducing sugar (18.56%), non reducing sugar (15.37%) and total sugar (17.28%) was observed in Var. Ganesh while Dholka showed minimum reduction in reducing sugar (5.94%), non reducing sugar (9.67%) and total sugar (7.41%).

Diseased leaves contain low sugar compared to healthy leaves it might be due to hydrolysis of the sugar. Our results are in agreement with Gupta *et al.* (2010). Waghmare *et al.* 2012 reported that the plant infected with leaf spot of rose caused by *Alternaria alternata* showed decreased in the quantity of sugar and reducing sugar compare to healthy plant.

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