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Sunil Kumar Pipliwal
Department of Plant Pathology, College of Agriculture, SKRAU, Bikaner, Rajasthan, India

SL Godara
Department of Plant Pathology, College of Agriculture, SKRAU, Bikaner, Rajasthan, India

Corresponding Author:
Sunil Kumar Pipliwal
Department of Plant Pathology, College of Agriculture, SKRAU, Bikaner, Rajasthan, India

Role of biochemical constituents in fruit rot of chilli

Sunil Kumar Pipliwal and SL Godara

Abstract
Anthracnose of chilli is a serious disease affecting fruit yield and quality of fruit. India is the world leader in chilli production followed by China, Thailand and Pakistan. The experiment was conducted at department of plant pathology, college of agriculture, Bikaner and find out effect of biochemical constituents in fruit rot of chilli. The experiment was conducted under controlled conditions applying CRD design and all the treatments were replicated thrice. Diseased fruits of resistant variety Pusa Sadabahar, moderate resistant (Mathaniya) and susceptible (Pusa Jawala) varieties showed more phenols content compared to phenols in healthy fruits. Maximum total sugars and non-reducing sugars content were increased in anthracnose infected fruits of susceptible variety followed by moderate resistant and resistant variety. Reducing sugar and total protein content were decreased in anthracnose infected fruits of susceptible variety followed by moderate resistant and resistant variety.

Keywords: Pusa Sadabahar, Pusa Jawala, Mathaniya, phenol, sugars, protein

Introduction
Chilli commonly known as *Capsicum annuum* L. is a prominent vegetable crop belonging to the family solanaceae. It is an important spice, vegetable as well as cash crop in India grown in *Kharif* season which gives good returns to the cultivators. Chilli is good source of vitamins like A, B, C and minerals like Ca, P, Fe, Na and Cu in trace amounts. It is grown in tropical and subtropical regions of the world for its pungent fruits which are used both green and ripe. Chilli is also used in pickles, sauces, ketchup, essence, oleoresins and is an inevitable ingredient in Indian dishes. Alkaloid capsaicin is extracted from chilli, which has medicinal values. These properties increase the demand for chillies all over the world. India is the world leader in chilli production followed by China, Thailand and Pakistan. In our country, dry and green chilli are cultivated on an area of about 840 thousand hectares with an annual production of 2096 MT and about 316 thousand hectares with an annual production of 3634 MT, respectively (Anonymous, 2016-17) [1]. The important chilli growing states in our country are Andhra Pradesh, Odisha, Maharashtra, West Bengal, Karnataka, Rajasthan and Tamil Nadu (Subbiah and Jaykumar, 2009) [2]. In Rajasthan, the areas under dry and green chilli are about 9.95 thousand hectares with annual production of 20.15 MT and 8.58 thousand hectare with annual production of 27.73 MT (Anonymous, 2019-20) [3]. In Rajasthan, the major chilli growing districts are Jaipur, Jodhpur, Swai Madhopur, Ajmer, Bhilwara, Sikar, Tonk, Pali and Nagaur (Anonymous, 2019-20) [3]. The disease has been observed to occur in two phases, which are (i) leaf spot and dieback (ii) fruit rot. Typical anthracnose symptoms on chilli fruit include sunken necrotic tissues with concentric rings of acervuli. The biochemical's *viz*., Total phenols, sugars, total soluble protein, oxidative enzymes etc. are known to play important role in the defense mechanisms / resistance against the biotrophic pathogens, hemi-biotrophic and necrotic plant pathogens (Saharan *et al*., 2001 [4]. and Hasabnis *et al*., 2004) [5].

Material and Methods
In the present study, role of the biochemical's *viz.,* Total phenols, sugars (total, reducing and non-reducing), total protein were exploited in relation to resistance, moderate resistant and susceptible varieties of chilli against *C. capsici*. These Biochemical's were estimated from the fruits of resistant, moderate resistant and susceptible varieties of chilli, applying the standard biochemical analysis methods. The experiment was conducted under controlled conditions applying CRD design and all the treatments were replicated thrice**.**

Estimation of total phenol content
The total phenol content was estimated by the method described by Thimmaiah (1999) [6].

One gram of sample was ground in mortar and pestle with 10ml of 80 per cent ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The supernatant was filtered and the residue was re-extracted with five-time volume of 80 per cent ethanol, supernatant was cooled and evaporated to dryness in water bath. The residue was dissolved in 5 ml of distilled water on aliquot 0.2 ml was transferred in test tube and volume was made to 3 ml with distilled water. Folin-ciocalteau reagent (0.5 ml) was added in each test tube. After three minutes, 2 ml of 20 per cent sodium carbonate was added in each tube and mix thoroughly. The tubes were then placed in boiling water for one minute. After cooling the absorbance was measured at 650 nm against a reagent blank. The standard curve was prepared by taking different concentrations of catechol. The phenol content was express as mg g^{-1} fresh sample.

Estimation of sugar content

Total sugar analysis: Total sugar content was determined by colorimetric method using anthrone reagent. One ml of diluted fruit sample (100 times), 4 ml of anthrone reagent was added, heated for 20 minutes in a water bath, cooled to room temperature and absorbance was measured at 630 nm on spectrophotometer. The amount of sugars present in the sample was plotted against standard curve prepared from glucose. The sugar content in fruit samples was expressed as mg g^{-1} sample (Dubois *et al*., 1956) [7].

Reducing sugars: Reducing sugar content was measured by following "Nelson's modification of "Somogyi's method" (Somogyi, 1952) [8] using arseno-molybdate colour forming reagent and two copper reagent "A" and "B". One ml of fruit sample (100 times diluted) was added with a mixture of 1 ml copper reagent prepared from 24 part of copper "B" solutions. This mixture in test tubes was heated in boiling water bath, cooled, added with the colour forming reagent and absorbance was measured at 620 nm with spectrophotometer. The value was plotted against a standard curve prepared from glucose. The figures were expressed in fruit samples were expressed as mg g^{-1} sample.

Non- reducing sugar: The amount of non-reducing sugars was determined by following the formula suggested by Loomis and Shull (1937) [9]. The amount of non-reducing sugar was obtained by subtracting reducing sugar from the amount of total sugars and multiplying the resultant with a constant factor 0.95.

Estimation of soluble protein

The protein content of the fruit samples was determined using the method of Lowry *et al*. (1951) [10]. One gram of fresh fruit sample was macerated in mortar and pestle with 5 ml 0.1 M sodium phosphate buffer (pH 7.0). The homogenate so obtained was centrifuged at 16,000 rpm for 20 minutes. The supernatant was used for estimation of soluble protein content. For this purpose 2 per cent sodium carbonate (an-hydrous) in 0.1N NaOH (solution A) was prepared similarly, 0.5 per cent copper sulphate ($CuSO_4$ $5H_2O$) in 1 per cent sodium potassium tartarate (freshly made) was prepared and was regarded as solution B. From there, solution C (alkaline cupper solution) was prepared by mixing 50 ml solution A with 1ml of solution B test before use. An aliquot of 0.1 ml of supernatant was taken in test tube and the volume was to 1ml with distilled water followed by addition of 5 ml solution C mix well and incubated at room temperature for 10 minutes, 5.0 ml of Folin-ciocalteau reagent was diluted with distilled water, mixed well and incubated at room temperature in dark for 30 minutes. The absorbance was taken at 660 nm against blank. Amount of protein in sample was determined from the standard curve prepared by using different concentrations bovine serum albumin. The total protein content in fruit samples was expressed as mg g^{-1} sample.

Results and discussion

Total phenol: The study was conducted to evaluate the role of total phenol in the fruits of contrasting chilli varieties during pathogenesis. Phenol content (Table 1 and Fig.1) in healthy and infected (*C. capsici*) fruits of chilli varieties was estimated at 75 DAT. More content of total phenols were recorded in diseased fruits than in healthy ones. Diseased fruits of resistant variety, Pusa Sadabahar showed more content of total phenol (1.21 mg /g sample) compared to total (0.94 mg/g sample) phenols in healthy fruits. Similar trend observed in moderate resistant and susceptible varieties, where diseased fruits recorded 1.04 mg/g sample and 0.85 mg/g sample against healthy fruits of 0.84 mg/g sample and 0.75 mg/g sample of total phenol content, respectively. However, the rate of increases in total phenols (+29.16%) were higher in resistant variety Pusa Sadabahar than that of the moderately resistant variety Mathaniya (+23.10%) and lower in susceptible variety Pusa Jawala (+13.38%). The total phenol content was increased from 13.38 to 29.16 per cent in infected fruits as compared to healthy ones. The increase in total phenol content in response to disease in resistant variety is in agreement with the observations of Prasath and Ponnuswami, (2008) [11]. Anand *et al*. (2009) [12]. has also been noted that total phenol content increased in ripe chilli fruits after the inoculation and thereafter the content started to decrease. Although, it is difficult to pin point the cause of increased level of phenol from the expression of the disease symptoms of different growth stages. Accumulation of phenol does occur due to several reasons, principally due to release of phenolic compounds which exist in the form of glycosides and esters (Saunders *et al.*, 1977) [13].

Soluble protein: There was significant decrease in soluble protein content at 75 DAT in diseased fruits as compared to healthy fruits in all three varieties of chilli (Table 1). Maximum reduction in soluble protein (-37.12%) due to *C. capsici* infection was observed in susceptible variety Pusa Jawala followed by Mathaniya (-22.40%). However, soluble protein reduced in diseased resistant variety Pusa Sadabahar about -8.08 per cent. The soluble protein was observed more in healthy fruits of resistant variety Pusa Sadabahar than moderately resistant variety Mathaniya. Healthy fruits of resistant variety were recorded higher protein content of 4.40 mg/g sample when compared to 4.04 mg/g sample in diseased fruits and in moderately resistant variety, where 4.27 mg/g sample protein content was recorded in healthy fruits and 3.31 mg/g sample in diseased fruits. In susceptible variety protein content was 4.20 and 2.64 mg/g sample were found in healthy and diseased fruits, respectively (Fig. 2). The present findings are in agreement with the earlier reports. Sunkad and Kulkarni (2006) [14] and Mathivanan *et al.* (2013) [15] were studied on biochemical changes *viz.*, sugar, phenol, ortho-dihydroxy phenol and protein than the resulting in total protein more

content in healthy plants as compared to infected plants of groundnut. These enzymes permitted the pathogen to use the host protein as source of nitrogen and amino acid for their growth and development. Decrease in protein content might be due to hydrolysis of protein by fungal proteolytic enzymes (Jamaluddin *et al*. 1977) [16].

Table 1: Change in total phenol and protein content in fruits of chilli varieties infected by *C. capsici*

Variety	Disease reaction	Phenol content (mg/g sample)		Protein content (mg/g sample)	
		Healthy	Diseased	Healthy	Diseased
Pusa Sadabahar	Resistant	0.94*	1.21* (+29.16) #	4.40*	4.04* (-8.08) #
Mathaniya	Moderate resistant	0.84	1.04 (+23.10)	4.27	3.31 (-22.40)
Pusa Jawala	Susceptible	0.75	0.85 (+13.38)	4.20	2.64 (-37.12)
	S.Em±		C.D. (P=0.05)	S.Em±	C.D. (P=0.05)
Variety	0.005		0.017	0.013	0.042
Healthy/Diseased	0.004		0.014	0.011	0.034
Variety×Healthy/Diseased	0.008		0.024	0.019	0.060

(+) or (-)= Per cent increase or decrease in phenol or protein content, *Mean of three replication, #Values in parentheses indicate per cent deviation in diseased fruits over healthy fruits of corresponding variety

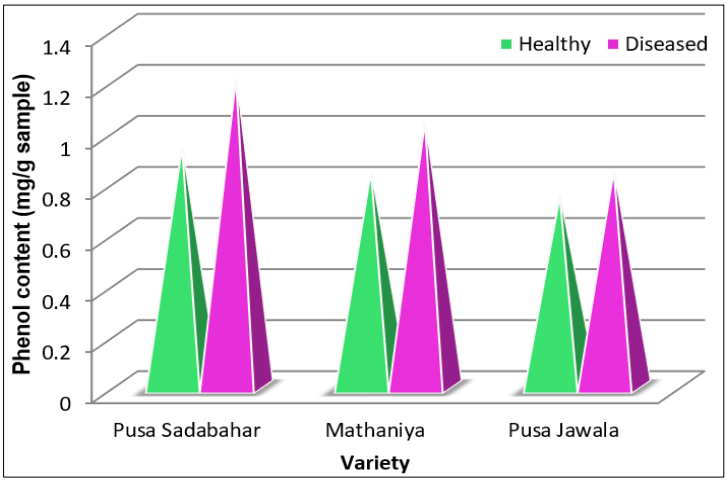

Fig 1: Effect on phenol content in healthy and diseased fruits of chilli

Sugars (Total, reducing and non-reducing)

Total sugar: Total sugar content was more in diseased fruits when compared to healthy fruits. Maximum total sugar content of 9.67 was recorded in diseased fruits of susceptible variety (Pusa Jawala) against 7.88 mg/g sample in healthy fruits (Table 2 and Fig. 3). While, the least total sugar content of 6.17 mg/g sample was recorded in healthy fruits of resistant variety (Pusa Sadabahar) when compared to 6.52 mg/g sample in diseased fruits. Maximum total sugar content was increased in anthracnose infected fruits of susceptible variety Pusa Jawala (+22.63%), followed by moderate resistant variety Mathaniya (+13.08%) and resistant variety Pusa Sadabahar (+5.71%).

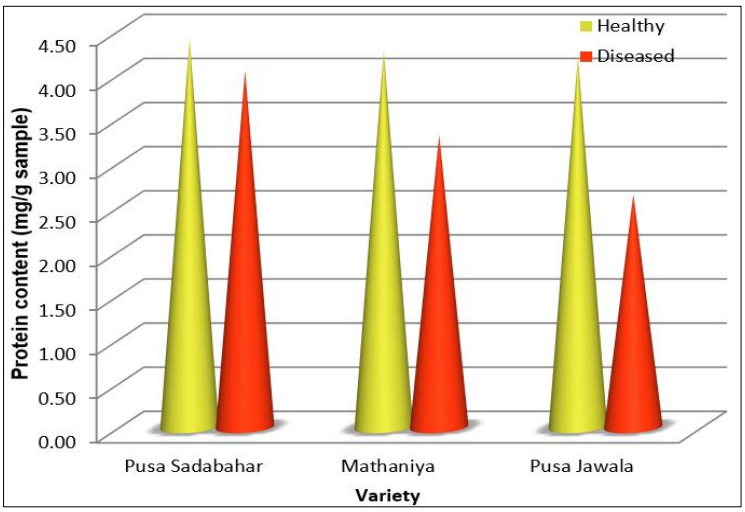

Fig 2: Effect on protein content in healthy and diseased fruits of chilli

Reducing sugar

Reducing sugar content in healthy and diseased fruits followed the reverse trend of results as observed in total sugars. Healthy fruits of susceptible variety were recorded higher reducing sugar content of 1.92 mg/g sample when compared to 1.28 mg/g sample in diseased fruits and in moderately resistant variety, where 1.63 mg/g sample reducing sugar was recorded in healthy fruits and 1.17 mg/g sample in diseased fruits. In the similar passion, in resistant variety, where 1.52 mg/g sample reducing sugar was recorded in healthy fruits and 1.31 mg/g sample in diseased one. Maximum reducing sugars content were reduced in anthracnose infected fruits of susceptible variety Pusa Jawala (-33.56%), followed by moderate resistant variety Mathaniya (-28.08%) and resistant variety Pusa Sadabahar (-13.98%). (Table 2 and Fig. 3).

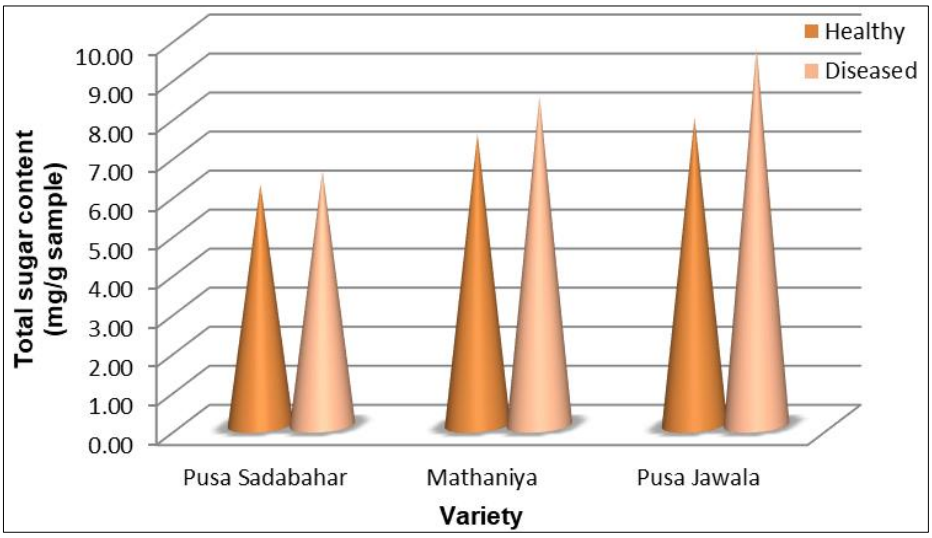

Fig 3: Effect on total sugar content in healthy and diseased fruits of chilli

Non-reducing sugar

Non–reducing sugar contents (Table 2) in healthy and diseased one varied greatly in the susceptible, moderately resistant and resistant varieties. Healthy fruits of resistant variety showed less content of non-reducing sugar of 4.42 mg/g sample compared to that in diseased fruits 4.96 mg/g sample of same variety and Mathaniya variety showed 5.53 mg/g sample compared to 6.90 mg/g sample in disease fruits. The same trend was followed in susceptible variety, where healthy fruits of susceptible variety were recorded 5.66 mg/g sample of non-reducing sugar compared to 7.97 mg/g sample in diseased. The maximum non reducing sugars were increased in susceptible variety (+40.74%) followed by moderate variety (+24.60%) and resistant variety (+12.13%). (Fig. 4).

The maximum increasing of total sugars and non-reducing sugars were found in susceptible variety Pusa Jawala followed by moderate variety Mathaniya and resistant variety Pusa Sadabahar. The similar trend was observed in case of reducing sugars, the maximum decreasing was found in susceptible variety Pusa Jawala followed by moderate variety Mathaniya and resistant variety Pusa Sadabahar.

The changes in chilli fruits infected by anthracnose pathogen as explained above are in confirmation to the results obtained by previous workers. Azad (1991) [17] estimated major chemical constituents in the tissue system of ripe fruits of resistant, moderately resistant and susceptible varieties of chilli, in both healthy and pathogenic conditions with *C. capsici* and reported that maximum percentage of sugar (reducing, non-reducing and total) was observed in susceptible variety than the resistant variety. Hegde and Anahosur (2001) [18]. Found higher content of total sugar in healthy chilli fruits than infected fruits. The present study revealed that the low amount of carbohydrates in resistant variety may be responsible for resistance of genotypes against anthracnose of chilli

Table 2: Change in total, reducing and non-reducing sugar content in fruits of chilli varieties infected by *C. capsici*

Variety	Disease reaction	Total sugar content (mg/g sample)		Reducing sugar content (mg/g sample)		Non-reducing sugar content (mg/g sample)	
		Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Pusa Sadabahar	Resistant	6.17*	6.52* (+5.71) #	1.52*	1.31* (-13.98) #	4.42*	4.96* (+12.13) #
Mathaniya	Moderate resistant	7.45	8.43 (+13.08)	1.63	1.17 (-28.08)	5.53	6.90 (+24.60)
Pusa Jawala	Susceptible	7.88	9.67 (+22.63)	1.92	1.28 (-33.56)	5.66	7.97 (+40.74)
	S.Em±		C.D. (P=0.05)	S.Em±	C.D. (P=0.05)	S.Em±	C.D. (P=0.05)
Variety	0.05		0.16	0.03	0.09	0.04	0.14
Healthy/Diseased	0.04		0.13	0.02	0.07	0.04	0.11
Variety×Healthy/Diseased	0.07		0.22	0.04	0.13	0.06	0.19

(+) or (-)= Per cent increase/decrease in sugars, *Mean of three replication, #Values in parentheses indicate per cent deviation in diseased fruits over healthy fruits of corresponding variety

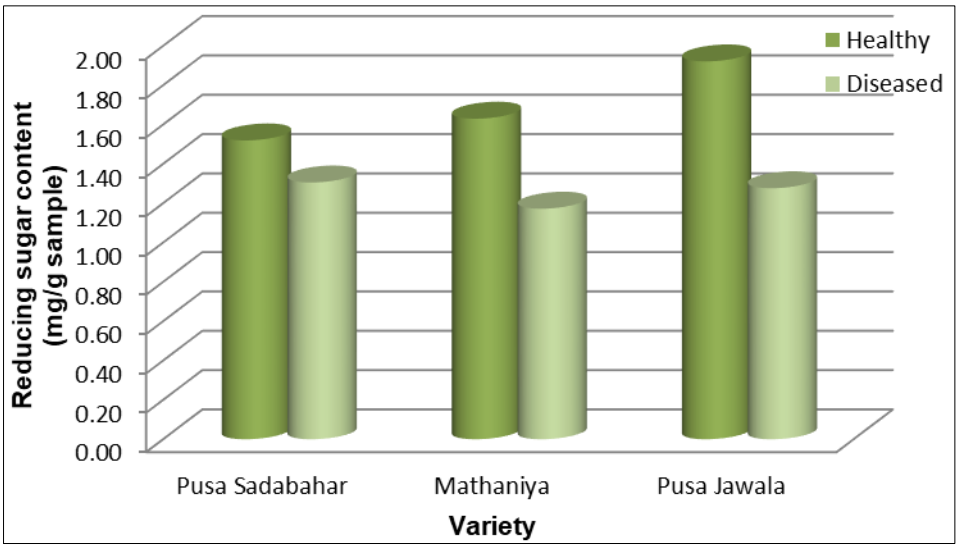

Fig 4: Effect on reducing sugar content in healthy and diseased fruits of chilli

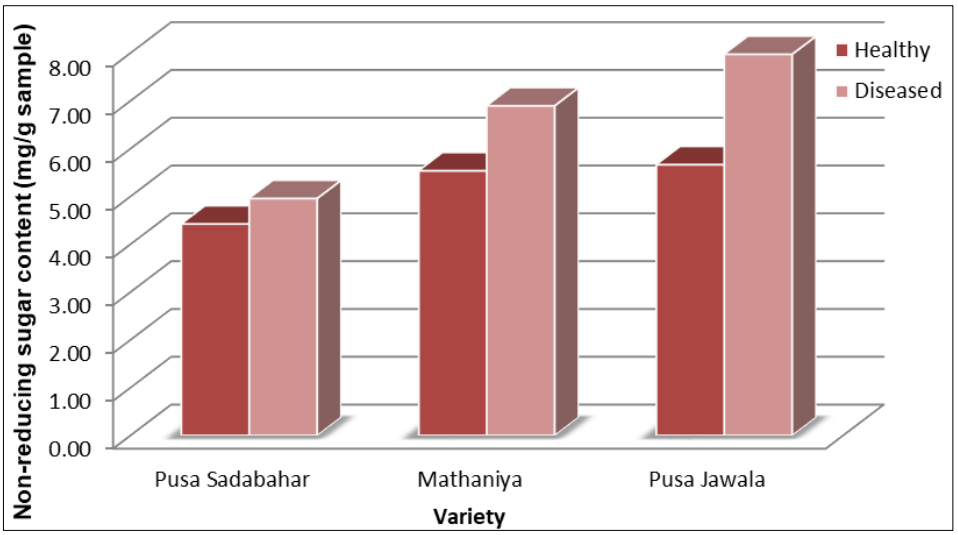

Fig 5: Effect on non reducing sugar content in healthy and diseased fruits of chilli

Conclusion

The biochemical studies indicated that more content of total phenols were recorded in diseased fruits than in healthy ones. Diseased fruits of resistant variety Pusa Sadabahar showed more phenols content compared to phenols in healthy fruits. Similar trend observed in moderate resistant (Mathaniya) and susceptible (Pusa Jawala) varieties. Maximum total sugars and non-reducing sugars content were increased in anthracnose infected fruits of susceptible variety followed by moderate resistant and resistant variety. Reducing sugar and total protein content in healthy and diseased fruits followed the reverse trend of results as observed in total sugars.

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