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Biochemical changes in coriander plant parts infected by stem gall disease caused by *Protomyces macrosporus*

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Abstract
Biochemical studies of plant pathogen interaction revealed that soluble sugar, starch, chlorophylls, phenolic and tannin content were observed higher level in healthy leaves compared to pathogen infected leaves. Only scanty information is available for biochemical changes in coriander plants during the growth of *Protomyces macrosporus*. The aim of this study was to increase knowledge regarding the physiological and biochemical changes during the occurrence of stem gall disease and try to define changes in phenols, flavonoids and tannins that might be related to the potential antioxidants and may be efficient as preventive agents in the pathogenesis of same disease. The experiments were conducted during 2016 at Plant Pathological laboratory and Vegetable Farm, Department of Vegetable Science, Narendra Deva University of Agriculture and Technology, Narendra Nagar, Kumarganj, Ayodhya (U.P.) with four treatments and three replication. Biochemical studies were revealed that total phenol content was highest in healthy plant parts (2.97-6.20 µg/g) as compared with infected plant parts (2.53-5.58 µg/g). Total tannin content was found highest in leaves (3.29-3.92 µg/g) and lowest in inflorescence (2.31-2.79 µg/g) in both stem gall infected and healthy plants, flavonoids content in healthy plant parts was (0.98-1.47µg/g) as compared to infected plant parts (0.77-1.07 µg/g). Whereas saponin content was 0.99-1.80% in infected plant and 1.93-2.16% in healthy plants of coriander.

Keywords: Biochemical, stem gall, *Protomyces* and coriander

Introduction
Stem gall disease caused by *Protomyces macrosporus* Unger is an important disease in all coriander growing area of Madhya Pradesh, Bihar, Uttar Pradesh and adjoining district of Rajasthan. The disease manifests itself in the form of galls on stems, branches, leaves, petioles and fruits, causes 15-20 per cent yield loss (Pandey and Dange, 1998) [19] and deteriorates quality of seeds (Lakra, 1993) [13]. Crop is grown in eastern part of Uttar Pradesh for both green leaves and seed yield. The crop is suffering number of biotic stresses *viz*; Stem gall, powdery mildew and wilt disease with. Among them, stem gall disease is causing 20.8 percent yield losses in eastern part of Uttar Pradesh. Recent past, incidence of stem gall disease has increased significantly and become a major limiting factor for successful cultivation of coriander. The genus *Protomyces macrosporus* Unger are obligate parasites within the *Apiaceae* family causing galls on stems, leaves, flowers and fruits. On the leaves galls are restricted to petiole, midrib, veins and veinlet's (Buren, 1915, 1922) [4, 3]. In these galls ascogenous cells are present. Ascogenous cells are spherical to sub spherical with thick and smooth walls, formed intercalary in the intercellular mycelium throughout the infected tissue (Gjaerum, 1964) [7]. The bio trophic phase of mycelium depends on the host tissue for nutrients to sustain its growth and development. The marked characteristic of the parasitic life style of plant pathogen is the unidirectional transfer of nutrients from the host to pathogen (Wei *et al.*, 2004) [23]. Biochemical studies of plant pathogen interaction revealed that soluble sugar, starch, chlorophylls, phenolic and tannin content were observed higher level in healthy leaves compared to pathogen infected leaves (Anjum *et al.* 2012) [1]. Only scanty information is available for biochemical changes in coriander plants during the growth of *Protomyces macrosporus*. The aim of this study was to increase knowledge regarding the physiological and biochemical changes during the occurrence of stem gall disease and try to define changes in phenols, flavonoids and tannins that might be related to the potential antioxidants and may be efficient as preventive agents in the pathogenesis of same disease.

Materials and Methods

Diseased samples were collected from the coriander crop grown at Vegetable Farm, Narendra Deva University of Agriculture and Technology, Narendra Nagar (Kumarganj), Ayodhya (U.P.) during 2016.

Biochemical study

Infected and healthy plant parts *viz*. leaves, stem, seed and inflorescence were collected from stem gall infected coriander field to study the biochemical changes such as total phenol, tannins, flavonoids and saponins. The methodology are described as under-

Total phenol

Procedure

The healthy and infected plant parts *viz*. leaves, stem, inflorescence, and seed were directly selected from the field for the estimation of total phenol. One gram of dried sample was weighted, cut into small pieces and then placed in smearing methanol until the green colour was extracted. Leaves tissues were homogenized after decanting the methanol. These homogenized tissues were again boiled in methanol for further 5 minute and then filtered. Residual material was washed with 80% acidified (0.1% HCL conc.) methanol. Methanol was evaporated using a rotavapour and the aqueous layer was collected to adjust the final volume as ml/g of weight with distilled water. The aqueous portion of the extract was then washed with nhexane to remove the green colour. Total phenols were estimated using Folin-Ciolcalteu reagent, according to the modified method. Place the tubes in boiling water for one minutes allowed to cool and measured the absorbance at 650 nm against a reagent blank. Standard curve was prepared using different known concentration of catechol. The absorbance was measured in a spectrophotometer at 650nm.

Calculation

From the standard curve find out the concentration of phenols in the test sample and express as µg phenols/g material. The total phenols are calculated by the following formula.

$$\mu g/g = \frac{\text{G.F. x O. D. x Total volume}}{\text{Aliquot taken x Weight of sample}} \times 1000$$

G. F. = Graph factors
O.D. = observance density

Estimation of tannin content

Sample collection and preparation of plant solution

Healthy and infected stem gall of coriander plant parts were collected at the time of flowering and fruiting. The crude powders of the leaves, stem, inflorescence and seed were prepared for photometric determination of tannins. The standard procedure was followed by folin-Denis method. Powdered material of each (0.05g) was transferred into 250ml conical flask and adds 75ml water. Heat the flask gently and boil 30 minutes. The solution was centrifuged at 2000rpm for 20 minutes and collects the supernatant in 100ml volumetric flask and make up the volume. Then transfer 1.0ml of extract sample into 100 ml volumetric flask containing 75ml distilled water, and add 5.0ml of Folin-Denis reagent and 10ml sodium carbonate solution (sodium carbonate, 350g was dissolved in 1000ml water at 70 0C temperature), solution was allowed to stand overnight and then it was filtered through glass wool and dilute it with 100ml of distilled water and shaken well. Read the absorbance at 700nm after 30minutes against blank (water).

Preparation of standard curve

10ml of standard tannic acid solution (100g of tannic acid was dissolved in 100 ml distilled water) was made up with distilled water. 1-10ml aliquots were taken in clean test tubes of 0.5ml Folin-Denis reagent and 100ml of sodium carbonate solution was added to each tube. Each tube was made up to 10.0ml with distilled water. The reagents add in each tube and after about 30 minutes read the absorbance at 700nm against blank reagent.

Estimation of flavonoids

Total flavonoids were determined according to the methods of (Nabavi *et al.* 2008) [16]. Powdered sun dried leaves, stem, inflorescence and seed (1.0g each) were extracted in a sox let extractor with 10 mL 80% methanol and shaking for 2 h. Total flavonoids extract (0.4 mL) was added to 4 mL of distilled water. Then 0.3 mL of 5% NaNO2 was added. After 5 min, 0.3 mL of 10% AlCl3 was added. After 6 min, 2 mL of 1M NaOH was added and the total volume was made up to 10 mL with distilled water. The absorbance was measured at 510 nm against a blank reagent. Catechin used to prepare the standard curve. The flavonoid content was calculated using the following linear equation.

$$A = 0.01069\ C - 0.00163,\ r = 0.9998$$

Where

A = is absorbance
C = is flavonoids content in µg.g-1

Estimation of Saponin

Sources of Materials

The healthy and infected leaves, stems, inflorescence and seed of coriander were collected in the months of March at maturity stage of crops. The leaves, stem, inflorescence and seed were sun dried for seven days. The dried samples were then crushed with mortar and pestle before grinding into fine powder using a manual grinder.

Qualitative Determination of Saponin

The homogenous sample of each of the samples of the leaves, stem, inflorescence, and seeds were used for qualitative determination of saponin according to the methods described by (Nyam *et al.* 2009) [18]. A measured weight (5g) of the powdered sample was mixed with 50 ml of 20% aqueous ethanol solution in a flask. The mixture was heated with periodic agitation in water bath for90 minutes at 55ºC; it was then filtered through Whatman filter paper (No 42). The residue was extracted with 50 ml of 20% ethanol and both extract were poured together and the combined extract was reduced to about 40 ml at 90ºC and transferred to a separating funnel where 40 ml of diethyl ether was added and shaken vigorously. Re extraction by partitioning was done repeatedly until the aqueous layer become clear in colour. The saponins were extracted, with 60 ml of normal butanol. The combined extracts were washed with 5% aqueous sodium chloride (NaCl) solution and evaporated to dryness in a pre-weighed evaporation dish. It was dried at 60ºC in the oven and reweighed after cooling in desiccators. The process was repeated two more times to get an average. Saponin content

was determined by difference and calculated as a percentage of the original sample as described by Harbone (1973).

$$\text{Saponin (\%)} = \frac{W2-W1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where-
W1 = Weight of evaporating dish
W2 = Weight of evaporating dish + sample

Statistical Analysis
The quantitative data obtained were statistically analyzed by calculating the mean of three replicates followed by calculation of the Sum of Square, Variance, Standard Deviation and Standard error. The results were presented as mean + standard error.

Result and Discussion
Biochemical changes in healthy and infected plant parts of coriander
Perusal of data revealed that total phenol content was highest in healthy plant parts (2.97-6.20µg/g)as compared with stem gall infected plant parts (2.53-5.58µg/g). The stem gall disease markedly decreased total phenol content in all parts of coriander. The reduction was 32.77% in stem, 14.80% in inflorescence, 10.00% in leaves and 8.44% in seeds. Total tannin content was significantly highest in leaves (3.29-3.92 µg/g) and lowest in inflorescence (2.31-2.79 µg/g) in both stem gall infected and healthy plants. Reduced tannin content was significant in all parts of infected plants. The reduction was markedly highest in inflorescence (17.20%) followed by stem (16.53%), leaves (16.07%) and seeds (15.07) over the healthy plant parts respectively. Healthy plants of coriander brought about significant enhancement of flavonoids content (0.98-1.47µg/g) as compared to infected plant parts (.77-1.07µg/g). Stem gall disease was markedly decreased the amount of flavonoids content (17.64-27.21%). The maximum reduction was observed in leaves (27.20%) and minimum in seeds (17.64%). Stem gall disease significantly decreased the saponin content (0.99-1.80%) as compared to healthy plants of coriander (1.93-2.16%). The maximum decrease was in inflorescence (48.70%) followed by leaves (23.61%), seed (14.50%) and stem (10.00%) plants are a rich source of thousands of secondary metabolites. These are low molecular weight compounds that are not essential for sustaining life but are crucial for survive of the organisms (Zhuang 1992) [24]. These compounds are phenol, tannins, flavonoids and saponins. Which lead to a range of defense responses in the host plants? In present study total phenol content was found highest in healthy plants and lowest in infected plants. Phenol compounds may be confirming resistance to a disease by limiting the growth of the pathogen (Isaac, 1992) [12]. Stem gall infection was severe on stem hence reduction of phenol substances was found maximum in stem and minimum in seed. The finding was supported by (Gogoi *et al.*, 2001) [8], was observed an immediate accumulation of phenol following pathogenic attack in resistant varieties, whereas susceptible varieties did not accumulate significantly higher phenol substances. Tannins content was significantly higher in leaves and lowest in inflorescence in both healthy and infected plants. Czech- Kozbowskee and Krywanasks, (1984) detected poly phenol oxides activity is higher in the infected resistant variety than in infected tissue of susceptible varieties. Tannins have been documented as antimicrobial compound because condensed tannins can inhibit basidiospore germination and also altered germ tube morphology of *C. perniciosa*, Brownlee *et al.*, 1990 [2] and Ndoumou *et al.*, 1996 [17]. Stem gall disease markedly decreased the flavonoid and saponin content in all infected parts, the maximum reduction was in leaves and inflorescence respectively. The high level of saponin in the root might be as results of the need to protect plant against soil borne pathogen attack. It has been also noted that many saponins are present in healthy plants in high concentration because of these antifungal properties Papadopoulou *et al.*, (1999) [20]. Saponin presence in stem and tem bark might to be serving as natural defense against viral, bacterial and fungal infection Geyter *et al.*, (2007) [6].

Table 1: Study of biochemical changes in healthy and infected plant parts of coriander.

S. No.	Treatment	Total Phenol (µg/g)			Tannin (µg/g)			Flavonoids (µg/g)			Saponin (%)		
		Healthy	Infected	% decrease	Healthy	Infected	% decrease	Healthy	Infected	% decrease	Healthy	Infected	% decrease
1.	Leaves	6.20	5.58	10.00	3.92	3.29	16.07	1.47	1.07	27.20	2.16	1.65	23.61
2.	Stem	4.18	2.81	32.77	3.87	3.23	16.53	1.26	0.99	21.42	2.00	1.80	10.00
3.	Seed	4.5	4.12	8.44	3.78	3.21	15.07	1.19	0.98	17.64	2.00	1.71	14.50
4.	Inflorescence	2.97	2.53	14.80	2.79	2.31	17.20	0.98	0.77	21.42	1.93	0.99	48.70
	SEm±	0.12	0.26		0.16	0.19		0.14	0.08		0.08	0.11	
	CD (0.5%)	0.40	0.89		0.55	0.64		0.47	0.28		0.29	0.39	

Conclusion
The study was conducted to biochemical changes check the different plant parts of coriander. Biochemical changes such as phenol, tannin content, flavonoids and saponin content was studies. It can be concluded maximum in healthy parts compare to infected plant parts.

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