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Role of non-conventional chemicals in biochemical defence against charcoal rot of sesame incited by *Macrophomina phaseolina* (Tassi) Goid

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Abstract
Charcoal rot of sesame (*Sesamum indicum*) incited by *Macrophomina phaseolina* (Tassi) Goid. is an economically important disease in India and worldwide. Seed and soil borne nature, non-specific, wide host range of pathogen, difficult to manage with fungicides and absence of complete genetic resistance impelled to biochemical defence analysis against this pathogen. Treatment with non-conventional chemicals induces defence against pathogen in the form biochemical changes in plant. Hence, the present investigation was carried out to know the impact of non-conventional chemicals on changes in defence biochemical parameters in plant. As the concentration of different non conventional chemicals increased from 50, 100 and 200 ppm there was reduction in incidence of charcoal rot disease. Maximum induction of resistance was recorded by the application of salicylic acid at 200 ppm concentration after challenge inoculation with *M. phaseolina* followed by indole acetic acid, indole butyric acid, acetyl salicylic acid, riboflavin and thiamine. The activity of total phenols and sugar content was reached at peak after six days of challenge inoculation with the pathogen and subsequently activity of these parameters slightly declined after six days of inoculation in all tested non-conventional chemicals. At each concentration, SA showed maximum induction of total phenol and total sugar followed by IAA, IBA, ASA, thiamine and riboflavin. However, sesame variety HT 2 showed higher induction of biochemical activities and lower disease incidence of charcoal rot as compared to HT 1.

Keywords: Sesame, charcoal rot, *Macrophomina phaseolina*, total phenol, sugar content

Introduction

Sesame (*Sesamum indicum* L.) is an important annual oilseed crop belongs to the family *Pedaliaceae*. Plant has erect, pubescent stem, arrangement of lower leaves opposite, alternate, flower hermaphrodite and fruit called as capsule. Sesame is grown mainly for seed purpose which has a good quality food, nutrition, health care and religious value. Due to potent medicinal properties in leaves, oil and seeds of sesame are used for treatment of ulcers, cough, asthma and many other diseases. It is a high valued crop due to its economical, nutritional and medicinal properties. Despite of high nutritive, economic value and large acreage, production and productivity of crop is low in sesame growing areas in state as well as in the country. Several biotic and abiotic stresses during crop growth stages resulted in low production and productivity in the country. Charcoal rot caused by *Macrophomina phaseolina* is one of the most destructive diseases and causes heavy losses. It is worldwide distributed pathogen and infects different parts of plant and causes root rot, stem rot and seedling blight (Moi and Bhattacharyya, 2008) [8]. The pathogen attacks mostly at the basal region of the plant (Kumar *et al*., 2011) [7].

Management of charcoal rot fungus is difficult due to its variable nature, long term survival and wider host range. A safe and eco-friendly approach for its management is done through resistance induction by using potential chemical elicitors. It involves artificial inoculation of pathogen to induce resistance in the chemical elicitors' pre-treated plant. It induces biochemical changes and enhances accumulation of total phenol and total sugar content imparting role in resistance of plant.

Induction of genetic resistance in plant, proper sowing time and proper seed treatment with non-conventional chemicals is an effective means for integrated disease management. Sharma *et al.* (2011) [13] observed that induction of phenols and defence enzyme increased in salicylic acid treated sesame after inoculation of *M. Phaseolina.* Sahar-Zayan (2016) [11] reported the positive effect of inducer chemicals *viz.*, salicylic acid, ascorbic acid, benzothiadiazole and bion on systemic resistance against charcoal rot and enhanced growth and yield of okra. Nie and Xu (2016) [10] observed riboflavin as a resistance elicitor to protect plants against various pathogens. Adrees *et al*. (2019) [2] reported that chemical elicitor increased the activities of defence related biochemical like total phenolics and related enzymes which confirmed the induced systemic resistance against charcoal rot of cotton. Non conventional chemicals can be used to trigger defence, since they also known to change the activity of important biochemical constituents for disease defence.

Materials and methods

The experiment was conducted under screen house conditions with two varieties HT 1 and HT 2. Fifteen days old plants were treated with six non-conventional chemicals @ 50, 100 and 200 ppm concentration each and after two days of treatment pots were inoculated with *M. phaseolina*. The leaves samples at various intervals (0, 2, 4, 6 and 8 days) were collected from treated and untreated control plants and immediately homogenized in liquid nitrogen for further study for analyzing total phenol content determined by using the method of Swain and Hills (1959) [14] and total sugar content by method of Dubosis *et al*. (1956).

Estimation of total phenol content

Leaves samples of 100 mg from each concentration (50, 100 and 200 ppm) were taken at required intervals, macerated with 10 ml of 80% ethanol in pre chilled pestle and mortar. The extract was centrifuged at 12,000 rpm for 15 minutes. The volumetric flasks with supernatant were kept on water bath at 80ºC for 30 minutes. Subsequently, 1 ml of extract was added with 4 ml of distilled water and made the final volume 5 ml in a test tube.

Reagents

1. Folin-Ciocalteau reagent: 1ml Folin-Ciocalteau reagent added with 1ml of distilled water.
2. Sodium carbonate (Na_2CO_3): Saturated Na_2CO_3 (20%) solution was prepared by dissolving 17.5 g Na_2CO_3 in 50 ml of distilled water and subjected to heating on water bath at 70ºC followed by cooling the content overnight and filtered.

Procedure:

Leaves extract of 0.5 ml containing 80 per cent ethanol was added with 5 ml of Folin-Ciocalteau reagent. After 3 minutes, 2 ml of saturated Na_2CO_3 solution was added in each test tube and shaken thoroughly. These test tubes were kept in dark and after sixty minutes absorbance was measured against reagent blank by UV spectrophotometer at 650 nm. The total phenol content was estimated by a calibration curve of (10-50 mg) tannic acid equivalent and expressed in mg phenol/g fresh weight of tissue.

Estimation of total sugar content

Total sugar content in leaves sample was determined by the method as described by Dubois (1956).

Extraction

Method of extraction of total sugar was similar to the extraction of total phenol content.

Reagents

1. Phenol (2%)
2. Concentrated sulphuric acid (95.5%)

Procedure

Leaves extract of 0.5 ml containing 80% ethanol was added with 2 ml phenol (2%) solution in test tubes and shaken thoroughly. After 3 minutes, extract was added carefully with 2.2 ml concentrated H_2SO_4 solution and mixed properly. Afterwards test tubes were kept in dark for 60 minutes for measurement of absorbance against blank reagent at 490 nm by using UV spectrophotometer. The total sugar content was estimated by a calibration curve of glucose and expressed as mg sugar/g fresh weight of tissue.

Results

The effect of non-conventional chemicals (50, 100 and 200 ppm) after challenge inoculation of *M. phaseolina* was studied to know the activity of total phenol content and total sugar content in leaves of sesame. The total phenol content was induced after challenge inoculation of *M. Phaseolina* and reached its peak on six days after inoculation (DAI) in pre-treated sesame varieties with six different non-conventional chemicals. However, after six DAI activity of phenol content was decreased in both varieties of sesame (Table 1and 2). Furthermore, total phenol content was comparatively more induced at higher concentration (200 ppm) as compared to the lower concentrations (50 ppm) of non-conventional chemicals. SA at higher concentration showed maximum induction of total phenol content (0.75 and 0.81 mg/g fresh weight) followed by IAA (0.66 and 0.70 mg/g fresh weight) on six DAI in varieties HT 1 and HT 2, respectively. However, after six DAI induction of phenol content was slightly decreased at higher concentration of SA (0.62 and 0.65 mg/g fresh weight) in varieties HT 1and HT 2, respectively. Among all treatments at higher concentration (200 ppm), riboflavin showed minimum induction of total phenol content (0.50 and 0.60 mg/g fresh weight) on six DAI in varieties HT 1 and HT 2, respectively.

The induction of total sugar content was enhanced in pre-treated sesame varieties by six non-conventional chemicals after challenge inoculation of pathogen and reached at peak on six DAI and subsequently declined after six DAI (Table 3 and 4). Salicylic acid (200 ppm) showed maximum induction of sugar content (3.69 and 3.71mg/g fresh weight) followed by IAA (3.40 and 3.43 mg/g fresh weight) and minimum by riboflavin (2.42 and 2.45 mg/g fresh weight) at six DAI in HT 1 and HT 2, respectively. However, it was decreased rapidly after six DAI (3.10 and 3.12 mg/g fresh weight) at higher concentration of SA in varieties HT 1and HT 2, respectively.

Table 1: Effect of non-conventional chemicals on phenol content in the leaves of sesame variety HT 1

Treatments	Concentration (ppm)	Phenol content (mg/g dry weight)					
		0 DAI	2 DAI	4 DAI	6 DAI	8 DAI	Mean
Salicylic acid		0.43	0.46	0.55	0.65	0.59	0.53
	100	0.46	0.48	0.57	0.69	0.60	0.56
	200	0.50	0.52	0.62	0.75	0.62	0.60
Acetyl salicylic acid	50	0.37	0.38	0.42	0.54	0.50	0.44
	100	0.38	0.39	0.44	0.56	0.52	0.45
	200	0.40	0.42	0.47	0.58	0.54	0.48
Indole acetic acid	50	0.41	0.43	0.50	0.62	0.56	0.50
	100	0.43	0.46	0.52	0.64	0.58	0.52
	200	0.47	0.50	0.54	0.66	0.60	0.55
Indole butyric acid	50	0.38	0.40	0.46	0.58	0.53	0.47
	100	0.41	0.44	0.48	0.59	0.55	0.49
	200	0.43	0.46	0.52	0.62	0.57	0.52
Riboflavin	50	0.30	0.32	0.37	0.46	0.41	0.37
	100	0.32	0.34	0.38	0.48	0.43	0.39
	200	0.36	0.38	0.42	0.50	0.45	0.42
Thiamine	50	0.35	0.37	0.41	0.48	0.47	0.41
	100	0.36	0.38	0.42	0.49	0.48	0.43
	200	0.38	0.40	0.45	0.52	0.50	0.45
Control		0.28	0.29	0.32	0.39	0.35	0.33
Mean		0.39	0.41	0.46	0.57	0.52	0.47
		Treatment		DAI		Treatment × DAI	
Sem±		0.003		0.001		0.005	
CD (p=0.05)		0.006		0.003		0.014 0.014	

Note: DAI – Days after inoculation

Table 2: Effect of non-conventional chemicals on phenol content in the leaves of sesame variety HT 2

Treatments	Concentration (ppm)	Phenol content (mg/g dry weight)					
		0 DAI	2 DAI	4 DAI	6 DAI	8 DAI	Mean
Salicylic acid		0.50	0.52	0.60	0.71	0.61	0.59
	100	0.54	0.55	0.65	0.79	0.62	0.63
	200	0.57	0.65	0.67	0.81	0.65	0.67
Acetyl salicylic acid	50	0.40	0.46	0.50	0.60	0.52	0.50
	100	0.41	0.48	0.51	0.65	0.54	0.52
	200	0.44	0.50	0.52	0.65	0.58	0.54
Indole acetic acid	50	0.47	0.50	0.53	0.67	0.59	0.55
	100	0.51	0.54	0.55	0.68	0.61	0.58
	200	0.51	0.57	0.58	0.70	0.64	0.60
Indole butyric acid	50	0.45	0.49	0.52	0.65	0.56	0.53
	100	0.46	0.50	0.54	0.67	0.57	0.55
	200	0.48	0.53	0.56	0.68	0.61	0.57
Riboflavin	50	0.32	0.36	0.43	0.52	0.46	0.42
	100	0.35	0.40	0.47	0.57	0.49	0.46
	200	0.38	0.42	0.50	0.60	0.53	0.49
Thiamine	50	0.40	0.44	0.48	0.59	0.51	0.48
	100	0.42	0.47	0.49	0.62	0.52	0.50
	200	0.45	0.48	0.51	0.64	0.54	0.52
Control		0.31	0.33	0.35	0.39	0.36	0.35
Mean		0.44	0.48	0.52	0.64	0.55	0.53
		Treatment		Treatment		DAI	
Sem±		0.006		0.003		0.013	
CD (p=0.05)		0.016		0.080		0.035	

Note: DAI – Days after inoculation

Table 3: Effect of non-conventional chemicals on sugar content in the leaves of sesame variety HT 1

Treatments	Concentration (ppm)	Sugar content (mg/g dry weight)					
		0 DAI	2 DAI	4 DAI	6 DAI	8 DAI	Mean
Salicylic acid	50	1.75	2.23	2.88	2.65	3.07	2.51
	100	2.09	2.31	2.95	3.57	3.12	2.81
	200	2.22	2.35	3.05	3.69	3.26	2.91
Acetyl salicylic acid	50	1.61	1.72	2.08	2.45	2.16	2.01
	100	1.73	1.78	2.14	2.52	2.26	2.09
	200	1.79	1.85	2.20	2.58	2.44	2.17
Indole acetic acid	50	1.84	2.09	2.42	3.22	2.89	2.49
	100	1.87	2.11	2.61	3.33	2.98	2.58

	200	2.08	2.19	2.60	3.40	3.10	2.67
Indole butyric acid	50	1.66	1.83	2.33	2.71	2.46	2.20
	100	1.75	1.90	2.40	2.79	2.56	2.28
	200	1.79	1.96	2.43	2.95	2.57	2.34
Riboflavin	50	1.36	1.45	1.93	2.21	1.86	1.76
	100	1.44	1.51	2.02	2.41	1.97	1.87
	200	1.50	1.53	2.13	2.42	1.98	1.91
Thiamine	50	1.49	1.66	2.01	2.39	1.99	1.91
	100	1.53	1.76	2.09	2.47	2.05	1.98
	200	1.62	1.82	2.19	2.65	2.12	2.08
Control		1.32	1.34	1.37	1.41	1.39	1.37
Mean		1.71	1.86	2.31	2.73	2.43	2.21
		Treatment		DAI		Treatment × DAI	
Sem±		0.036		0.019		0.081	
CD (p=0.05)		0.101		0.053		0.226	

Note: DAI – Days after inoculation

Table 4: Effect of non-conventional chemicals on sugar content in the leaves of sesame variety HT 2

Treatments	**Concentration (ppm)**	**Sugar content (mg/g dry weight)**					
		0 DAI	**2 DAI**	**4 DAI**	**6 DAI**	**8 DAI**	**Mean**
Salicylic acid	50	1.90	2.26	2.91	3.44	3.10	2.72
	100	2.11	2.33	2.97	3.59	3.14	2.83
	200	2.24	2.37	3.07	3.71	3.27	2.93
Acetyl salicylic acid	50	1.64	1.75	2.10	2.48	2.19	2.03
	100	1.76	1.81	2.16	2.55	2.28	2.11
	200	1.82	1.89	2.22	2.61	2.47	2.20
Indole acetic acid	50	1.86	2.06	2.44	3.24	2.91	2.50
	100	1.89	2.13	2.63	3.34	3.01	2.60
	200	2.10	2.21	2.63	3.43	3.12	2.70
Indole butyric acid	50	1.68	1.85	2.35	2.74	2.48	2.22
	100	1.77	1.92	2.42	2.83	2.59	2.31
	200	1.82	1.98	2.46	2.97	2.73	2.39
Riboflavin	50	1.38	1.45	1.96	2.23	2.05	1.82
	100	1.44	1.51	2.02	2.41	2.12	1.90
	200	1.53	1.56	2.16	2.45	2.25	1.99
Thiamine	50	1.52	1.69	2.05	2.41	2.09	1.95
	100	1.55	1.73	2.12	2.50	2.17	2.01
	200	1.63	1.80	2.21	2.66	2.33	2.13
Control		1.35	1.36	1.39	1.45	1.42	1.39
Mean		1.74	1.88	2.33	2.79	2.51	2.25
		Treatment		DAI		Treatment × DAI	
Sem±		0.002		0.001		0.004	
CD (p=0.05)		0.005		0.002		0.010	

Note: DAI – Days after inoculation

Discussion

Phenolic contents have antifungal properties restrict the growth of pathogen due to rapid accumulation of phenol content at the site of infection. Accumulation of phenol content is higher in resistant than susceptible genotypes in different crop plants. Results of present experiment showed maximum accumulation of phenol content with treatment of salicylic acid followed by indole acetic acid, indole butyric acid, acetyl salicylic acid, thiamine and riboflavin as compared to control at six DAI consequently declined in both varieties. However, phenol content was higher in HT 2 as compared to HT 1 variety of sesame. Hayat *et al.* (2010) [6] reported that salicylic acid is an important signalling molecule and involved in local and systemic disease resistance in plants against various pathogenic attacks. Sharma *et al.* (2011) [13] observed induction of phenol content in sesame by the inoculation of charcoal rot fungus *M. Phaseolina.* Abdel-Monaim (2011) [1] observed phenol content increased and reached at peak on six days after inoculation with *M. phaseolina* in pre-treated soybean with thiamine as compared to riboflavin and control. Ngadze *et al.* (2012) [9] reported total phenol content imparting resistance against soft rot pathogen in potato and found significantly higher total phenol content in resistant variety than susceptible. Yadav *et al.* (2015) [15] found maximum induction of total phenol content in salicylic acid treated Indian mustard on five days after challenge inoculation of Alternaria blight pathogen. Geat *et al.* (2016) [5] evaluated salicylic acid, indole acetic acid, indole butyric acid and carbendazim at 5 mM concentration against *Colletotrichum capsici* found more accumulation of total phenol in resistant chilli variety Sadabahar than susceptible Pusa jwala, after 48 hours of challenge inoculation of pathogen.

Sugars act as precursor for synthesis of phenolics, phytoalexin, lignin, and cellulose which play an important role in defence mechanism of plant against invading pathogen. In present finding, total sugar content was enhanced with treatment of SA followed by IAA, IBA ASA, thiamine and riboflavin as compared to control at six DAI and subsequently declined in both varieties. Basarkar *et al.* (1988) [3] reported that higher sugar content present in resistant genotypes against spot blotch disease than susceptible. Saini *et al*. (1988) [12] observed higher sugar content in wheat resistant variety (CPAN 1333) as compared to susceptible variety (Sonalika).

Summary and conclusion

The impact of non-conventional chemicals at different concentrations after challenge inoculation of *M. phaseolina* was observed in total phenol content and total sugar content of sesame leaves. The induction of these biochemical constituents was increased with increase in concentrations and reached at peak on six days after inoculation (DAI) in both varieties HT 1 and HT 2 treated with six different non-conventional chemicals. However, after six DAI, these biochemical activities were declined at each concentration. The salicylic acid showed maximum induction of total phenol content and total sugar content at six DAI followed by IAA and IBA in both varieties. However, riboflavin showed minimum induction of these activities at six DAI among all treatments. Induction of these biochemical activities was found higher in variety HT 2 as compared to HT 1 at each concentration after challenge inoculation with *M. phaseolina*.

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