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Influence of Phenolic Compounds Imparting Resistance to Cotton (*Gossypium hirsutum*) against *Helicoverpa armigera*

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ABSTRACT: *A pot experiment was conducted on cotton during kharif 2013 to study the effect of insect damage (ID) and mechanical damage followed by insect damage (MDFID) on expression of phenolic compounds like total phenol, condensed tannin and gossypol content in Sahana and Laxmi genotypes at different stages of crop. The results revealed that the total phenol and condensed tannin were significantly higher in MDFID than ID and gossypol was significantly higher in ID than MDFID.*

Key words: *Phenolic compounds, Total Phenol, Condensed Tannin and Gossypol*

INTRODUCTION

Cotton is one of the most ancient and important commercial crop next only to food grains and is the principal raw material for a flourishing textile industry. Cotton, although under pressure from synthetic fibers, has made resurgence worldwide and remains as the most improved crop species producing lint plus oil and meal from seed [11].

Cotton is the backbone of our sprawling textile industry contributing 7.00% to our Gross domestic product (GDP), fetching an export earning besides providing employment in the production, promotion, processing and trade. It accounts for 45% of the world fiber and supplies 10% world edible oil [13].

Many factors are responsible for low productivity of cotton but the magnitude of insect pests that damage the crop from sowing to maturity is most important. More than 1326 species of insects have been reported in commercial cotton fields worldwide, but only a small proportion of 162 pests are differing from season to season and between different regions [17].

Of the 30 pests of cultivated cotton, the most important are the caterpillars of *Helicoverpa armigera*, Pink bollworm (*Pectinophora gossypiella) and* Spotted bollworm (*Earias vitela).* [5].

The mechanisms of host plant resistance in response to insect infestation consists of a series of biochemical events, including increased production of phenolics, mediated by the activity of such enzymes as phenylalanine ammonia-lyase, tyrosine ammonia-lyase, peroxidase and polyphenoloxidase. The primary metabolites include carbohydrates and proteins, which are exploited by the herbivores for their growth and development. These primary metabolites also function as precursors of secondary substances, which are major elements of resistance in plants. The secondary substances determine the suitability of the substrate for colonization and exploitation by the herbivores and thus govern host preferences and acceptability. Age correlated biochemical profiles of host tissues also significantly influence infestation patterns.

Metabolites play a major role in the adaptation of plants to the changing environment and in overcoming stress constraints. This flows from the large complexity of chemical types and interactions underlying various functions: structure stabilizing, determined by polymerization and condensation of phenols and quinones, or by electrostatic interactions of polyamines with negatively charged loci in cell components; as well as aromatic nuclei and unsaturated aliphatic chains and signal transduction, several plant-biotic and abiotic stress stimuli systems,

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multiplicity of biochemical mechanisms involved in the protective role by metabolites [6].

MATERIALS AND METHODS

A pot experiment was conducted on cotton at Agriculture Research Station, Dharwad during *kharif 2013*. To study the effect of insect damage and mechanical damage followed by insect damage on expression of phenolic compounds like total phenol, condensed tannin and gossypol. The experiment was conducted in pot for maintaining the pest and disease free plants in the glass house condition. One tolerant genotype *viz*., Sahana and one susceptible genotype *viz*., Laxmi were used in the study. Seeds of each genotype were sown in 12″ X 8″ pots containing soil and compost mixture in 2:1 proportion and were watered regularly. In each genotype pots were maintained under controlled condition using cages lined with nylen net to prevent from pest and the infection from pests and air borne pathogens to maintain healthy seedlings.

The trifoliate leaves from middle portion of the plants were collected from the control plants of two genotypes for estimation of biochemical constituents which is referred as control and on the day of estimation, the other plants of same genotypes were allowed to ID (*Helicoverpa armigera* L.) by releasing five larvae of 3rd instars per plant and MDFID (releasing five larvae of 3rd instars per plant after mechanical damage using needle) by avoiding inter plant larval movement. After 48hr of releasing larvae, leaves were used for biochemical analysis. The leaf samples collected in brown paper packets with holes for ventilation and brought to the laboratory after 45 DAS, 85 PAS, 125 DAS.

Rearing of *Helicoverpa armigera*

Field collected eggs of *H. armigera* were allowed to hatch in multi-cell well plates with semi-synthetic diet at 27±1 ^{0}C and 75% relative humidity.

Sample Preparation for Allelochemical Estimation

Damaged leaves, three to five leaves each, per plant were collected from five plants of each treatment. Leaf samples were also collected from control plants that were not subjected either to mechanical injury or prior herbivory. Samples were prepared from 1 g of dried leaves homogenized using appropriate standard methods. The data were statistically analyzed (factorial CRD) and the least significant differences were calculated according to Gomez and Gomez [7].

RESULTS AND DISCUSSION

Total Phenol

Sahana recorded significantly higher total phenol content in (6.25 mg/g dry weight) than Laxmi (5.60 mg/g dry weight). Significantly higher total phenol content was observed in MDFID (6.26 mg/g dry weight) followed by ID (6.07 mg/g dry weight) and control (5.46 mg/g dry weight).Very high amount of phenol content accumulation was observed in Sahana at 45 DAS in ID treatment (7.67 mg/g dry weight), which is on par with MDFID treatment (7.22 mg/g dry weight) and differed significantly with control (6.55 mg/g dry weight). Lower phenol content accumulation (4.10 mg/g dry weight) was observed in Laxmi at 125 DAS under control (Table 1).

Condensed Tannin

Laxmi, recorded significantly lower condensed tannin content in control (6.40 mg/g dry weight) differed significantly from other two treatments ID (7.43 mg/g dry weight) and MDFID (7.86 mg/g dry weight). Very high amount of condensed tannin content accumulation was observed in Sahana at 45 DAS 11.37, 10.75 and 8.17 mg/g dry weight MDFID, ID and control treatments respectively, which differed significantly with each other. Lower condensed tannin content accumulation (5.46 mg/g dry weight) was observed in Laxmi at 125 DAS under ID treatment (Table 2).

Gossypol

Sahana recorded significantly higher gossypol content (1.62 mg/g dry weight) than Laxmi (1.30 mg/g dry weight). Higher gossypol content was observed in ID (1.85 mg/g dry weight) from other two treatments MDFID (1.34 mg/g dry weight) and control (1.21 mg/g dry weight).Very high amounts of gossypol content accumulation was observed in Sahana at 45 DAS 2.35, 1.57 and 1.43 mg/g dry weight in ID, MDFID and control respectively. Lower gossypol content accumulation (1.14 mg/g dry weight) was observed in Laxmi at 125 DAS under control and MDFID treatments (Table 3).

Induced resistance to herbivores is observed in many crop plants and is being studied intensively in several agricultural systems including soybean, tomato, potato, wheat and more recently, in cotton. The role of induced biochemicals has been extensively studied in plant pathogen interactions in cotton. The area of herbivore induced biochemical changes in cotton is relatively new. It is important to determine if prior herbivory or mechanical damage cause changes in cotton in terms of its susceptibility to insect pests. While the former occurs naturally in field situations, the latter is relatively rare.

Among all the biochemical constituents of different hosts, phenols stand out as most important component in imparting resistance to several plant pests. Phenols directly or indirectly interfere with several metabolic systems of an organism. Phenols have been found to play an important role in determining resistance or susceptibility of a host to parasitic infection. High concentration causes an instant lethal action by a general tanning effect while, low concentration causes gradual effect on the cellular constituent of the parasite [1].

Role of total phenols in pest tolerance in cotton, total phenol estimation was done in bollworm tolerant (Sahana) and susceptible (Laxmi) genotypes. Presence of significantly higher amount of total phenol in bollworm tolerant genotype (Sahana) in all the stages and treatments than Laxmi indicates its positive role in pest tolerance. Presence of lesser number of survived larvae in Sahana than Laxmi indicates its biochemical basis of bollworm tolerance and it has recorded higher total phenols content than Laxmi in both the treatments. Laxmi has recorded 10.40 per cent decrease in total phenol content compared with Sahana genotype. Results of the experiment are in agreement with the findings of Chakrabarty *et al.* [3] he opined that rapid accumulation of phenolic compounds occur in incompatible (resistant) host than the compatible (susceptible). Shahida *et al.*[14] observed a direct correlation between insect attack and phenol production, says that resistant cotton variety Ravi showed 1.00 to 1.20 mg more phenolics than susceptible varieties at all growth stages. Borthakuar and Addy, [2] observed that a decrease in the phenol content ranging from 18.39 to 22.07 per cent, 17.79 to 18.39 per cent and 18.39 to 19.71 per cent when the susceptible genotypes were compared with resistant genotypes to *H. armigera*. The total phenol content under both the situations (healthy and infected) increased from 90 to 120 DAS. Rao and Panwar, [12] reported that phenol production in groundnut against *S. litura* and *H. armigera* induced pest resistance in the crop. Vamadevaiah, [18] reported that the genotype Abadhita and Sahana have expressed highest total phenol content in leaf (>13.00 %) which are known for their pest tolerance followed by RAH 3 and genotype CPD 818 has possessed 8.49 percent of total phenols in squares. Biochemical analysis indicated that G.cot 16 had lower reducing sugar content than Sahana. It indicates higher amount of reducing sugar makes genotypes susceptible to sucking pests incidence. Even though BCS-23 (*G.barbadense*) showed higher reducing sugar content, the incidence of leaf minor was low. It indicates that genotypes belong to *G.barbadense* may show resistance reaction to leaf minor.

There was significant induction of tannins in plants subjected to mechanically damaged. Tannins are reported to be potent in their antibiotic effect and adversely affect the growth and development of lepidopteran larvae [9].

Positive role of condensed tannin in pest tolerance in cotton, condensed tannin estimation was done in bollworm tolerant (Sahana) and susceptible (Laxmi) genotypes. Presence of significantly higher amount of condensed tannin in pest tolerant genotype (Sahana) in all the stages and treatments than Laxmi was observed. The phenomenon of increase of condensed tannin content has been reported by many workers attributing various reasons. Singh and Agarwal, [16] reported the incidence of *A. biguttula biguttula* was negatively correlated with the amount of tannins in the leaves of resistant cotton genotypes. *Sharma et al. [15] opined that the ex*pression of resistance to pod borer, *Helicoverpa armigera* in wild relatives of pigeon pea was also associated with high amounts of tannins. Rao and Panwar, [12] studied the influence of tannin content production in groundnut against *S. litura* and *H. armigera.* Kranthi and Kranthi, [9] opined that significant induction in concentration of tannin content soon after mechanical damage in cotton plants. Chan *et al.* [4] reported the effect of condensed tannin isolated from flower buds of cotton (*Gossypium hirsutum* L., Tx254) retards larval growth of the tobacco budworm (*Heliothis virescens* F.) when added to artificial diet.

Positive role of gossypol in pest tolerance in cotton, gossypol estimation was done in bollworm tolerant (Sahana) and susceptible (Laxmi) genotypes. Presence of significantly higher amount of gossypol in bollworm tolerant genotype (Sahana) in all the stages and treatments than Laxmi was observed. The presence of lesser number survived larvae in Sahana than Laxmi indicates its biochemical basis bollworm tolerance. The phenomenon of increase of gossypol content has been reported by many workers attributing various reasons. Singh and Agarwal [16] and Hedin and McCarty, [8] opined that, in cotton a high level of gossypol, flavanols, silica and low sugar contents were reported to have some role in insect resistance. Kranthi and Kranthi, [9] opined that

Table 1
Effect of insect damage and mechanical damage followed by insect damage on accumulation of total phenol content in cotton leaf at different stages of crop growth

Genotypes	Total phenol (mg/g dry weight)																		
	Control						Insect damage						Mechanical damage followed by insect damage						
	45 DAS	85 DAS	125 DAS	Percent decrease in 85 DAS over 45 DAS	Percent decrease in 125 DAS over 45 DAS	Mean	45 DAS	85 DAS	125 DAS	Percent increase or decrease in 85 DAS over 45 DAS	Percent increase or decrease in 125 DAS over 45 DAS	Mean	45 DAS	85 DAS	125 DAS	Percent decrease in 85 DAS over 45 DAS	Percent decrease in 125 DAS over 45 DAS	Mean	Grand mean
Sahana	6.55	5.66	4.65	-13.59	-29.01	**5.62**	7.67	6.38	6.10	-16.82	-20.47	**6.72**	7.22	6.38	5.63	-11.63	-22.02	**6.41**	**6.25**
Laxmi	6.31	5.47	4.10	-13.31	-35.02	**5.29**	4.66	5.54	6.05	18.88	29.83	**5.42**	6.70	5.11	6.50	-23.73	-2.99	**6.10**	**5.60**
Percent increase or decrease over laxmi	-3.66	-3.36	-11.83			-5.87	-39.24	-13.17	-0.82			-19.35	-7.20	-19.91	15.45			-4.84	-10.40
Grand mean	**6.43**	**5.57**	**4.38**			**5.46**	**6.17**	**5.96**	**6.08**			**6.07**	**6.96**	**5.74**	**6.07**			**6.26**	

	S.Em±	CD at 1%
Factor G	0.05	0.19
Factor T	0.06	0.23
Factor D	0.06	0.23
G x T	0.08	0.33
G x D	0.08	0.33
T x D	0.10	0.40
G x T x D	0.15	0.56

G: Genotype, T: Treatment, D : Days after sowing

Table 2

Effect of insect damage and mechanical damage followed by insect damage on accumulation of condensed tannin content in cotton leaf at different stages of crop growth

Genotypes	Condensed tannin (mg/g dry weight)																		
	Control						Insect damage						Mechanical damage followed by insect damage						
	45 DAS	85 DAS	125 DAS	Percent decrease in 85 DAS over 45 DAS	Percent decrease in 125 DAS over 45 DAS	Mean	45 DAS	85 DAS	125 DAS	Percent decrease in 85 DAS over 45 DAS	Percent decreas e in 125 DAS over 45 DAS	Mean	45 DAS	85 DAS	125 DAS	Percent decrease in 85 DAS over 45 DAS	Percent decrease in 125 DAS over 45 DAS	Mean	Grand mean
Sahana	8.17	6.69	5.67	-18.12	-30.60	**6.84**	10.75	9.21	6.88	-14.33	-36.00	**8.95**	11.37	9.28	7.22	-18.38	-36.50	**9.29**	**8.36**
Laxmi	7.89	5.74	5.56	-27.25	-29.53	**6.40**	8.95	7.88	5.46	-11.96	-38.99	**7.43**	9.46	7.97	6.14	-15.75	-35.10	**7.86**	**7.23**
Percent increase or decrease over laxmi	-3.43	-14.20	-1.94			-6.43	-16.74	-14.44	-20.64			-16.98	-16.80	-14.12	-14.96			-15.39	-13.52
Grand mean	**8.03**	**6.22**	**5.62**			**6.62**	**9.85**	**8.55**	**6.17**			**8.19**	**10.42**	**8.63**	**6.68**			**8.57**	

	S.Em±	**CD at 1%**
Factor G	0.01	0.04
Factor T	0.01	0.05
Factor D	0.01	0.05
G x T	0.02	0.07
G x D	0.02	0.07
T x D	0.02	0.08
G x T x D	0.03	0.12

G: Genotype, T: Treatment, D : Days after sowing

Table 3
Effect of insect damage and mechanical damage followed by insect damage on accumulation of gossypol content in cotton leaf at different stages of crop growth

Genotypes	Gossypol (mg/g dry weight)																		
	Control						Insect damage						Mechanical damage followed by insect damage						
	45 DAS	85 DAS	125 DAS	Percent increase or decrease in 85 DAS over 45 DAS	Percent decrease in 125 DAS over 45 DAS	Mean	45 DAS	85 DAS	125 DAS	Percent decrease in 85 DAS over 45 DAS	Percent decrease in 125 DAS over 45 DAS	Mean	45 DAS	85 DAS	125 DAS	Percent increase or decrease in 85 DAS over 45 DAS	Percent decrease in 125 DAS over 45 DAS	Mean	Grand mean
Sahana	1.43	1.28	1.02	-10.49	-28.67	**1.25**	2.35	2.13	2.06	-9.36	-12.34	**2.18**	1.57	1.44	1.34	-8.28	-14.65	**1.45**	**1.62**
Laxmi	1.17	1.18	1.14	0.85	-2.56	**1.17**	1.75	1.55	1.22	-11.43	-30.29	**1.51**	1.25	1.30	1.15	4.00	-8.00	**1.24**	**1.30**
Percent increase or decrease over laxmi	-18.18	-7.81	11.76			-6.40	-25.53	-27.23	-40.78			-30.73	-20.38	-9.72	-14.18			-14.48	-19.75
Grand mean	**1.30**	**1.23**	**1.08**			**1.21**	**2.05**	**1.84**	**1.64**			**1.85**	**1.41**	**1.37**	**1.25**			**1.34**	

	S.Em±	CD at 1%
Factor G	0.003	0.01
Factor T	0.004	0.02
Factor D	0.004	0.02
G x T	0.006	0.02
G x D	0.006	0.02
T x D	0.007	0.03
G x T x D	0.010	0.04

G: Genotype, T: Treatment, D : Days after sowing

significant induction of gossypol occurred at 48 h after semilooper and mechanical damage in cotton plants. Niles, [10] reported that the nature of biochemical resistance against the insect pests ascribed to "high gossypol", increasing gland density in cotton plant appeared to result in increasing concentration of the toxic compounds.

In summary, the higher production of secondary metabolites like Total phenol, Condensed tannin and Gossypol in Sahana play a defensive role against the insect pest. It is probably due to production of secondary metabolites, such as Total phenol, Condensed tannin and Gossypol.

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