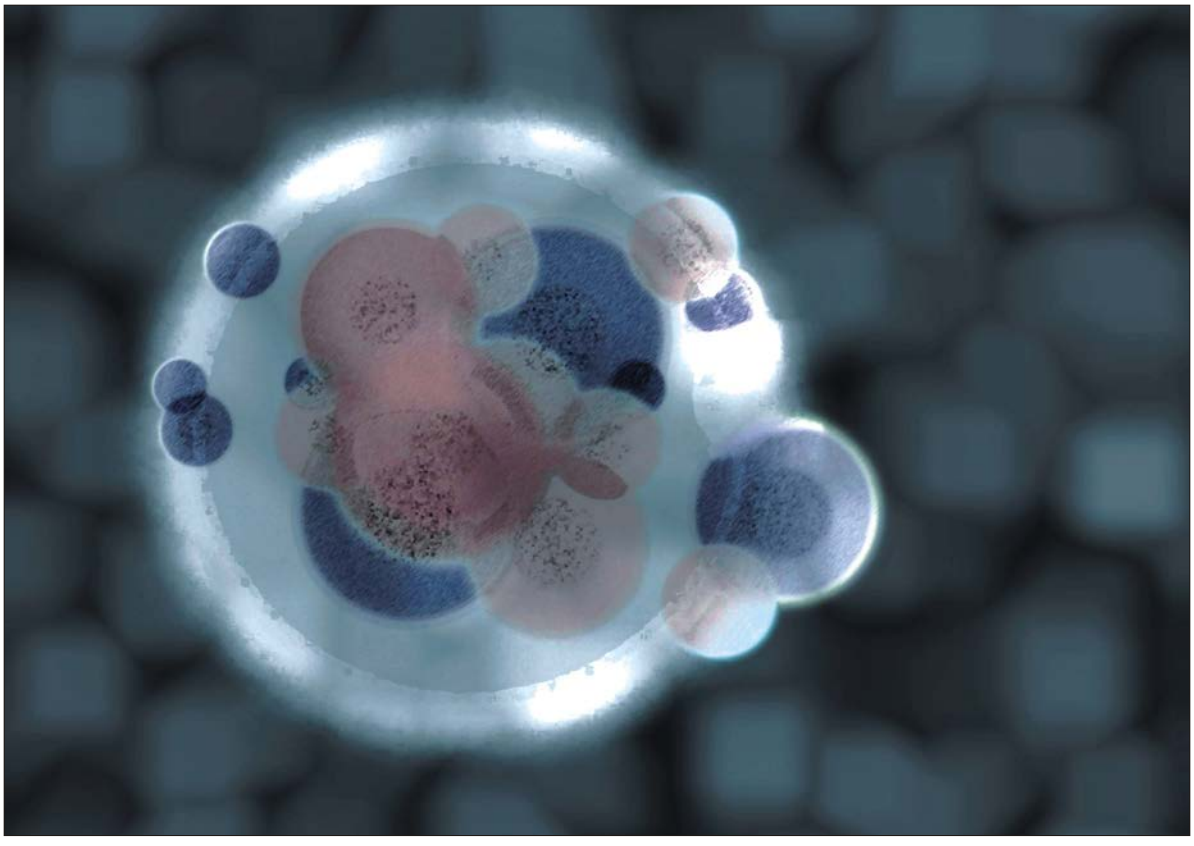

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HPLC Analysis of Cotton Phenols and Their Contribution in Bollworm Resistance

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Abstract: Considering the role of phenolics in plant defensive strategies, some cotton varieties with varied degree of insect resistance and susceptibility were analyzed through HPLC for their phenolic content. Out of 15 major peaks, 9 were identified as galic acid, protochatechuic acid, chlorogenic acid, p.hydroxy benzoic acid, syringic acid, p.coumaric acid, gentisic acid, benzoic acid and ferulic acid. Major quantitative differences were seen for syringic and ferulic acids being significantly higher in resistant variety Ravi and present only in traces in more susceptible varieties, NIAB-26N and S-12. Individual effect of some phenolic acids on growth and survival of cotton bollworm also showed extreme retardation of larval weight (99.42% to 99.71%),100% mortality(in 1st week) and no pupation in diet incorporated with these two acids syringic and ferulic acids. p-hydroxy benzoate, chlorogenic, sinapic and cinnamic acids showed 45 to 80% retarded weights, 16 to 33% mortality in 1st week and 61 to 94% mortality in 2nd week and 5 to 40% pupation with 2 to 4 days delay.

Key words: Biochemical basis of resistance, phenols,, bollworm, *Gossypium,* inbuilt resistance, antifeedants

Introduction

Cotton varieties grown in Pakistan are mainly narrow gene based varieties originated by the intra specific crosses of *G. hirsutum* varieties. A crop with narrow genetic base is more prone to insects and diseases (Iqbal *et al.*, 1997). Chemical means of insect control being less effective due to multiple insecticide resistance (Snodgrass, 1996) must be replaced by developing varieties that can shoo away insects due to their natural potential or inbuilt resistance (Henneberry *et al.*, 1980). There are some cotton varieties which are comparatively less prone to insect attack than others which are highly susceptible (Yein, 1983, Zummo *et al.*, 1984). This insect count variability may depend upon certain inherited morphological and chemical characteristics of the plants that can determine their insect resistance or susceptibility. Phenolic compounds are considered to play an important role in plant defense. Phenols are found in plants in the form of glycosides, which act as mobilized defense system can be translocated by plants and enzymatically converted to defensive substances at the site of attack (Reichardt *et al.*, 1988). Butter *et al.* (1992) determined a negative correlation of tannins and phenols with white fly population densities. Similarly Felton *et al.* (1992) reported that oxidized form of chlorogenic acid is a potent alkylator of dietary protein and can exert a strong anti nutritive effect upon larvae through chemical degradation of essential amino acids. Guerra *et al*. (1990) and Corcuera (1993) also described phenols as important biochemical basis of insect resistance. Considering the role of phenolics in plant defense, some cotton varieties of varied degree of resistance and susceptibility were analyzed for their phenolic compounds to see the varietal differences regarding insect resistance or susceptibility.

Materials and Methods

HPLC Analysis Of Cotton Phenols: Phenols or phenolic acids of cotton varieties were extracted and analyzed by following the method described by Lege *et al.* (1995). Leaves (80 days after planting) were picked from the upper part of the plant and dried in Labconco freeze dry system. 200 mg leaf powder was extracted in 12 ml of 2% (v/v) glacial acetic acid placed in a boiling water bath for 10 minutes and centrifuged at 10,000 rpm for 15 minutes. The supernatant was filtered using 0.22 μm filters, then hydrolyzed with 12 ml of 1N HCl in a boiling water bath for 1 hr. After cooling the reaction mixture, 20 ml diethyl ether was added. Ether layer was separated and dried under nitrogen gas stream. Residue was dissolved in 0.5 ml of the solvent consisted of 2.5% butanol: 12.5% methanol: 2% glacial acetic acid: 10% ammonium acetate: 73% water. The dissolved samples were filtered using 0.2 μm filters. Some available standards were prepared at 1 mg/ml concentrations and also filtered similarly before analysis. 20 μl of each sample and standard was subjected to HPLC analysis by isocratic elusion following Oszmianski and Sapis (1989). Mobile phase was 11% acetonitrile (CH_3CN) in water at 0.7 ml/mm flow rate. PH, 2.6 was maintained by adding few drops of phosphoric acid (H_3PO_4) in mobile phase. Reversed phase ODS column (4.6 mmX15 cm) was used. Column oven temperature was kept constant at 30°C. HPLC pump was LC-10AT (Shimadzu) controlled by SCL-10A. Detector was SPD-10AV (Shimadzu) U.V-VIS. Detection was made at 280 nm wavelength and 0.02 AUFS. Recorder used was C-R5A chromatopac at attenuation '7' and chart speed 10 mm/min. Column was washed with 100% acetonitrile after each run. Phenols were identified and quantified by comparing the peak area obtained on similar retention time of the standard peak area with known concentrations.

Effects of some phenolic acids on cotton bollworm larvae (*Helicoverpa armigera*): Individual effect of phenolic acids, cinnamic, sinapic, chlorogenic, p-hydroxy benzoate, syringic and ferulic acids was evaluated on larval growth and survival of cotton bollworm. These chemicals were added in larval artificial diet. The bollworm diet was prepared by following the method described by Burton and Perkins (1972) and modified by Ahmed *et al.* (1998). Biotests were performed, following Guerra *et al.* (1990) with some modification. Tissue culture multi-well plates with covers having 6 flat bottom wells of area/well 9.62 cm^2 were used for biotest. The compounds were poured separately in these wells at the concentration of 0.2% of the diet. The semi solid diet was quickly added into the wells by a syringe (10 ml) and mixed quickly for a uniform composition of the compounds in diet. When the diet became solidified and cooled to 24 ± 2°C, the second instar larvae of *Heliothis armigera* (initial weight 0.0025-0035 g) were put on the surface of the diet in each well (one larvae/well or 6 larvae/treatment). The larvae were taken from the IRAC laboratories, NIBGE, Faisalabad. Diets with out added chemicals served as controls. The multi cells were then covered and placed in controlled environment growth chamber at 60-70% humidity and 27 ± 1^0C temperature. Temperature and humidity fluctuations were monitored by a

Table 1: Qualitative and quantitative composition of phenolics in cotton varieties

Retention time (min)	Phenol (mg mL^{-1})*	Varieties					
		Ravi	SP-16	NIAB-86	NIAB-78	NIAB-26N	S-12
2.30	Unknown	0.0046	0.0073	0.0095	0.0058	0.0050	0.0035
3.40	Galic acid	0.0098	0.0404	0.567	0.0264	0.0322	0.0224
4.60	Unknown	0.6798	0.5152	0.6008	0.5250	0.5996	0.6021
6.00	Unknown	0.0084	0.0188	0.0085	0.0072	0.0184	0.0076
7.30	Unknown	0.0834	0.0660	0.0504	0.1250	0.0666	0.0336
9.05	Protocatechuic acid	0.0554	0.0351	0.0251	0.0062	0.0001	0.0006
9.76	Chlorogenic acid	0.0115	0.0388	0.0248	0.0242	0.0270	0.0196
10.71	P-hydroxy benzoate	0.0031	0.0053	0.0050	0.0097	0.0103	0.0102
12.69	Unknown	0.0020	0.0118	0.0248	0.0119	0.0056	0.0022
14.10	Syringic acid	0.2132**	0.0562	0.0246	0.0662	0.0202	0.0110
15.90	Gentisic acid	0.0078	0.0038	0.0063	0.0030	0.0049	0.0031
19.44	P-coumaric acid	0.0342	0.0383	0.0130	0.0132	0.0353	0.0380
21.44	Benzoic acid	0.0598	0.0309	0.0301	0.0300	0.0327	0.0254
23.70	Unknown	0.0016	0.0066	0.0052	0.0085	0.0030	0.0042
37.61	Ferulic acid	0.2181**	0.0404	0.0257	0.0402	Traces	Traces
	Total Phenols	1.3918	0.9149	0.9105	0.8965	0.8609	0.7813

* Concentrations of identified phenols were calculated by comparing the peak area of samples with mean peak area of standards at known concentration

** Numbers differentiated indicate obvious quantitative difference of phenols in resistant cotton variety Ravi

Table 2: Effect of some phenolic acids on growth and survival of cotton bollworm larvae (*Heliothis armigera*)

Treatment*	Mean** larval weight (g)	+SD	% Weight*** retardation	% Mortality 1st week	% Mortality 2nd week	Mean days to Pupation	% Pupation
Control (Diet without additive)	0.4885a	0.0762	0	0	0	14	100
Cinnamic acid	0.263b	0.0773	45.97	16.6	61.1	16	39.9
Sinapic acid	0.2047b	0.0180	58.09	16.6	66.6	18	33.4
Chlorogenic acid	0.1459c	0.0480	70.13	27.8	88.8	18	11.2
P-hydroxy benzoic acid	0.0938c	0.0564	80.79	33.3	94.4	18	5.6
Syringic acid	0.0028d	0.0015	99.42	100	-	No pupation	0
Ferulic acid	0.0014d	0.0010	99.71	100	-	No pupation	0

Means followed by the similar letters are similar at 0.05% probability level by DMRT.

* Each treatment at 0.2% concentration of diet

** Mean larval weight of 18 larvae 2nd instar at 7th day of experiment, Larval weight in syringic and ferulic acid treatments was observed just after mortality.

*** Percent weight retardation calculated with reference to control larval weight as standard.

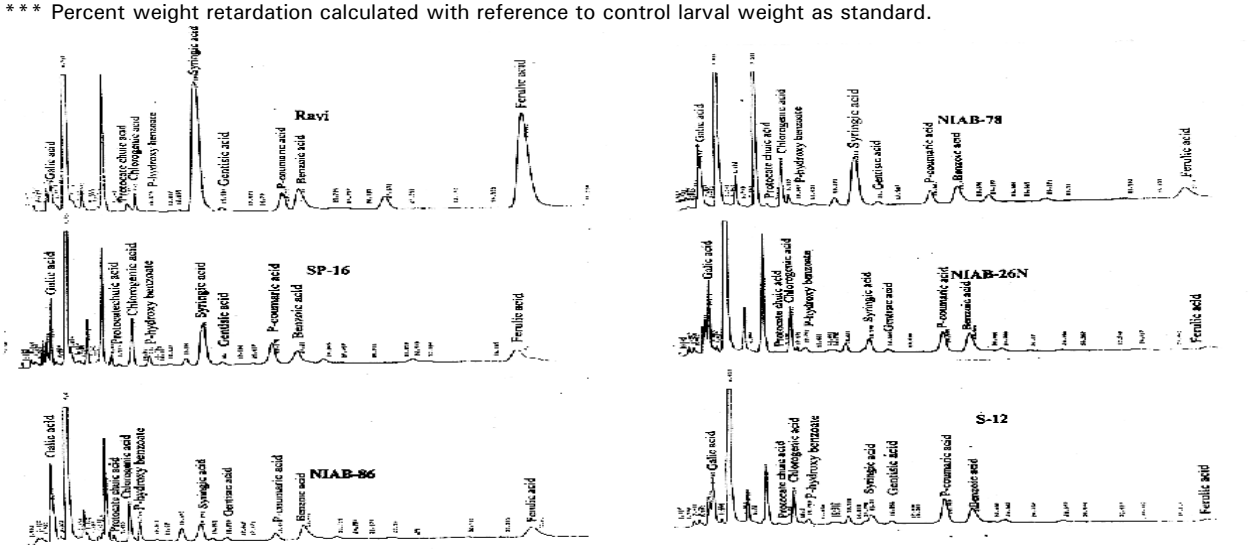

Fig. 1: HPLC profile of cotton phenols. Showing obvious quantitative differences for syringic and ferulic acids in resistant and susceptible varieties

Fig. 2: Effect of phenols on growth and survival of cotton bollworm larvae at 0.2% conc. of diet (at 7th day of experiment), (a). Control (diet with out phenolics) (b) Cinnamic acid and © Sinapic acid showing moderate degree of retardation in larval weight (45.97% than to 58.09%) (d) Chlorogenic acid showing phagodeterrency and retarded growth (70.13%) with severity than cinnamic and sinapic acids, (e) Syringic acid and (f Ferulic acid showing extreme retardation of larval weight and 100% mortality

hygrothermograph. Larvae were checked daily for mortality. Larval weights were recorded (for each larvae at 1st, 3rd and 7th day of the experiment started). Developmental time to pupation was also recorded. Data of larval weights was subjected to analysis of variance followed by DMRT on microcomputer using MSTATE software package.

Results and Discussion

HPLC determination of phenols: Results presented (Table 1, Fig. 1) indicate the qualitative and quantitative composition of phenols in various cotton varieties. Out of 15 major peaks, 9 were identified. Phenols eluted at the same retention times as standard peaks were referred to by the standard phenol name as gallic acid, protochatechuic acid, chlorogenic acid, p-hydroxy benzoic acid, syringic acid, p-coumaric acid, gentisic acid, benzoic acid and ferulic acid. Major quantitative differences were seen for two acids, syringic and ferulic acids being higher in Ravi, 0.2132 mg mL^{-1} syringic acid and 0.2181 mg mL^{-1} ferulic acid. SP-16 possessed 0.0562 mg of syringic and 0.0404 mg mL^{-1} of ferulic acid. NIAB-86 showed 0.0246 mg syringic and 0.0257 mg mL^{-1} of ferulic acid. Syringic acid was present in S-12 and NIAB-26N at very low concentration being 0.0202 and 0.0110 mg mL^{-1} while ferulic acid was found only in traces. NIAB-78 showed these two acids comparatively in greater concentration among the susceptible varieties having 0.0662 mg syringic and 0.0402 mg mL^{-1} ferulic acid. NIAB-78 is considered a variety having tolerance against leaf curl virus. This greater potential for phenols, especially syringic and ferulic acids may be a contributory factor to the tolerant behavior of this variety against leaf curl virus. Insect susceptibility of NIAB-78 even with equal penolics to semi resistant varieties indicates the involvement of some other factors, including anatomical factors and nutritive value. p-coumaric acid, p-hydroxy benzoic acid, gentesic and gallic acid were found at almost similar concentrations in all the varieties with few minor exceptions. Low amount of chlorogenic acid was observed in Ravi while all other varieties possessed similar amount of chlorogenic acid. Trace amounts of protochatechuic acid were present in susceptible varieties than resistant one. Total phenol concentration (sum of concentrations of all peaks) was also observed to be higher in resistant variety

Ravi,1.3918 mg mL^{-1}. The semi resistant varieties SP-16 and NIAB-86 and susceptible variety NIAB-78 showed 0.9149, 0.9105 and 0.8965mg mL^{-1} phenols NIAB-26N and S-12 showed least phenolics, 0.8609 and 0.7813 mg mL^{-1} respectively. Lege *et al.* (1995) also found more phenolics in resistant varieties.

Effect of phenols on cotton bollworm: Obvious growth inhibition and mortality can be observed in various phenolic treatments (Table 2, Fig. 2). Growth and survival of bollworm larvae was equally affected by cinnamic acid and sinapic acid with 0.2639 and 0.2047g larval weight in comparison to 0.4885g in control diet (45.97 to 58.09% weight retardation). Percent mortality was 16.6 in 1st week of experiment started and 61.1 and 66.6% before pupation (2^{nd} week of experiment). Pupation was delayed by 2-4 days than control with 39.9 and 33.4% pupation respectively. Chlorogenic acid and p-hydroxy benzoic acid showed severe effect on growth as well as survival with 70.13 to 80.79% larval weight retardation and 88.8 to 94.4% mortality. Percent pupation was only 5.6 to 11.2% in both phenolic acids. Syringic and ferulic acids, both severely effected the survival of larvae. In first 12 hrs, the larval weight seemed to be retarded and in 24 hrs all the larvae died. These two acids also showed almost similar effect at 0.1% concentration of diet. Ravi is considered to be the more resistant variety and the only variety having immunity against leaf curl virus. It is also reported that *G. arboreum* have a high degree of natural resistance to bollworm but inter species transfer of such a character, diploid to tetraploid species is restricted. Transgenic cotton having Bt toxin is considered to be highly resistant against lepidopteran insects (Buehler, 1993). Monogenic endo toxins exert a high selection pressure on the insects, which may lead to the development of new resistant insect biotypes. Due to the concern of development of resistances by the insects for monogenic endotoxin like Bt protein, it is necessary to supplement genetic engineering with induced mutation by enhancing the poly gene based resistance or secondary plant compounds having deleterious effects. As far as biochemical basis of insect resistance in cotton is concerned the major difference was observed for phenolics, syringic and ferulic acids in Ravi (the variety more resistant against insects and leaf curl virus) so enhancement of these phenols may strongly contribute to the plant resistance against insects and leaf curl virus.

References

Ahmed, K., F. Khalique and B.A. Malik, 1998. Modified artificial diet for mass rearing of chickpea pod borer, *Helicoverpa* (*Heliothis*) *Armigera* (Hubn). Pak. J. Biol. Sci., 1: 183-187.

Buehler, R.E., 1993. The development of transgenic cotton containing a Bt gene for control of lepidopteran pests. ICAC Recorder, 2: 3-4.

Burton, R.L. and W.D. Perkins, 1972. WSB, a new laboratory diet for the corn earworm and the fall armyworm. J. Econ. Entomol., 65: 385-386.

Butter, N.S., B.K. Vir, K. Gurdeep, T.H. Singh and R.K. Raheja, 1992. Biochemical basis of resistance to whitefly *Bemisia tabaci* Genn. (Aleyrodidae: Hemiptera) in cotton. Trop. Agric., 69: 119-122.

Corcuera, L.J., 1993. Biochemical basis for the resistance of barley to aphids. Phytochemistry, 33: 741-747.

Felton, G.W., J. Workman and S.S. Duffey, 1992. Avoidance of antinutritive plant defense: Role of midgut pH in Colorado potato beetle. J. Chem. Ecol., 18: 571-583.

Guerra, D.J., T.J. Cothren and J.R. Phillips, 1990. Influence of selected phenolic compounds on development of bollworm (Lepidoptera: Noctuidae) larvae. J. Econ. Entomol., 83: 2115-2118.

Henneberry, T.J., L.A. Bariola and D.L. Kittock, 1980. Integrated methods for control of the pink bollworm and other cotton insects in the Southwestern United States. Technologies Bulletin No. 1610, U.S. Department of Agriculture, USA., July 1980, pp: 1-45.

Iqbal, M.J., N. Aziz, N.A. Saeed, Y. Zafar and K.A. Malik, 1997. Genetic diversity evaluation of some elite cotton varieties by RAPD analysis. Theoret. Applied Genet., 94: 139-144.

Lege, K.E., J.T. Cothreen and C.W. Smith, 1995. Phenolic acid and condensed tannin concentrations of six cotton genotypes. Environ. Exp. Bot., 35: 241-249.

Oszmianski, J. and J.C. Sapis, 1989. Fractionation and identification of some low molecular weight grape seed phenolics. J. Agric. Food Chem., 37: 1293-1297.

Reichardt, P., T.P. Clausen and J.P. Bryant, 1988. Phenol Glycosides in Plant Defense against Herbivores. In: Biologically Active Natural Products: Potential Use in Agriculture, Cutler, H.G. (Ed.). 2nd Edn., Chapter 10, American Chemical Society, USA., ISBN-13: 9780841215566, pp: 130-142.

Snodgrass, G.L., 1996. Insecticide resistance in field populations of the tarnished plant bug (Heteroptera: Miridae) in cotton in the Mississippi Delta. J. Econ. Entomol., 89: 783-790.

Yein, B.R., 1983. Relative susceptibility of some cotton cultivars to insect pests. J. Res. Assam Agric. Univ., 4: 141-147.

Zummo, G.R., J.C. Segers and J.H. Benedict, 1984. Seasonal phenology of allelochemicals in cotton and resistance to bollworm (Lepidoptera: Noctuidae). Environ. Entomol., 13: 1287-1290.