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# Biochemical Basis of Insect Resistance in Cotton

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**Abstract:** Considering the role of phenolics in plant defense, some cotton varieties were investigated for their total phenols at various growth stages, change in phenolics as a result of insect exposure and their effect on growth and survival of cotton boll worm larva. More resistant variety Ravi showed 1.00 to 1.20 mg more phenolics than susceptible varieties at all growth stages. A direct correlation was observed between insect attack and phenol production. Bollworm diet incorporated with tannic acid and phenolic mixture at 0.1% concentration showed phagodeterrency, 61 to 96% weight retardation, 44 to 50% mortality in 1st week of experiment and 94 to 100% mortality in 2nd week of experiment. Phenols showed 5.05% pupation (delayed) while no pupation in tannic acid treatment. Methanolic extract of Ravi showed more effect on bollworm larvae than susceptible variety S-12.

**Key words:** Insect resistance, phenols, tannins, bollworm, *Gossypium*

## Introduction

Host plant insect interaction is a dynamic system and co evolutionary process, which involves development of the defense mechanisms by the plants and counter adaptations by the insects. Defense mechanisms involve either morphological barriers or elaborative array of phytochemicals, which act as repellents, phagodeterrents and oviposition deterrents, thus exhibiting resistance. Poly phenols (tannins) and phenols are considered to play an important role in plant defense mechanisms. Tobacco budworm and cotton bollworm survival was found negatively correlated with the tannin content of *Gossypium* (Zummo and Segers, 1984). Poly phenols react through hydrogen bonding with protein (Swain, 1965) forming a protein- tannin complex (Jones and Morgan, 1977) thereby reducing the nutritive value of the ingested foods through chemical degradation of essential amino acids (Felton *et al*, 1992). Cotton phenols and tannins were also found negatively correlated with white fly population densities (Butter *et al*, 1992). Some resistant and susceptible cotton varieties were evaluated for their phenolic contents which could be related to insect resistance. Effects of tannins (tannic acid), phenolic mixture and methanolic extracts of cotton leaves of S-12 and Ravi were also observed on cotton bollworm larvae.

## Materials and Methods

Experimental fields were planted with the cotton varieties NIAB-86, NIAB-78, NIAB-26N, S-12, SP-16 and RAVI (an old world cotton *G. arborium* having high degree of natural resistance against insects and leaf curl virus) at Nuclear Institute for Agriculture & Biology Faisalabad. Varieties were planted in 3 beds. Each bed containing 6 varieties and each variety consisted of 5 rows of plant (20 plants in each row). The cotton varieties were grown at alternate sequence in each bed. One field was sprayed and other was kept unsprayed. Sprayed field was considered to be control where there was no insect attack or least attack while unsprayed field was considered as fully exposed to the insect attack

**Spectrophotometric Estimation of Total phenols:** Total phenols of cotton were estimated by taking 0.1 gm of foliage powder in 9 replicates for each variety at 50, 80 and 110 days old plants in sprayed and unsprayed conditions. Powder was extracted three times with 2 ml of methanol and twice with 2 ml of 80 percent acidified methanol by boiling (Bray & Thrope, 1954). Total extract (10 ml) was then evaporated under reduced pressure. Dilution was made with 5 ml. of water and filtered. Chlorophyll remained in flask un dissolved. 0.2 ml of the extract was used for total phenol estimation by adding 4 ml of 2% Sodium Carbonate ($Na_2CO_3$) and then 0.2 ml of Folin's reagent in each test tube at one-minute difference. After 30 minutes the absorbance was measured at 750 nm accordingly. The spectrophotometeric observations were kept time scheduled otherwise readings show much variation. Comparative quantities (mg/100mg dry weight) were calculated from the standard curve of chlorogenic acid.

**Mechanical Injury and Phenol Production:** To see any difference of phenol production in mechanically injured cotton leaves, as compared to insect infested leaves, an experiment was designed. Three leaves of 60 days old SP-16 cotton plant were pricked (50 pricks/leaf) with common pin and three leaves of opposite side were used as control (un pricked). Total 3 plants were selected for mechanical injury. Experiment was repeated with difference in number of pricks (100 pricks/leaf). After 24 hours the leaves were picked and processed for phenol estimation as described earlier.

**Effects of phenolics and cotton extracts on cotton bollworm larvae (*Helicoverpa armigera*):** Effect of phenols, tannins (tannic acid) and methanolic extracts of cotton varieties S-12 and Ravi 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, by following Daniel *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 biotests. The compounds, tannic acid, phenolic mixture, and methanolic extract of cotton variety S-12 and Ravi (separately) were poured in these wells at the concentrations of 0.1% 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 and extracts in diet. When the diet became solidified and cooled to $24 \pm 2^{0}C$, the second instar larvae of *Heliothis armigera* (initial weight 0.0025-0035g) 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. Each treatment was replicated 3 times. 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°C temperature. Temperature and humidity fluctuations were monitored by a hygrothermograph. Larvae were checked daily for mortality. Larval weights were recorded (for each larvae at 1st.day, 3rd. day and 7th day of the experiment started). Developmental time to pupation was also recorded. The data was subjected to analysis of variance followed by DMR.test on microcomputer using MSTATE soft- ware package.

## Results and Discussion

Results of total phenols under control or sprayed condition (Table 1a) obviously indicate that resistant and semi resistant cotton varieties possessed significantly greater phenolic content at all growth stages. The more resistant variety Ravi showed about 1.00 to 1.20mg more phenolics than susceptible varieties. Lege *et al.* (1995) also mentioned the direct correlation of phenolic compounds and insect resistance. The seasonal increase in phenolics from 50 to 80 days was also found to be higher in resistant varieties having 0.54 to 0.55 mg more phenolics at 80 days while susceptible varieties showed 0.32 to 0.36 mg more phenolics. Results under unsprayed conditions (Table 1b) clearly show the increased phenolics in all varieties at all growth stages in comparison to their sprayed counter parts. As plants under unsprayed conditions are fully exposed to insect attack, so this increase in phenolics may be the result of defensive strategies of the plants against the insects. The increase in resistant and semi resistant varieties was found to be less, 0.04 to 0.08mg while susceptible varieties contained 0.31 to 0.48 mg more phenolics than their sprayed counter parts, indicating the positive correlation between insect attack and phenol production.

Daniel *et al.* (1990) also reported the increased phenolics in infested cotton leaves than un infested leaves. The seasonal decrease in phenolic content from 80 to 110 days was also found to be less in unsprayed plants by retaining about 0.1 mg more phenolics than their sprayed counter parts at 110 days.

Table 1: Total phenolic content (mg/100 mg) of cotton varieties at various growth stages under (a)sprayed & (b) unsprayed conditions.

| Varieties | 50-Days | 80-Days | 110-Days |
|---|---|---|---|
| a) Sprayed | | | |
| Ravi | 2.84 a | 3.39 a | 2.88 a |
| SP-16 | 2.30 b | 2.84 b | 2.29 b |
| NIAB-86 | 2.25 b | 2.80 b | 2.27 b |
| NIAB-78 | 1.80 c | 2.16 c | 1.82 c |
| NIAB-26 N | 1.88 c | 2.20 c | 1.85 c |
| S-12 | 1.76 c | 2.11 c | 1.78 c |
| b)Unsprayed | | | |
| Ravi | 2.88 a | 3.48 a | 3.10 a |
| SP-16 | 2.36 b | 2.94 b | 2.56 b |
| NIAB-86 | 2.33 b | 2.89 b | 2.47 b |
| NIAB-78 | 2.15 b | 2.60 b | 2.30 b |
| NIAB-26 N | 2.19 b | 2.62 b | 2.33 b |
| S-12 | 2.10 b | 2.56 bc | 2.28 b |

Means followed by the similar letters in a column (for both sprayed and unsprayed conditions do not differ significantly at 5% probability level by Duncan's multiple range test.

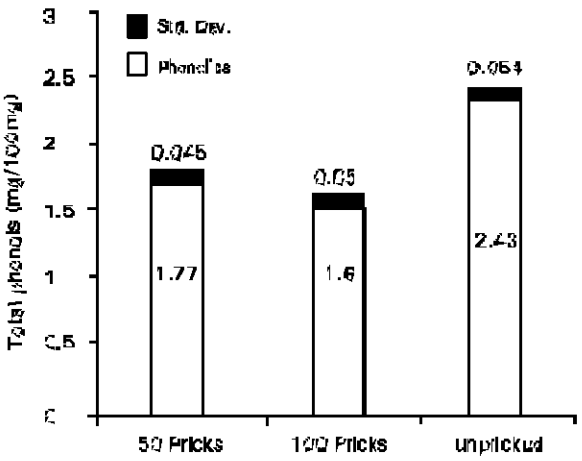


Fig. 1: Mechanical injury and phenol production

Feeding activities of herbivorous insects often result in physiological, morphological and chemical changes by accumulation of the compounds having defensive properties. According to Kogan and Paxton(1983) many changes that occur following herbivory result in accumulation of phenolic compounds. Our results also revealed by these findings. It is also obvious from the results that even the phenolic status of the susceptible varieties became higher in unsprayed condition yet resistant varieties contained more phenols. Less phenolic production in resistant varieties clearly indicate the less attack of insects on these varieties due to already greater phenolic content. This greater phenol concentration in resistant varieties may contribute to the inhibited herbivory due to the toxic or phagodeterrent effect of phenolics towards insects. Similarly increase in phenolic content of susceptible varieties as a result of insect attack may be due to increased herbivory on less toxic plants.

The results showing elevated phenols as a result of insect attack also revealed by the result of experiment "mechanical injury and phenol production" (Fig.1). This is generally thought that mechanical injury may produce phenolic compounds. Result indicated that mechanically injured (pricked) leaves possessed 0.5-0.7 mg less phenolics as compared to the control (un pricked) leaves. So it can be concluded that increase in phenols in unsprayed field is the result of induced resistance by herbivorous insects. Rose *at el.* (1996) also reported that plants damaged with blade did not produce chemical compounds in comparison to caterpillar infested plants. Similarly Alborn and his coworker (1996) described about the systemic induction of feeding deterrents in cotton plants in response to feeding of *spodoptera* larvae. Significant differences were observed in larval weight of bollworm fed on cotton extracts, mixture of phenolic acids and tannic acid as compared to larvae fed on control diet (Table 2, Fig. 2,3).

Tannic acid showed significant growth retardation , 96.72% with mean larval weight 0.0166g ± 0.0077g while mean larval weight in control was 0.5072g. ± 0.05400g. Phenolic mixture also showed growth retardation, 61.88% with larval weight 0.1933g ± 0.0241g. Methanolic extract of cotton variety Ravi, which contained more phenols showed

Table 2: Influence of some phenolics and cotton extracts 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) | 0.5072a | 0.0500 | 0 | 0 | 0 | 13 | 100 |
| S-12 extract | 0.3915b | 0.0434 | 22.81 | 0.00 | 11.11 | 14 | 88.90 |
| Ravi extract | 0.3006c | 0.0493 | 40.73 | 11.11 | 27.80 | 16 | 72.20 |
| Phenols | 0.1933d | 0.0241 | 61.88 | 44.50 | 94.50 | 18 | 5.50 |
| Tannins (Tannic acid) | 0.0166e | 0.0077 | 96.72 | 50.00 | 100 | No Pupation | 0 |

Means followed by the different letters for larval weight are significantly different at 0.05% probability level by DMRT.

* Diet incorporated with methanolic extract of cotton leaves at 0.1% conc. of diet.

* Diet incorporated with phenolic mixture (cinnamic acid, ferulic acid, chlorogenic acid,sinapic acid) and tannic acid at 0.1% conc. of diet.

** Mean weight of 18 larvae at 7th day of experiment started.

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


Fig. 2: Showing effects of chemicals and cotton extracts on growth and survival of cotton bollworm larvae at 7th day of experiment (a) Control, diet without any additive showing normal larval growth and feeding (b) S-12 extract with slightly retarded larval weight than control (22.81%), no mortality and no feeding inhibition. (c) Ravi extract showing 40.73% weight retardation, 11.11% mortality and comparative phagodeterrency than S-12 and control. (d) Phenolic acid with inhibited feeding, 61.88% growth retardation and 44.5% mortality. (e) Tannic acid showing severe phagodeterrency, 50% mortality and 96.88% weight retardation.


Fig. 3: Showing degree of pupation and mortality in each treatment (a) Control with 100% pupation and no mortality (b) S-12 extract showing 11.11% mortality and 88.9% pupation (c) Ravi extract with 27.8% mortality and 72.2% pupation (delayed) (d) Phenolic mixture with 94.50% mortality and 5.50% delayed pupation. (e) tannic acid showing complete phagodeterrency, 100% mortality and no pupation

significant effect on larval growth and survival than extract of more susceptible variety S-12 that possessed least concentration of phenols. Ravi showed 40.73% weight retardation while larval weights were retarded up to only 22.81% in S-12. Differences in larval growth at 7th day experiment are obvious (Fig.2) in treated and control diets. At 7th day S-12 and control showed no mortality while Ravi, phenols and tannic acid showed 11.11, 44.5,and 50% mortality. Percent mortality in 2nd week of experiment was 100% in tannic acid while 94.5, 27.8 and 11.11% mortality was observed in phenols, Ravi and S-12 extracts respectively. High degree of phagodeterrency was observed in phenolic treatments. Mean days to reach pupation (Table 2, Fig. 2) showed 3 to 5 days delay in Ravi with 72% pupation and phenols with 5.5% as compared to control (100% pupation). S-12 showed normal pupation period (89% pupation), while no pupation was observed in tannic acid.

## References

Ahmed, K., F. Khalique and B.A. Malik, 1998.Modified artificial diet for mass rearing of chick pae pod borer, *Helicoverpa (Heliothis) armigara(Hubn)*. Pakistan Journal of Biological Sciences, 1: 183-187.

Alborn, H.T., U.S.R. Rose, H.J. McAuslane, 1996.Systemic induction of feeding deterrents in cotton plant by feeding of *spodoptera spp*.larvae. J Chem Ecol., 22:919-932

Bray, H. G. and W.V. Thrope, 1954. Analysis of phenolic compounds of interest in metabolism. Methods of Biochemical Analysis (David Glich (eds), 1: 27-52.

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

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

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

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

Jones,W.T. and J.L. Morgan, 1977. Complexes of the condensed tannins of sainfoin with fraction 1 leaf protein, submaxillary mucoprotein and their reversal by poly ethylene glycol. J.Sci.Food & Agric., 28: 126-136.

Kogan, M. and J. Paxton, 1983. Natural inducers of plant resistance to insects. 'Plant Resistance to insects'. ACS symposium Series. P.A.Hedin Eds., pp: 153-171.

Lege, K.E., J.T. Cothren and C.W. Smith, 1995. Phenolic acid and condensed tannin concentrations of six cotton genotypes. Environmental and Experimental Botany, 35:241-249.

Rose, U.S.R., A. Manukian, R.R. Heath and J.H. Tumlinson, 1996. Volatile Semiochemicals released from undamaged cotton leaves: A systematic response of living plants to caterpillar damage. Plant Physiol., 111: 487-495.

Swain, T., 1965. The tannins. Plant Biochemistry. Ed. by Bonner J. Varner J. E. Academic press. New York, pp: 552-579.

Zummo, J.H. Benedict and J.C. Segers ,1984. Effect of the plant growth regulator Mepiquate chloride on host plant resistance in cotton to bollworm *(Lepidoptera: Noctuidae)*. J. Econ. Entomol., 77: 922-924.