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Biochemical Basis for Antibiosis Mechanism of Resistance in Sugarcane to Early Shoot Borer, *Chilo infuscatellus* Snellen

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ABSTRACT. *Studies on biochemical basis of resistance of sugarcane to early shoot borer, Chilo infuscatellus Snellen in different sugarcane genotypes, viz., four least susceptible (LS), four moderately susceptible (MS) and four highly susceptible (HS) genotypes in comparison with one susceptible check were carried out under in vitro conditions at Regional Agricultural Research Station, Anakapalle, Andhra Pradesh, India during 2009-10. The results revealed reduced larval survival (26-30%), weight (79.9-84.6 mg/larva), and prolonged larval period (21.32-22.26 days) on LS genotypes compared to HS genotypes (64-84.2%; 149.3-179.9 mg/larva; 16.56-18.78 days, respectively). The lowest mean growth index was found on LS genotypes (1.53-1.73) compared to HS genotypes (3.51-4.71). Results indicated the presence of antibiosis mechanism in the shoot tissues of LS genotypes either by accumulation of toxins or by the absence of very essential nutrients or feeding stimulant or by the presence of some feeding deterrent. Biochemical analysis indicated that low total sugar (5.34-6.38%), reducing sugar (0.078-0.089%) and higher phenol (11.20-12.30 mg/g), neutral detergent fiber (24.09-27.20%), acid detergent fiber (54.79-57.26 %), lignin (6.61-7.09%), silica (1.33-1.53%) and cellulose (25.95-28.58%) contents in the shoot tissues of LS genotypes (Co 0110, 98 A 165, Co 6806, 98 A 125) increased its resistance to early shoot borer by influencing the biology, establishment of early shoot borer and played an important role in the antibiosis mechanism.*

Key words: *Biology, survival, phenols, silica and total sugars*

INTRODUCTION

Early shoot borer (ESB), *Chilo infuscatellus* Snellen (Crambidae; Lepidoptera) is a serious pest in peninsular regions of India and a vital pest in early crop growth stages of sugarcane causing an economic loss (Avasthy & Tiwari, 1986). It destroys 26-65% of mother shoots (Khan & Krishnamurthy Rao, 1956) and causes losses of cane yield (22-33%), sugar recovery (12%) and jaggery (27%) (Patil & Hapse, 1981). It infests rainfed sugarcane crop causing 70% shoot loss (Prasad Rao *et al.*, 1991). In Andhra Pradesh, maximum yield loss could reach 42% when the incidence was at 60 day-old crop (Lakshminarayana, 1983). It destroys 58% of shoots in different states, causing reduction of 10.1-34.4 t/ha in cane yield and 0.25-3.0 units in sugar recovery (Chaudhary, 1973).

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Management strategies of early shoot borer include the use of resistant varieties. Knowledge on resistance mechanisms and associated factors involved is essential for effective utilization of sources of resistance in the future breeding programmes.

This study was carried out with the objectives of investigating the response of different genotypes and exploring the mechanism and biochemical basis of host plant resistance in different susceptible groups of genotypes against the early shoot borer, *C. infuscatellus* in sugarcane.

MATERIALS AND METHODS

Screening of sugarcane genotypes against ESB under field conditions

Preliminary study on screening of 147 sugarcane genotypes under natural and artificial infestation was carried out to identify the less susceptible genotypes against ESB at Regional Agricultural Research Station (R.A.R.S.), Anakapalle, Andhra Pradesh, India during 2008-09. Based on the per cent cumulative incidence of ESB, genotypes were graded according to Seshagiri Rao and Krinshnamoorthy (1973) and 12 sugarcane genotypes showing low to high susceptibility to ESB along with the susceptible check (93 A 145) were selected and utilized for further studies.

Category		Genotypes
Least Susceptible (LS)	:	98 A 125, 98 A 165, Co 0110 and Co 6806
Moderately susceptible (MS)	:	97 A 85, 2000 A 105, 99 A 5 and Co 62175
Highly susceptible (HS)	:	93A 11, 99 A 33, 94 A 124 and Co M 9902

These twelve genotypes along with susceptible check (93 A 145) were planted in a randomized block design with three replications at R.A.R.S during 2009-2010. The maximum number of shoots and the total number of dead hearts produced at 60 days after planting (DAP) in different genotypes were recorded and percent incidence of early shoot borer (% deadhearts) calculated. The data were subjected to analysis of variance.

Survival and development of ESB in the artificial diet compounded with dry shoot powder of different sugarcane genotypes

The experiment was conducted in IPM laboratory, R.A.R.S. during March to May, 2010. Antibiosis component of resistance was measured by impregnating dry shoot powders of twelve selected genotypes and a susceptible check 93 A 145 into the artificial diet under *in vitro* conditions. Ten neonate larvae were released on to the artificial diet impregnated with respective shoot powder of selected sugarcane genotypes. Each treatment was replicated three times. In the rearing room, temperature was maintained at 28±1 °C, 60-70% relative humidity (RH) and photo period of 12 h.

For the preparation of shoot powder, shoots of sugarcane (93 A 145) were collected from 60 -day old plants raised under field conditions and dried in a hot air oven at 80 °C for two days and powdered to <80 mesh size. The different ingredients utilized for preparation of the artificial diet were listed in Table 1. All the ingredients of fraction A (Table 1), except the sugarcane shoot powder, were blended for 1 min. Field bean seeds (100 g) were soaked in water for 24 h and extruded manually from the seed coat and cooked before use. They were

mixed with other ingredients of the medium (Fraction-A) except agar-agar, ascorbic acid, sugarcane shoot powder and ground to a paste. Agar was melted in 80 ml of water (Fraction-B) and sugarcane shoot powder was added to it and the mixture was poured into the blender containing Fraction-A. All the constituents were blended for three minutes followed by autoclaving. Ascorbic acid was dissolved in 30 ml of water and formaldehyde was added to medium just before it began to solidify. Medium was poured into plastic containers of 250 ml and also into plastic rearing trays having 10 cups of 25 ml capacity.

Table 1. List of ingredients of artificial diet used for rearing of ESB in the laboratory

Sample No.	Ingredients	Quantity
1	Shoot powder	50.0 g
2	Field bean	100.0 g
Fraction- A		
3	Casein	50.0 g
4	Yeast	16.0 g
5	Vitamin capsules	8.0 g
6	Salt mixture	5.0 g
7	Ascorbic acid	1.0 g
8	Sorbic acid	0.5 g
9	MPH	1.0 g
Fraction –B		
9	Agar agar	8.0 g
10	Formalin	1.0 ml
11	Distilled water	470 ml

Source: Mukunthan & Jayanthi, 2001

After cooling the medium for 2-3 h on the laboratory table, neonate larvae were released in to each cup using a fine brush. The plastic containers were kept in the rearing room in the dark for 2 days, because neonate larvae have a strong photopositive behaviour and settle better on the diet in darkness. Rearing rooms conditions were maintained as 28±1 °C, 60-70% RH and 12 h light period per day.

There were three replications for studying larval development and measuring larval survival at 15 days after infestation (250 ml capacity cups having 150 g diet). Observations on larval survival and larval mass at 15 days after inoculation, duration of larval development were recorded and calculated the growth index of *C. infuscatellus* on artificial diet impregnated with different sugarcane genotypes.

$$\text{Growth index} = \frac{\text{Mean percentage of larvae pupated (n)}}{\text{Mean larval period (p)}}$$

Statistical Analysis

The data were analyzed using ANOVA. Treatment means were compared using least significance difference (LSD) at $P<0.05$.

Studies on biochemical parameters of different sugarcane genotypes

Biochemical differences among twelve selected genotypes and one known susceptible check 93 A 145 were examined using samples of sugarcane at 60 DAP. Collected leaf sheath and shoot samples were washed 3-4 times with distilled water. The washed samples were kept at 80 °C in an electrical hot air oven for 48 h and grounded into powder for the estimation of neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose, silica and lignin contents in shoot tissues. Fresh samples were utilized for estimation of total phenols, reducing and non-reducing sugars. Extractions were prepared following the method suggested by Mahadevan and Sridhar (1986).

Estimation of total phenols

Total phenols were determined by adopting the method suggested by Mallick and Singh (1980). Ethanol extract (1 ml) was taken into a graduated test tube and 1 ml of Folin-Ciocalteu reagent and 2 ml of 20% Sodium carbonate were added. The mixture was heated in a water bath for about one minute and then cooled under running water. The sample was diluted to 25 ml with distilled water. The absorbance of the resultant blue colour solution was read at 650 nm in a Spectronic-20. A reagent blank was maintained with 1 ml of distilled water in place of ethanol-extract. Total phenols were calculated from Catechol standard curve and expressed in mg/g of sample.

Estimation of total sugars

Total sugar content was estimated using the method suggested by Dubois *et al.* (1956). Ethanol extract (0.2 ml) was taken into a volumetric flask and 1.8 ml of distilled water and 0.25 ml of 80% phenol were added. The sample was shaken and 5 ml of conc. H_2So_4 was added immediately and allowed to stand for 30 min. Per cent absorbance at 490 nm was measured using Spectronic-20 using a blank without sugar extract. The absorbance scale and sample values were corrected for the mean of three reaction blanks. Standard curve of glucose concentration versus absorbance was used to convert sample absorbance to sugar percentage. The average value of plant sample was recorded as the total sugar in mg/g of sample.

Estimation of reducing Sugars

Reducing sugar content of the plant samples was estimated by adopting Nelson and Somogyi method (Krishnaveni *et al.*, 1984). Ethanol extract (1 ml) was mixed with Copper reagent (1 ml). The mixture was heated for 20 min. in a water bath, cooled and arsenomolybdate (1 ml) reagent was added to the mixture. The intensity of blue colour of the mixture was read against a blank in Spectronic-20 at 520 nm and was expressed in terms of glucose equivalents. Standards with different concentrations (*i.e.* 25, 50, 75, 100 and 125) were prepared from the working standard and their absorbance was read by taking one ml aliquots.

Percent of total soluble sugars was calculated by using the formula:

$$\text{Total soluble sugars} = \frac{\text{Conc. of Std.}}{\text{Absorbance of Std.}} \times \text{Absorbance of 1 ml extract} \times \frac{1}{1000000} \times \frac{3\text{ ml}}{0.1\text{ g}} \times 100$$

Neutral detergent fibre (NDF) and Acid detergent fibre (ADF)

NDF content of sugarcane genotypes was measured by using the method suggested by Goering and Van soest's (1970) and calculated by using the following formula.

$$\text{NDF (\%)} = \frac{\text{Wt. of oven dry crucible with sample} - \text{Wt. of oven dry crucible without sample}}{\text{Wt. of sample}} \times 100$$

Air dried sample (1 g) was taken into a beaker of the refluxing apparatus, and acid detergent solution (100 ml) and decahydro-naphthalene (2 ml) were added. The ADF was extracted and estimated by Goering and Van soest's (1970) method.

Cellulose, lignin and silica

A crucible (sintered) containing ADF was filled with H_2SO_4 (72%) and stirred with glass rod to smoothen the paste and break the lumps. H_2SO_4 (72%) was added again and stirred at hourly interval till acid drained away. Thereafter, the contents were washed with hot water to make these free from acid. The residue in crucible was dried overnight at 100 °C, weighed and then kept in muffle furnace at 500-550 °C for 3 h. On cooling, the crucible was removed and the weight was measured. The cellulose, lignin and silica percentage were calculated as follows:

$$\text{Cellulose (\%)} = \frac{\text{Loss with 72\% } H_2SO_4}{\text{Weight of sample}} \times 100$$

$$\text{Lignin (\%)} = \frac{\text{Loss upon ignition after 72\% } H_2SO_4}{\text{Weight of sample}} \times 100$$

$$\text{Silica (\%)} = \frac{\text{Weight of crucible with silica - Wt. of oven dry empty crucible}}{\text{Weight of sample}} \times 100$$

Experimental design was completely randomized design (CRD) and the data were analyzed using ANOVA.

RESULTS AND DISCUSSION

Field screening of different sugarcane genotypes against ESB

The results of field screening of different genotypes for resistance to ESB revealed that the LS genotypes had less than 15% dead hearts (DH) *viz.*, Co 0110 (8.49%), 98 A 165 (9.87%), Co 6806 (9.58%) and 98 A 125 (8.01%) and the MS genotypes 97 A 85 (22.5%), 2000 A 105 (24.29%), 99 A 5 (24.23%) and C0 62175 (26.33%) had 15-30 %, whereas the HS genotypes 93A 11 (31.86%), 99 A 33 (37.54%), 94 A 124 (34.98%), Co M 9902 (41.77%) and the susceptible check, 93 A 145 (39.22%) had more than 30% incidence of ESB at 60 DAP. The plant samples of these thirteen genotypes were collected at 60 DAP and utilized for further studies in the present investigation (Table 2).

Effect of sugarcane genotypes incorporated in artificial diet on ESB

Effect of least susceptible sugarcane genotypes incorporated in artificial diet on larval survival, larval mass, duration of larval development of *C. infuscatellus* in comparison with susceptible check, 93 A 145 are presented in Table 3.

Table 2. Percent germination and incidence of ESB (% DH) in different genotypes at 60 DAP -2009-2010

Genotype	Germination (%) at 35 DAP	Total shoots At 60 DAP	No. of Dead hearts at 60 DAP	DH (%) at 60 DAP (*)
LS genotypes				
Co 0110	100.00	161.00	13.70	8.49 (16.92)
98 A 165	92.90	125.00	12.30	9.87 (18.25)
Co 6806	92.10	135.70	13.00	9.58 (18.00)
98 A 125	95.90	104.00	8.30	8.01 (16.42)
MS genotypes				
97 A 85	95.40	146.70	33.00	22.50 (28.28)
2000 A 105	97.50	70.00	17.00	24.29 (29.72)
99 A 5	99.40	263.3	63.8	24.23 (29.25)
Co 62175	91.30	93.70	24.70	26.33 (30.85)
HS genotypes				
93 A 11	86.30	166.30	53.00	31.86 (34.35)
99 A 33	90.40	97.70	36.70	37.54 (37.78)
94 A 124	90.00	223.00	78.00	34.98 (36.25)
Co M 9902	100.00	135.70	56.70	41.77 (40.30)
93 A 145 (Susceptible check)	92.90	145.30	57.00	39.22 (38.78)
CD (5%)	NS	15.8420	6.3425	1.7950
CV%		9.46	7.53	5. 69

LS: least susceptible; MS: moderately susceptible; HS: highly susceptible
Figures in parenthesis are transformed values

Effect on larval survival

Larval survival was significantly low on artificial diet incorporated with LS genotypes (26% on 98 A 125 to 34% on Co 6806) and more on HS genotypes (64-84.2%) as compared to susceptible check (84%) at 15 DAI. This clearly indicates the adverse effect of LS genotypes on larval survival of ESB (Table 3). The LS genotype, 98 A 125 affected larval survival in artificial diet and recorded the lowest survival at 15 DAI (26%). Similar results have been obtained by earlier workers with reference to *C. partellus* on maize (Durbey & Sarup 1985; Sharma & Chatterji 1971a) who inferred that the mortality of *C. partellus* on resistant maize genotypes might be due to the accumulation of toxins or lack of very essential nutrients or lack of feeding stimulant including the possibility of presence of some feeding deterrent and

indicating the presence of antibiosis mechanism in the shoot tissues. Similar results have been obtained by earlier workers with reference to larval survival on sorghum (Kalode & Pant, 1967a; Lal & Sukhani, 1979; Lal & Pant, 1981; Singh & Verma, 1988; Taneja & Woodhead, 1989) who reported that mortality of *C. partellus.*

Table 3. Effect of artificial diet impregnated with different genotypes on larval survival and larval mass of ESB-2009-2010

Genotype	Larval survival (%) at 15 DAI	Larval mass (mg)* at 15 DAI	Larval period (days)	Growth index (n/p)
LS genotypes				
Co 0110	30.00	83.0	21.98	1.73
98 A 165	29.80	82.2	21.32	1.69
Co 6806	34.00	84.6	21.14	1.61
98 A 125	26.00	79.9	22.26	1.53
MS genotypes				
97 A 85	50.00	94.8	21.38	2.39
2000 A 105	52.00	99.2	20.40	2.67
99 A 5	56.00	117.2	21.14	2.74
Co62175	60.00	124.3	20.10	2.94
HS genotypes				
93 A 11	64.00	149.3	18.78	3.51
99 A 33	72.00	152.0	17.72	3.72
94 A 124	76.00	157.8	17.22	3.89
CoM 9902	84.20	179.9	16.56	4.71
93 A145 (Susceptible check)	84.00	178.4	16.64	4.51
CD (5%)	5.9911	1.7935	0.2342	0.3546
CV%	8.22	1.16	0.70	0.48

DAI : days after inoculation; * mean of ten individuals
LS: least susceptible; MS: moderately susceptible; HS: highly susceptible

Effect on larval mass

Larval mass of ESB was considerably low, ranging from 79.9 to 84.6 mg per larva when reared on artificial diet impregnated with the shoot powders of four LS genotypes (98 A 125, 98 A 165, Co 0110 and Co 6806) at 15 DAI whereas 178.4 mg per larva was recorded in the susceptible check, 93 A 145 at 15 DAI (Table 3). The difference was two times more than that of the susceptible check, 93 A 145. The HS genotype, Co M 9902 recorded highest larval mass (179.9 mg per larva) and it was on par with the susceptible check. Larvae reared on MS genotypes (97 A 85, 2000 A 105, 99 A 5 and Co 62175) too recorded significantly lower mass compared to HS genotypes and susceptible check, 93 A 145 ($P<0.05$). All four LS genotypes have been found to exercise adverse effect on larval mass of early shoot borer when they were incorporated in artificial diet in the laboratory conditions. Similar results,

with reference to *C. partellus* on sorghum have been reported by Jotwani *et al.* (1978) and Taneja and Woodhead (1989).

Effect on larval period

There were highly significant differences of larval period among the resistant sugarcane genotypes and the susceptible check. In general, duration of early shoot borer larvae reared on the diet incorporated with powdered shoots of LS and MS genotypes was longer compared to the HS genotypes and the susceptible check, 93 A 145 (Table 3). On artificial diet incorporated with the LS genotypes, 98 A 125, the larval duration has been significantly prolonged, 6 days longer than in the susceptible check. However, in the other LS genotypes it ranged from 4-5 days longer than in the check as compared to the susceptible check, 93 A 145. The four MS genotypes, 97 A 85, 2000 A 105, 99 A 5 and Co 62175 also showed significantly prolonged larval duration when compared to susceptible check, 93 A 145. On artificial diet incorporated with four HS genotypes, the larval duration has been shortened and it ranged 20.56 - 21.78 days. The HS genotype, Co M 9902 has recorded 20.56 days of larval duration which was significantly on par with the susceptible check, 93 A 145. The present findings are in agreement with Kalode and Pant (1967a), Sharma and Chatterji (1971a), Jotwani *et al.* (1978) and Durbey and Sarup (1984, 1985).

Effect on growth index

The LS genotype, 98 A 125 showed the lowest growth index (1.53) whereas the HS genotype, Co M 9902 showed the highest growth index (4.71) which was twice that of Co 0110 (Table 3). The MS genotypes also had significant adverse effect on growth index and recorded 2.39 to 2.94 as against the susceptible check, 93 A 145. Higher mean larval period (p) and lower number of larvae pupated (n) might have contributed to lower growth index in LS genotypes. However, p-factor had been more contributive than n-factor. Sharma *et al.* (2007a) reported that in case of sugarcane top borer, *Chilo auricilius*, mean larval survival was lowest on LS group (57.91) compared to 62.08 in MS and 76.67 per cent in HS resulting into highest mean growth index on HS (1.32) followed by MS (0.96) and LS genotypes (0.83).

Biochemical analysis of sugarcane genotypes

The studies were undertaken on the major chemical constituents of leaf sheath and shoot tissues of LS, MS and HS genotypes of sugarcane at 60 DAP to determine the biochemical basis which may be associated with resistance to ESB and data were analyzed to find correlations between various chemical constituents and susceptibility to ESB in different sugarcane genotypes and the results are presented hereunder (Tables 4, 5, 6).

Total sugar and reducing sugar

The amounts of total sugar in shoot tissues of all genotypes were higher than in leaf sheath tissues irrespective of genotype. It was highest (8.98%) in shoot tissues of HS genotype, Co M 9902 and was lowest (5.34%) in LS genotype, 98 A 125 as compared to 8.95% in the susceptible check, 93 A 145 (Table 4). Total sugars in leaf sheath also showed significant difference among genotypes and the differences were ranged from 4.18 to 4.97% in LS. HS genotypes had 4.85% in susceptible check. An increasing trend of total sugar contents of both shoot and leaf sheath tissues of different genotypes was observed with increase in

susceptibility of hosts to ESB and correlated positively with the ESB susceptibility (r=0.96 and 0.88 in shoot and leaf sheath, respectively) (Table 6 and Fig. 1).

A similar trend was observed with respect to reducing sugars (Table 4). The shoots of LS genotypes contained significantly less (0.078-0.089%) amount of reducing sugars and it increased in MS genotypes (0.096-0.112%). It was the highest (0.105-0.173%) in HS genotypes and 0.179% in the susceptible check, 93 A 145. Similar trend was also observed in case of reducing sugars in leaf sheaths (Table 6). The differences in reducing sugar content in shoot and leaf sheath tissues among the different genotypes were significant and showed positive correlation with ESB susceptibility (r=0.89 and 0.82) (Table 6).

Table 4. Total sugars, reducing sugars and phenol contents in the leaf sheath and the shoot of tissues of different genotypes at 60 DAP -2009-10

Genotype	Total sugars (%)		Reducing Sugars (%)		Phenol (mg/g)	
	Shoot	Leaf sheath	Shoot	Leaf sheath	Shoot	Leaf sheath
LS genotypes						
Co 01 10	5.85	4.19	0.081	0.073	11.76	12.82
98 A 165	6.38	4.24	0.083	0.072	11.20	11.86
Co 6806	6.25	4.22	0.089	0.069	11.78	11.9
98 A125	5.34	4.18	0.078	0.063	12.30	13.03
MS genotypes						
97 A 85	7.40	4.19	0.096	0.068	10.27	11.77
2000 A 105	7.85	4.67	0.109	0.073	10.14	10.94
99 A 5	7.34	4.29	0.098	0.070	9.9	10.93
Co 62175	6.93	4.68	0.112	0.077	9.92	11.09
HS genotypes						
93 A 11	8.57	4.74	0.135	0.084	9.12	10.85
99 A33	7.82	4.55	0.105	0.079	9.2	12.24
94 A124	8.83	4.82	0.157	0.079	8.94	11.03
Co M 9902	8.98	4.97	0.173	0.082	7.68	9.43
93 A 145 (susceptible check)	8.95	4.85	0.179	0.079	8.87	9.22
CD (5%)	0.0284	0.0304	0.001	0.001	0.0588	0.012
CV%	0.23	0.40	0.50	0.84	0.35	0.06

DAP : Days after planting
LS: least susceptible; MS: moderately susceptible; HS: highly susceptible

Similar relation was reported by Rao and Rao (1961) for *C. auricilius*. Kalode & Pant (1967b) found that sorghum varieties susceptible to *Chilo zonellus* Swinhoe contained higher percentage of sugars than resistant ones. Sharma & Chatterji (1971b) and Knapp *et al.* (1965) observed 50% more reducing sugars in susceptible varieties to corn earworm. Since, sugar is

considered as one of the vital nutrients in plants and also sugar contents reflect the metabolic state of the sugarcane shoot, the differences in the relative amounts of sugars between different genotypes with differential susceptibilities to ESB indicate that these compounds might act as phagostimulants to *C. infuscatellus* feeding on sugarcane.

Total Phenols

Leaf sheaths contained higher total phenols than the shoot tissues (Table 4). The LS genotypes contained higher shoot tissue phenols (11.2-12.3 mg/g) than MS genotypes (9.9-10.27 mg/g) and HS genotypes (7.68-9.2 mg/g) as against 8.87 mg/g in susceptible check, 93 A 145. The LS genotype, 98 A 125 contained maximum amount (12.3 mg/g) and minimum was in HS genotype, Co M 9902 (7.68 mg/g). In leaf sheath tissues also, maximum phenol content was recorded in LS genotypes and minimum in HS genotypes. Phenol contents of both shoot and leaf sheath tissues showed significant differences among different genotypes and also showed a significantly inverse correlation with ESB, *C. infuscatellus* susceptibility. The LS genotypes recorded higher phenol contents than MS and HS genotypes (Table 4 & Fig. 1).

Phenol contents of both shoot (r=-0.97) and leaf sheath tissues (r=-0.80) showed a significantly inverse correlation with ESB, *C. infuscatellus* susceptibility (Table 6). Phenolic compounds have been implicated in the resistance of a number of plant species to various insect- pests like shoot borer in sugarcane. Phenols are one of the most important group of plant defense chemicals responsible for antifeedant and/or antibiotic effects on insects (Kennedy & Nachiappan, 1992; Shroff, 1996). Aphid in wheat (Niraz *et al.*, 1987), whitefly *in cotton* (Buttler *et al.*, 1992), aphid on mustard (Sachan & Achan, 1991) and stem borer *C. partellus* in maize (Kabre & Ghorpade, 1998) have also recorded *similar* observation in relation to stem resistance.

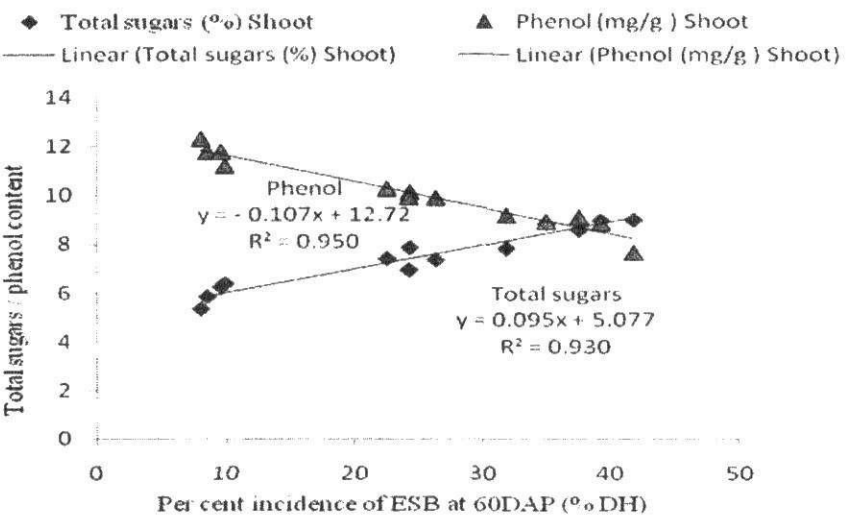

Fig. 1. Correlation between total sugars and phenol contents in shoot tissues of different sugarcane genotypes and ESB incidence at 60 DAP NDF and ADF

NDF was higher (54.79-57.26%) in shoot tissue of least susceptible genotypes (Table 5). It was 47.21-50.65% in MS and 45.07-50.11% in HS genotypes. The genotype, 98 A 125 had the highest NDF (57.26%) while the lowest (46.42%) was in susceptible check, 93 A 145. The differences in NDF content in shoot tissues among the susceptibility groups were more apparent, witnessing a gradual decline in NDF content with increase in susceptibility of hosts to ESB indicating significant inverse relationship (r=-0.92) between NDF and susceptibility of the shoots to ESB infestation (Table 6 and Fig. 2).

The ADF content in shoot tissue varied from a lowest of 20.7% in HS genotype, Co M 9902 to the highest of 27.2% in LS genotype, 98 A125 as compared to 20.83% in the susceptible check, 93 A 145 (Table 5). The ADF contents in shoot tissue of least, moderately and highly susceptible genotypes were estimated at 24.09-27.20%, 20.51-22.81% and 20.70-22.11%, respectively. A gradual decline in ADF content with increase in susceptibility of hosts to ESB indicated a significantly negative correlation with susceptibility. The percentage of ADF and the NDF were found to be much lower in genotypes of high susceptibility and therefore, showed a significantly negative correlation (r=-0.86) with ESB infestation (Table 6 & Fig. 2).

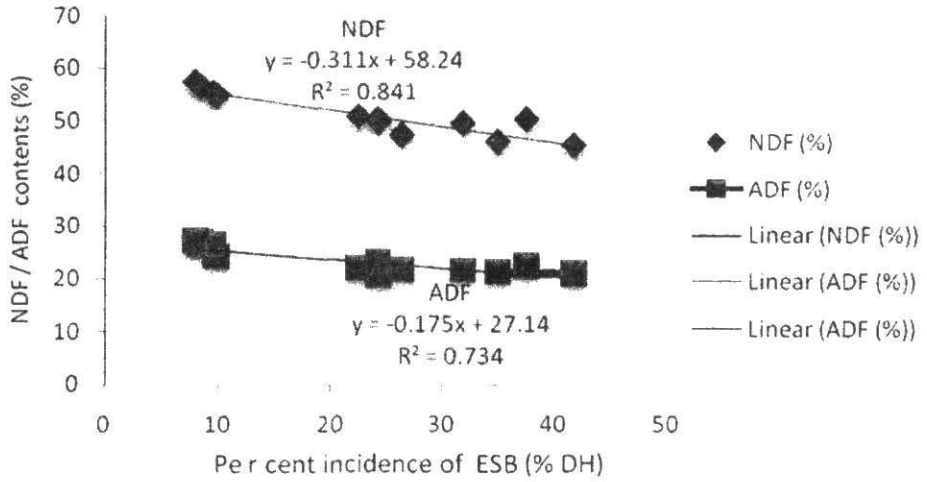

Fig. 2. Correlation between NDF, ADF contents (%) of different sugarcane genotypes and ESB incidence at 60 DAP

Cellulose, lignin and silica

Cellulose, lignin and silica contents in shoot tissues at 60 DAP revealed that the stalk tissue of least susceptible genotype, 98 A 125 recorded relatively higher amount of cellulose (28.58%) than other LS genotypes (Table 5). The MS genotypes contained 22.43-25.10% cellulose and HS genotypes had 23.95-18.97%. This indicates a decreasing trend in cellulose content in shoot tissues in genotypes with the susceptibility showing a negative correlation (r=-0.90) with ESB susceptibility (Tables 5 and 6).

Similarly, lignin content of shoot was maximum (7.09%) in LS genotype, 98 A 125 and minimum (4.96%) in HS genotype, Co M 9902 (Table 5). The lignin percentage in shoot tissue of LS, MS and HS genotypes varied from 6.61 to 7.09; 5.99 to 6.38 and 4.96 to 5.69, respectively as compared to 5.11% in the susceptible check, 93 A 145 indicating a negative correlation between lignin content and ESB susceptibility (r=-0.94) (Table 6).

Table 5 revealed that the silica content in shoot tissues of LS genotypes was significantly high (1.33-1.53%) as compared to the susceptible check, 93 A 145 (0.79%). The shoot tissues of MS genotypes also contained significantly more silica content (1.09-1.24%) than HS genotypes (0.75-0.98%). The differences in silica content in different groups of genotypes were significant ($P<0.05$) and showed decreasing trend with susceptibility and exhibited significantly inverse relationship with ESB susceptibility (r =-0.96) (Table 6).

Table 5. Effect of NDF, ADF, cellulose, lignin and silica contents in shoot tissues of different genotypes on the incidence of ESB at 60 DAP-2009-2010

Genotype	NDF (%)	ADF (%)	Cellulose (%)	Lignin (%)	Silica (%)
LS genotypes					
Co 0110	56.40	26.82	27.81	6.95	1.47
98 A 165	54.79	24.09	28.38	7.06	1.51
Co 6806	55.29	26.41	25.95	6.61	1.33
98 A125	57.26	27.20	28.58	7.09	1.53
MS genotypes					
97 A 85	50.65	21.82	24.74	6.38	1.24
2000 A105	49.60	20.51	25.10	6.31	1.21
99 A 5	47.21	21.61	22.43	5.99	1.09
Co 62175	50.11	22.81	23.82	6.25	1.19
HS genotypes					
93 A 11	50.11	22.11	22.48	5.49	0.92
99 A33	49.41	21.47	23.95	5.69	0.98
94 A124	45.84	21.07	20.62	5.36	0.87
Co M 9902	45.07	20.70	18.97	4.96	0.75
93 A 145 (Susceptible check)	46.42	20.83	19.06	5.11	0.79
CD (5%)	0.0442	0.0287	10.3744	0.0107	0.0104
CV%	0.05	0.07	19.46	0.14	0.54

NDF :Neutral detergent fibre ADF: Acid detergent fibre DAP : Days After Planting
LS: least susceptible; MS: moderately susceptible; HS: highly susceptible

Table 6. Correlation between biochemical constituents of different genotypes and ESB incidence at 60 DAP -2009- 2010

Biochemical constituent	Correlation with ESB incidence (%DH) at 60 DAP	
	Leaf sheath	Shoot
Reducing sugar (%)	0.89*	0.82*
Phenol (mg/g)	- 0.97*	- 0.80*
Silica (%)	--	-0.96*
Cellulose (%)	--	-0.90*
Lignin (%)	--	-0.94*
Neutral detergent fibre (NDF) (%)	--	-0.92*
Acid detergent fibre (ADF) (%)	--	-0.86*

*Significant at 5 % level

The LS genotypes had a relatively higher amount of cellulose, lignin and silica, the basic constituents of NDF and ADF that had a supportive function in cell wall thickening and hardening of plant tissues, thereby, help to avoid attack by the ESB. Rao (1962) reported similar results: a negative correlation between silica content and shoot borer incidence. Varieties with a high number of silica cells per unit area in the leaf-sheath portion, 2-3 inches from the base of the shoots are resistant to shoot borer infestation. Khurana and Verma (1983) with reference to *C. partellus* in sorghum observed that natural detergent fibre, acid detergent fibre, cellulose, lignin, tannin and total phenols in 50 day old plants had negative correlation with susceptibility to *C. partellus* in sorghum. Similarly, Sharma *et al.* (2007b) reported negative association between biochemical constituents like the NDF, ADF, phenol, cellulose and lignin content in leaf sheath and stalk with susceptibility to top borer *C. auricilius* in sugarcane whereas positive correlation had been reported between total sugars, reducing sugars and top borer susceptibility.

CONCLUSIONS

The present study revealed reduced larval survival, reduced larval mass and prolonged larval developments of ESB in least susceptible genotypes compared to highly susceptible genotypes as well as susceptible check, 93 A 145. This is mainly due to the presence of antibiosis mechanism in the shoot tissues of LS genotypes either by accumulation of toxins or lack of essential nutrients or lack of feeding stimulant including the possibility of presence of some feeding deterrents.

Biochemical analysis of different sugarcane genotypes indicated that lower total sugar, reducing sugar contents and higher phenol, NDF, ADF, cellulose, lignin, silica and cellulose contents in the shoot tissues of LS genotypes (Co 0110, Co 6806, 98 A 125, and 98 A 165) has increased its resistance to early shoot borer by influencing the biology, establishment of early shoot borer and played an important role in the antibiosis mechanism.

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