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Biochemical resistance in sugarcane, tentative conclusions and new research directions-II

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Abstract
Sugarcane genotypes selected on the basis of dead hearts formation were studied for biochemical based characters of resistance. Variations in biochemical parameters were recorded especially amount of total and reducing sugars at 45 days after planting (DAP), the least susceptible genotypes contained less, whereas highly susceptible group had more total and reducing sugars. The highest amount of total sugar was estimated in highly susceptible variety CoJ 64 (7.9%) and lowest in genotypes Co 87263 (4.9%). At 60 DAP; highly susceptible genotypes recorded the highest total and reducing sugar contents; average content of total sugars were 5.1, 5.7 and 7.2 per cent and of reducing sugars 0.06, 0.094 and 0.192 per cent in least moderately and highly susceptible entries. Tannin contents at 45 DAP decreased gradually with the borer susceptibility. The average tannin content of least, moderately and highly susceptible entries were estimated to be 12.06 mg, 11.71 mg and 10.22 mg per gm dry weight of tissue, respectively. Highly susceptible genotypes recorded highest average nitrogen content (1.6%) as against least (1.35%) and moderately susceptible (1.42%). Phosphorus contents accounted with an average content of 0.39 per cent in least susceptible, 0.36 per cent in moderately susceptible and 0.3 per cent in highly susceptible genotypes. Lowest average potassium content (1.67%) was evident in highly susceptible entries in comparison with least and moderately susceptible, which recorded 2.4 and 2.2 per cent potassium contents, respectively. Highly significant positive association of nitrogen level and highly significant negative correlation of potassium and phosphorus levels with varietal susceptibility have been observed.

Keywords: Sugarcane, early shoot borer, antibiosis, resistance, biochemical characters

Introduction
A number of insect pests and diseases attack sugarcane crop owing to long duration crop, luxurious growth, variety of niche etc., which results in declines of sugarcane production by approximately 20.0 per cent by insect-pests. India was the second largest producer of sugar in the world after Brazil in 2015-16 [1]. India produced around 350 million tonnes of sugarcane and 25 million tonnes of sugar [2]. In 2016-17, area was 49.27 lakh ha with average cane productivity of 70.7 t/ha and production was 348.45 million tones [3]. Out of more than half dozen lepidopterous tissue borers; these lepidopterous species, inflict severe losses in cane yield as well as in sugar recovery [4]. One of these, Shoot borer, *Chilo infusatellus* Snellen (Crambidae: Lepidoptera) commonly known as shoot borer in North Indian sugarcane belt and as early shoot borer in peninsular India is chronic and of wide occurrence, infesting the *eksali* or spring planted crop during its early stages of growth (March to June) and *adhsali* crop during September to October, every year. The crambid moth borer eat their way along the spindle/stems length by consuming soft tissues thus killing shoots, impairing growth, destroying meristematic tissues of mother axixultimately alleviate breakage of canes. In parts of Rajasthan, it also infests millable canes particularly in years of drought or scanty rainfall. Damage done by *C. infuscatellus* results in killing of mother shoot causing formation of dead hearts and thereby creating a gap in sugarcane field that ranges from 30.0 to 75.0 per cent in the early stage of the crop (May-June) in subtropical India [5].
Owing to unscrupulous use of chemical pesticides increased exposure to high sugared and high yielding genotypes caused imbalances in the biotic system resulting into development of resistance by insect-pests, fall in bio-diversity especially parasites and predators compounding with resurgence of pests and secondary pest outbreaks at the same time arising problems of environmental deterioration, contamination of food chain, human and animal health hazards [6].

An epitome shift in pest control strategies from unilateral chemical approach to integrated pest control has, therefore, become imperative. Selection of resistant varieties for cultivation is premier maneuver in Integrated Pest Management because, being environment friendly, very flexible, economical and practical mean to contain insect-pests and diseases. An experiment was laid to estimate the factors of antibiosis resistance in sugarcane.

Materials and Methods

Field experiment was laid down at Research Farm, CCS HAU, Regional Research Station, Uchani, Karnal on the second fortnight of March, 2014 with the objective to analyse the impact of the biochemical characters of sugarcane plant imparting some sort of resistance against arthropods especially shoot borer infesting sugarcane crop. Ten sugarcane genotypes were selected on the basis of incidence of shoot borer assessed as the per cent dead hearts at 60 and 90 days after planting and categorized on a three degree scale i.e. 1= least susceptible, 2= moderately and 3= highly susceptible [7]. Three budded sets of sugarcane genotypes selected for study were planted in randomized block design with three replicates each in furrow drawn at a spacing of 75 cm. Dead hearts counts due to *Chilo infuscatellus* was recorded at 60 and then at 90 days after planting (DAP) along with other field's observation by adopting standard procedure of observation to estimate their correlation with the different biochemical characteristics. The estimations of biochemical constituents were made twice at 45 and 60 days after planting (DAP). The oven dried samples cooled and ground in Willey Grinding Mill with 1mm screen. Total sugars [8], reducing sugars [9], total phenol [10] using Folin Denis reagent [11], tannin [12], Nitrogen, phosphorus and potassium [13] were calculated by adopting procedures of standard methods of estimation.

Results and Discussion

Host plant resistance holds an integral part in integrated pest management of field crops. The statement of Wilson and Huffakar reflects much of a paradigm upon which IPM is built "…. biological controls, together with plant resistance are the core around which pest control in crops and forests should be built" [14]. It has become increasingly important to understand the interactions between insect-pests and host plants for building foundation of breeding programme for resistance.

Keeping in the view some of their morphological characters in relation to borer incidence as young virgin moth of *Chilo infuscatellus* have been reported to oviposit generally on the ventral side of the third and fourth leaf from the base of shoot [6]. The data of sugarcane genotypes chosen to investigate for their biochemical characteristics as well are presented in tables at column 1.

Total sugars and reducing sugars

As the evident from the data at 45 DAP (Table 1, column 2 & 3), the least susceptible genotypes contained less amount of total and reducing sugars, whereas highly susceptible group had more total and reducing sugars (Fig.1). The highest amount of total sugar was estimated in highly susceptible variety CoJ 64 (7.9%) and lowest in genotypes Co 87263 (4.9%). Likewise, highest percentage of reducing sugar was recorded in highly susceptible entries Co 1148 and CoH 70 (0.24%) and minimum in variety CoS 767 (0.065%). Thus total and reducing sugars have hown a significant positive correlation with borer attack.

At 60 DAP, highly susceptible genotypes recorded the highest total and reducing sugar contents (Table 2, column 2 & 3). The average content of total sugars were 5.1, 5.7 and 7.2 per cent and of reducing sugars 0.06, 0.094 and 0.192 per cent in least, moderately and highly susceptible entries (Fig. 2). This indicated an increase in sugar levels with increased borer susceptibility. Total sugars in different susceptibility group ranged from 5.1 per cent in least susceptible Co 87263 to 7.9 per cent in highly susceptible CoJ 64 and reducing sugars from 0.06 per cent in least susceptible variety CoS 767 to 0.23 per cent in highly susceptible Co 1148. Total sugars and reducing sugars varied greatly between genotypes. Mean sugar contents of young shoots of highly susceptible genotypes were greater than moderately or least susceptible genotypes. Such differences were more pronounced for reducing sugars ($r = 0.8244$, 45 DAP). Significant positive association of total and reducing sugars with borer incidence was, thus, apparent as similar relation was earlier reported [15]. In case of sugarcane mite, *Aceria sacchari* and stalk borer, *Chilo auricilius* the total sugar content were also highest in susceptible varieties [16], [17]. This relationship was also found true in other crops as well. A positive correlation of aphid, *Lipaphis erysimi* population with sugar contents of Indian mustard varieties [18]. Contrarily, in chickpea Gram pod borer, *Helicoverpa armigera* resistant cultivar had higher amount of non reducing sugars compared to the susceptible cultivars [19], as also in cotton against whitefly [20]. Since sugar is considered one of the vital nutrients in the plants, and also sugar contents reflect the metabolic state of the young sugarcane shoot, the differences in the relative amount of sugars between genotypes with differential susceptibilities indicate that these compounds might act as phagostimulants to *C. infuscatellus* feeding on sugarcane.

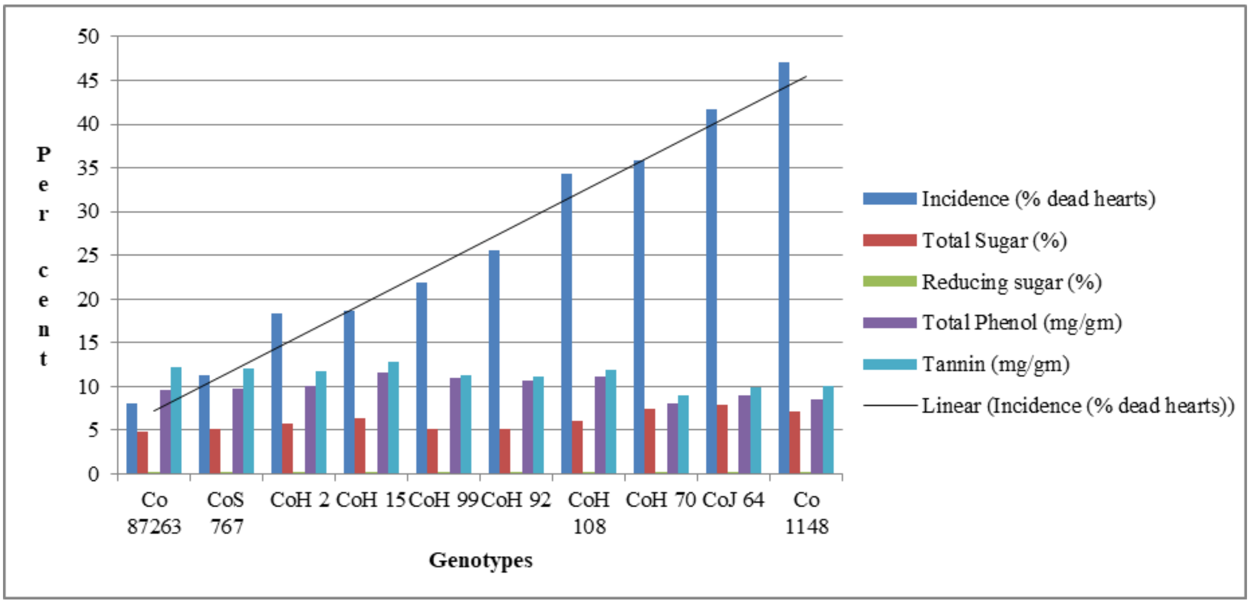

Fig 1: Total and reducing sugar, total phenol and tannin contents of sugarcane genotypes at 45 days after planting

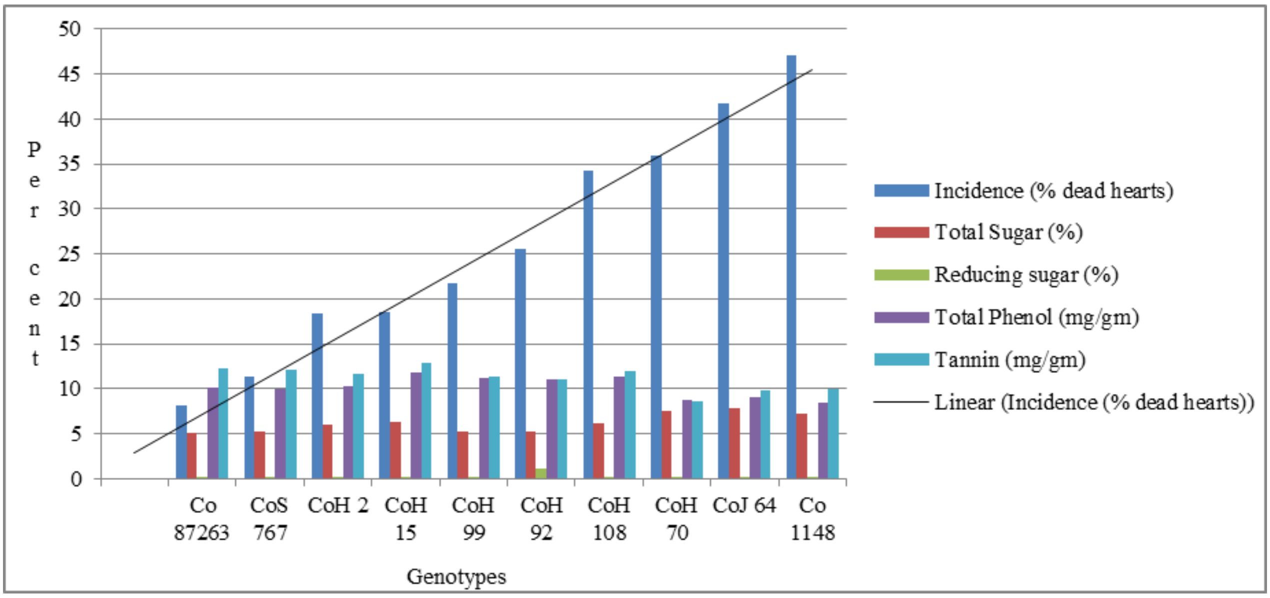

Fig 2: Total and reducing sugar, total phenol and tannin contents of sugarcane genotypes at 60 days after planting

Total phenols and tannins

Tannin contents at 45 DAP decreased gradually with the borer susceptibility (Table 1, column 4 & 5). The phenolic constituents showed irregular trend, the average content being maximum in moderately susceptible genotypes. The average tannin content of least, moderately and highly susceptible entries were estimated to be 12.06 mg, 11.71 mg and 10.22 mg per gm dry weight of tissue, respectively (Fig. 3). Significantly less phenolic constituents were recorded in highly susceptible than moderately or least susceptible. Both total phenols and tannins indicated a significantly inverse correlation with borer incidence.

A gradual decrease in tannin contents at 60 DAP was observed with increased shoot borer incidence (Table 2, column 4 & 5). Highest tannin contents were recorded in least susceptible group (average 12.18 mg/gm) as against moderately (av. 11.71 mg/gm) and highly susceptible genotypes (av. 10.11 mg/gm) (Fig. 4). The phenolic constituents in highly susceptible cultivars were also less than the moderately susceptible and least susceptible groups. Interestingly, in the moderately susceptible entries were found to contain higher phenolic contents than least susceptible. In present studies, the higher phenolic and tannin contents seem to have significant deterrent effect on borer incidence. A wide variations were evident in relative amount of total phenol and tannin content between genotypes, wit increase in borer susceptibility, a steady decrease in phenol and tannin contents was evident but for entry CoH 99, a moderately susceptible cultivar, which showed higher phenol content than low susceptible genotypes. In general, there was significant inverse correlation between phenol and tannin contents and borer susceptibility. Phenolic compounds have been implicated in the resistance of a number of plant species to various insect-pests. Significantly higher phenolic contents were reported in resistant/less susceptible varieties of wheat to aphid [21], cotton to whitefly [20], Indian mustard to aphid [18] and sugarcane to shoot borer and stalk borer [22], [17] than their susceptible counterpart. A correlation between tannin contents of sorghum and midge resistance has also been suggested [23], [24], [25], [26].

The phenols and tannins constitute one of the most important groups of plant defense chemicals. These secondary metabolites are known to be responsible for anti feedant and/or antibiotic effects on some insects in soybean [27], cotton [28], [29], sorghum [30], [31], [26] and sugarcane [17]. Antibiosis to shoot borer in less susceptible cultivars of sugarcane [32] also appears partly a consequences of feeding deterrence due to occurrence of relatively higher levels of phenols and tannins in the cultivars.

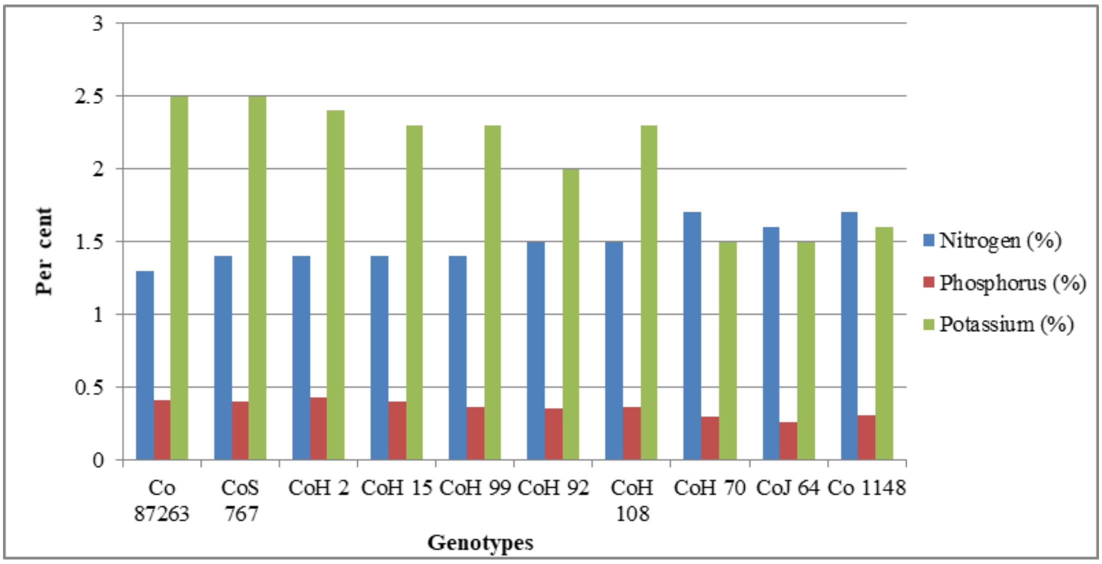

Fig 3: Nitrogen, phosphorus and potassium contents of sugarcane genotypes at 45 days after planting

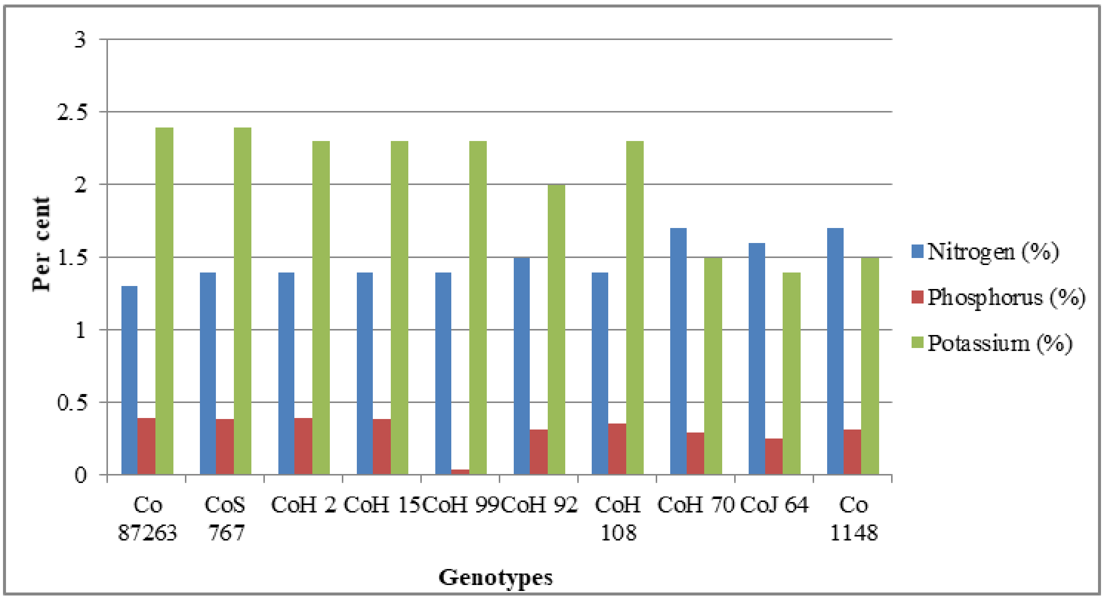

Fig 4: Nitrogen, phosphorus and potassium contents of sugarcane genotypes at 60 days after planting

Nitrogen, phosphorus and potassium

At 45 DAP, the highly susceptible genotypes contained the highest average nitrogen content (1.62 per cent) with a maximum of 1.70 per cent in Co 1148 and CoH 70 (Table 3, column 2, 3 & 4). A steady decrease in shoot nitrogen was evident with increased resistance, the least susceptible entry Co 87263 containing the lowest nitrogen level of 1.3 per cent. Nitrogen content of moderately susceptible entries ranged from 1.4 per cent (CoH 2, CoH 15, CoH 99) to1.5 per cent (CoH 92). A highly significant direct correlation was clear between borer incidence and shoot nitrogen level. Contrarily, both phosphorus and potassium showed a decreasing trend with susceptibility, the least susceptible containing the highest average amount of 0.405 per cent and 2.5 per cent potassium as against 0.30 per cent and 1.72 per cent, respectively, recorded in highly susceptible entries. Thus, both phosphorus and potassium showed a significant negative correlation with susceptibility. An inverse correlation was also apparent between shoot nitrogen level and potassium and phosphorus levels.

The contents of nitrogen, phosphorus and potassium at 45 and 60 DAP showed negligible differences. Highly susceptible genotypes recorded highest average nitrogen content (1.6 per cent) as against least (1.35 per cent) and moderately susceptible (1.42 per cent). Phosphorus contents ranged from 0.4 per cent in least susceptible Co 87263 and moderately susceptible CoH 2 to 0.25 per cent in highly susceptible cultivar CoJ 64, with an average content of 0.395 per cent in least susceptible, 0.36 per cent in moderately susceptible and 0.3 per cent in highly susceptible genotypes. Lowest average potassium content (1.67 per cent) was recorded in highly susceptible entries in comparison with least and moderately susceptible, which recorded 2.4 and 2.2 per cent potassium contents, respectively. Highly significant positive association of nitrogen level and highly significant negative correlation of potassium and phosphorus levels with varietal susceptibility have been observed.

A gradual decrease in shoot nitrogen was evident with increased resistance, the least susceptible entry Co 87263 recording the lowest nitrogen level. Contrarily, the potassium and phosphorus contents showed negative correlation and regression coefficient with *C.infuscatellus* resistance. A correlation was suggested between nitrogen and potassium contents of cane stalks and borer resistance [22], [17]. High potassium and low nitrogen increased resistance to the pyralid. Positive correlation of nitrogen and inverse relationship of potassium and phosphorus with resistance have been established in rice against brown plant hopper [33] and yellow stem borer [34]. Susceptible and resistant varieties of sorghum to shoot fly, *Atherogona soccata* and stem borer, *Chilo partellus* have also been shown to contain high and low of nitrogen, respectively [35]. Comparatively low amount of nitrogen in genotypes of low susceptibility to shoot borer may have a direct relation on the nutritional status to shoot borer larvae at the susceptible stage and it may answer for a slow development observed on least susceptible cultivar CoS 767 [32]. Nitrogen is also one of the important nutrients which a host plant should be provided with for its better growth. However, high nitrogen contents are expected to make plant tissues softer than those containing low amount of nitrogen. The affinity of shoot borer for high nitrogen containing sugarcane genotypes thus seems to be linked with softness of spindles as a consequence of greater accumulation of nitrogen content. Greater amount of phosphorus and potassium have been reported to impart resistance in plants against insects [36]. These are also considered vital elements for the growth and development of *Chilo partellus* on sorghum [37]. In the present studies, however, it has not been feasible to link greater amount of phosphorus and potassium with development of shoot borer [32].

Table 1: Biochemical parameters of sugarcane genotypes at 45 DAP in relation to shoot borer, *C. infuscatellus* incidence

Genotype	Incidence (% dead hearts) 1	Total Sugar (%) 2	Reducing sugar (%) 3	Total Phenol (mg/gm) 4	Tannin (mg/gm) 5
Least Susceptible					
Co 87263	8.10 (16.58)	4.90 (12.80)	0.070 (1.54)	9.65	12.13
CoS 767	11.30 (19.38)	5.10 (13.09)	0.065 (1.45)	9.73	11.99
Mean		5.0	0.067	9.69	12.06
Moderately Susceptible					
CoH 2	18.40 (25.43)	5.80 (13.90)	0.090 (1.78)	10.12	11.70
CoH 15	18.60 (25.55)	6.30 (14.55)	0.100 (1.81)	11.51	12.83
CoH 99	21.80 (27.83)	5.20 (13.23)	0.100 (1.79)	10.94	11.28
CoH 92	25.60 (30.37)	5.20 (13.20)	0.100 (1.82)	10.72	11.06
Mean		5.6	0.097	10.82	11.71
Highly Susceptible					
CoH 108	34.30 (35.88)	6.10 (14.30)	0.100 (1.83)	11.14	11.95
CoH 70	35.90 (36.82)	7.40 (15.80)	0.240 (2.81)	8.03	8.91
CoJ 64	41.70 (40.22)	7.90 (16.30)	0.220 (2.70)	8.95	9.92
Co 1148	47.00 (43.28)	7.10 (15.45)	0.240 (2.81)	8.45	10.11
Mean		7.1	0.20	9.14	10.22
SEm	2.287	0.118	0.028	0.035	0.017
CD(P=0.05)	6.794	0.351	0.083	0.104	0.053
r(P=0.05)		0.6377	0.8244	-0.3965	-0.6336

Figures in parentheses are angle transformed values

Table 2: Biochemical parameters of sugarcane genotypes at 60 DAP in relation to shoot borer, *C. infuscatellus* incidence

Genotype	Incidence (% dead hearts) 1	Total Sugar (%) 2	Reducing sugar (%) 3	Total Phenol (mg/gm) 4	Tannin (mg/gm) 5
Least Susceptible					
Co 87263	8.10 (16.58)	5.10 (13.07)	0.065 (1.49)	10.11	12.28
CoS 767	11.30 (19.38)	5.20 (13.19)	0.060 (1.41)	9.98	12.09
Mean		5.15	0.062	10.04	12.18
Moderately Susceptible					
CoH 2	18.40 (25.43)	6.00 (14.10)	0.090 (1.75)	10.28	11.73
CoH 15	18.60 (25.55)	6.30 (14.55)	0.090 (1.75)	11.78	12.82
CoH 99	21.80 (27.83)	5.20 (13.16)	0.095 (1.78)	11.26	11.29
CoH 92	25.60 (30.37)	5.30 (13.31)	1.100 (1.79)	11.03	11.02
Mean		5.70	0.094	11.08	11.71
Highly Susceptible					
CoH 108	34.30 (35.88)	6.20 (14.42)	0.100 (1.79)	11.33	11.98
CoH 70	35.90 (36.82)	7.50 (15.90)	0.220 (2.71)	8.72	8.63
CoJ 64	41.70 (40.22)	7.90 (16.30)	0.220 (2.69)	9.13	9.85
Co 1148	47.00 (43.28)	7.20 (15.55)	0.230 (2.77)	8.49	9.98
Mean		7.20	0.192	9.41	10.11
SEm	2.287	0.028	0.024	0.046	0.020
CD(P=0.05)	6.794	0.085	0.071	0.136	0.076
r(P=0.05)		0.6479	0.8353	-0.4395	-0.6327

Figures in parentheses are angle transformed values

Table 3: Biochemical parameters of sugarcane genotypes at 40 DAP in relation to shoot borer, *C. infuscatellus* incidence

Genotype	Incidence (% dead hearts) 1	Nitrogen (%) 2	Phosphorus (%) 3	Potassium (%) 4
Least Susceptible				
Co 87263	8.10 (16.58)	1.30 (6.70)	0.41 (3.68)	2.50 (8.98)
CoS 767	11.30 (19.38)	1.40 (6.80)	0.40 (3.65)	2.50 (9.02)
Mean		1.35	0.405	2.5
Moderately Susceptible				
CoH 2	18.40 (25.43)	1.40 (6.84)	0.43 (3.76)	2.40 (8.89)
CoH 15	18.60 (25.55)	1.40 (6.89)	0.40 93.61)	2.30 (8.74)
CoH 99	21.80 (27.83)	1.40 (6.84)	0.36 (3.45)	2.30 (8.76)
CoH 92	25.60 (30.37)	1.50 (7.01)	0.35 (3.370	2.00 (8.14)
Mean		1.42	0.38	2.2
Highly Susceptible				
CoH 108	34.30 (35.88)	1.50 (6.90)	0.36 (3.40)	2.30 (8.76)
CoH 70	35.90 (36.82)	1.70 (7.40)	0.30 (3.12)	1.50 (7.10)
CoJ 64	41.70 (40.22)	1.60 (7.32)	0.26 (2.92)	1.50 (7.00)
Co 1148	47.00 (43.28)	1.70 (7.51)	0.31 (3.19)	1.60 (7.16)
Mean		1.62	0.30	1.72
SEm CD(P=0.05)	2.287 6.794	0.038 0.114	0.040 0.121	0.025 0.075
r(P=0.05)		0.8140	-0.7769	-0.7903

Table 4: Biochemical parameters of sugarcane genotypes at 60 DAP in relation to shoot borer, *C. infuscatellus* incidence

Genotype	Incidence (% dead hearts) 1	Nitrogen (%) 2	Phosphorus (%) 3	Potassium (%) 4
Least Susceptible				
Co 87263	8.10 (16.58)	1.30 (6.62)	0.40 (3.65)	2.40 (8.94)
CoS 767	11.30 (19.38)	1.40 (6.75)	0.39 (3.59)	2.40 (8.96)
Mean		1.35	0.395	2.4
Moderately Susceptible				
CoH 2	18.40 (25.43)	1.40 (6.84)	0.40 (3.62)	2.30 (8.77)
CoH 15	18.60 (25.55)	1.40 (6.82)	0.39 (3.55)	2.30 (8.67)
CoH 99	21.80 (27.83)	1.40 (6.76)	.035 (3.40)	2.30 (8.72)
CoH 92	25.60 (30.37)	1.50 (6.98)	0.31 (3.20)	2.00 (8.06)
Mean		1.42	0.36	2.2
Highly Susceptible				
CoH 108	34.30 (35.88)	1.40 (6.86)	0.35 (3.37)	2.30 (8.76)
CoH 70	35.90 (36.82)	1.70 (7.35)	0.29 (3.08)	1.50 (6.97)
CoJ 64	41.70 (40.22)	1.60 (7.30)	0.25 (2.86)	1.40 (6.90)
Co 1148	47.00 (43.28)	1.70 (7.43)	0.31 (3.20)	1.50 (7.03)
Mean		1.6	0.30	1.67
SEm CD(P=0.05)	2.287 6.794	0.035 0.104	0.044 0.130	0.030 0.089
r(P=0.05)		0.8407	-0.7726	-0.7907

Conclusion

Sugarcane with certain plant biochemical *viz.*, total phenols, tannins, nitrogen, phosphorus and potassium present in resistant plant have an adverse biological effects *i.e.*, disturbing the number of eggs, oviposition period, larval and pupal weight, development period, adult emergency, longevity and fertility; behavioral effects on feeding, oviposition behaviour, locomotor system and reducing or increasing the reproduction that ultimately effect on growth, survival and reproduction of insect. Preponderanthly, these biochemical constituents bring about changes in the insect biology, physiology, behaviour and demographic parameters which cut the chances of individuals or populations survival, owing to starvation or semi-starvation joined with unfavourable environmental factors. Biochemical content as such directly associated with resistance, therefore, could be used as an easy selection criterion in breeding for resistance to shoot borer.

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