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

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Abstract
Sugarcane genotypes selected on the basis of dead hearts formation were studied for biochemical based characters of resistance. Variations were recorded in the NDF and ADF contents of the shoots of low and high susceptibility across the stages of shoot development. However, in general, at 45 and 60 days after planting, the neutral and acid detergent fibres were higher in least susceptible genotypes than that of highly susceptible ones. At 45 DAP, the least susceptible variety CoS 767 showed 71.9 percent NDF and 42.8 percent ADF, while CoJ 64, the highly susceptible variety recorded 66.7 percent NDF and 37.6 percent ADF. Both, NDF and ADF showed a significant inverse relationship to borer susceptibility. At 60 DAP, the average NDF of least susceptible (70.8 percent) and moderately susceptible (68.2 percent) genotypes were more than that of highly susceptible genotypes (66.8 percent). There were considerable differences in the contents of cellulose, lignin and silica of spindle in 45 and 60 days old shoots of low, moderate and high susceptible genotypes. Higher levels of cellulose, lignin and silica were observed at 60 DAP than at 45 DAP with significant differences in contents of least, moderately and highly susceptible groups. Both, cellulose and lignin contents were found to be lower where the borer incidence was higher. Compared to cellulose; the lignin content showed a sharper contrast between entries of different susceptibility. The lignin contents ranged from 5.4 percent (CoJ 64) to 6.9 percent (CoS 767), while the cellulose content varied between 31.8 percent (CoJ 64) to 34.0 percent (CoS 767). All three biochemical constituents thus revealed a significant increase relationship with the incidence, with lignin being of a higher order (r = -0.861, 45 DAP) than cellulose (r = -0.746, 45 DAP) and silica (r = - 0.465).

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 percent 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 43. 89 lakh ha with average cane productivity of 69.88t/ha and production was 3067.2 lakh tones [3]. Out of more than half dozen lepidopterous tissue borers; these lepidopterous species, inflict severe losses in cane yield and sugar recovery [4] as they eat their way along the spindle/stems killing shoots, impairing growth, destroying buds and facilitating breakage of canes; the crambid moth 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. 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 percent in the early stage of the crop (May-June) in subtropical India [5].

The increased vulnerability of most of the high sugared and high yielding genotypes owing to unscrupulous use of chemical pesticides have brought imbalances in the biotic system resulting into development of resistance by insect-pests, loss of bio-diversity especially parasites and predators combining with resurgence of pests and secondary pest outbreaks simultaneously arising problems of environmental pollution, contamination of food chain, human and animal health hazards [6].

A paradigm shift in pest control strategies from unilateral chemical approach to non-chemical methods such as biological control, ecological management or host plant resistance has, therefore, become imperative. 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 percent 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 (Fig. 3) counts due to *Chilo infuscatellus* was recorded at 60 and then at 90 days after planting along with other field's observation by adopting standard procedure of observation to estimate their correlation with the different biochemical characteristics. The estimations were made twice at 45and 60 days after planting (DAP). The oven dried samples cooled and ground in Willey Grinding Mill with 1mm screen. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) determined according to standard methods [8]. For estimation of cellulose, lignin and silica, a crucible (sintered) containing ADF was filled with 72% H_2SO_4 and stirred with glass rod to smoothen the paste and break the lumps and then refilled with 72% H_2SO_4 and again stirred at hourly interval till acid drains away. Thereafter, the contents were washed with hot water to make these free from acid. The residue in crucible was dried overnight at 100 ^{0}C, weighed and then kept in muffle furnace at 510±5 ^{0}C. On cooling the crucible was removed and weighed again. The cellulose, lignin and silica percentage were calculated as under;

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

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

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

Results and discussion

Plant resistance occupies prime place in integrated pest management of field crops. 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 table 1.

Neutral detergent fibre and acid detergent fibre: At 45 DAP, the neutral and acid detergent fibres in general was higher in least susceptible genotypes than that of highly susceptible ones. The least susceptible variety CoS 767 showed 71.9 percent NDF and 42.8 percent ADF, while CoJ 64, the highly susceptible variety recorded 66.7 percent NDF and 37.6 percent ADF. Both, NDF and ADF showed a significant inverse relationship to borer susceptibility.

At 60 DAP (Table 2), the average NDF of least susceptible (70.8 percent) and moderately susceptible (68.2 percent) genotypes were more than that of highly susceptible genotypes (66.8 percent). The minimum NDF was recorded in highly susceptible genotypes CoH 70 while the highest in least susceptible cultivar CoS 767, thus indicating a decreasing trend in NDF with susceptibility. Like NDF, acid detergent fibre of highly susceptible genotypes was also significantly lower that of the least and moderately susceptible genotypes.

Considerable variations were recorded in the NDF and ADF contents of the shoots of low and high susceptibility across the stages of shoot development. The percentage of ADF and the NDF were found to be much lower in genotypes of high susceptibility. Maximum contents of these were found in the least susceptible cultivar CoS 767 at 45 as well as 60 days after planting and minimum in highly susceptible CoJ 64 and CoH 70. Both ADF and NDF showed a significantly negative correlation with the borer incidence. This relation was shown by the fairly large number of test genotypes. A similar correlation between ADF and NDF contents and borer susceptibility has been suggested for *Heliothis armigera* in chickpea [9], *Spodoptera frugiperda* in sorghum [10] and *Chilo auricilius* in sugarcane [11]. For top borer of sugarcane, however, this correlation was not found to hold true [12]. Since both, ADF and NDF are expected to provide hardness to the spindle and underlying tissues apart from their significant contribution to the dry matter. The higher quantities of NDF and ADF may impede the feeding and boring activities of pests. In case of shoot borer an extra hardness may responsible be expected to offer more resistance to an easy entry of the larva as also actively be able to restrict the feeding activity.

Cellulose, lignin and silica: At 45 DAP, as evident from data in table 1 and fig. 1; the cellulose and lignin content were found to be higher in least susceptible cultivar compared to moderately and highly susceptible ones. The least susceptible variety CoS 767 contained 33.8 percent and 6.6 percent cellulose and lignin, respectively, whereas most susceptible variety CoJ 64 showed 31.4 percent lignin. The levels of these compounds in other genotypes were generally found to be in accordance with their level of resistance. Highly significant inverse correlation was observed between shoot borer incidence and lignin and cellulose contents. The level of silica in shoot did not reveal a specific trend, though the highly susceptible genotypes with average content of 1.0 percent had significantly less silica than the moderately or least susceptible entries, thereby, indicating it's negative association with susceptibility.

Higher levels of cellulose, lignin and silica were observed at 60 DAP (table 2 and fig. 2) than at 45 DAP with significant differences in contents of least, moderately and highly susceptible groups. Both, cellulose and lignin contents were found to be lower where the borer incidence was higher. Compared to cellulose, the lignin content showed a sharper contrast between entries of different susceptibility. The lignin contents ranged from 5.4 percent (CoJ 64) to 6.9 percent (CoS 767), while the cellulose content varied between 31.8 percent (CoJ 64) to 34.0 percent (CoS 767).

Like cellulose, silica contents also showed a steady decrease from least to highly susceptible, save for moderately susceptible CoH 99, which contained highest silica contents. All three biochemical characteristics showed significantly negative association with shoot borer incidence in sugarcane.

There were considerable differences in the contents of cellulose, lignin and silica of spindle in 45 and 60 days old shoots of low, moderate and high susceptible genotypes. The least susceptible genotype CoS 767 and Co 87263 generally contained relatively higher amount of cellulose, lignin and silica than moderately and highly susceptible entries, except for CoH 99 which showed highest silica contents. While lignin contents presented a sharper contrast between genotypes of differential susceptibility, the cellulose and silica contents decreased steadily with increase in *Chilo infuscatellus* incidence. All three biochemical constituents thus revealed a significant increase relationship with the incidence, with lignin being of a higher order (r = -0.861, 45 DAP) than cellulose (r = -0.746, 45 DAP) and silica (r = -0.465). The association of cellulose, lignin and silica with resistance has been speculated long back [13] against *C. Infuscatellllus*, *Scirpophaga novella* [14] and *Chilo auricilius* in sugarcane [11] as observed in this study. A high percentage of cellulose, hemicelluloses and lignin are believed to inhibit pod damage in chickpea by *H. armigera* [9] and high silica content in sorghum varieties have been implicated in imparting resistance against shoot fly, *Atherigona soccata* [15, 16, 17] that's all supports the result achieved in context of all the biochemical constituents during the course of this study. In rice varieties also a significant positive correlation between silica contents and brown plant hopper resistance was also pointed out [18].

Cellulose, the most abundant structural polysaccharide in plants, is a polymer of glucose molecule that joins together by a β (1→4) glucosidic linkage to form long unbranched chains held together linearly by hydrogen bonds. The cellulose, lignin (a polymer of aromatic alcohol) and the silica are basic constituents of acid detergent fibre (ADF) in plants. These constituents are, infact, supportive in function as these cause thickening of cell walls and subsequent hardening of plant tissue. Thus, it seems plausible that high cellulose contents with increased lignifications and silica deposition impart strength to the tissue to protect young cane shoots from attack by *C. infuscatellus.*

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

Cultivar/accession	Incidence (% dead hearts)*	NDF (%)	ADF (%)	Cellulose (%)	Lignin (%)	Silica (%)
	1	2	3	4	5	6
Least Susceptible						
Co 87263	8.10 (16.58)	70.40 (57.06)	41.90 (40.35)	33.20 (35.20)	6.40 (14.67)	2.30 (8.78)
CoS 767	11.30 (19.38)	71.90 (57.99)	42.80 (40.85)	33.80 (35.56)	6.60 (14.90)	2.40 (8.93)
Mean		71.15	42.35	33.5	6.50	2.35
Moderately Susceptible						
CoH 2	18.40 (25.43)	68.10 (55.61)	39.90 (39.15)	31.70 (34.29)	5.90 (14.07)	2.30 (8.61)
CoH 15	18.60 (25.55)	68.70 (55.99)	39.70 (39.05)	31.60 (34.19	5.70 (13.80)	2.40 (8.90)
CoH 99	21.80 (27.83)	69.50 (56.45)	40.20 (39.34)	31.60 (34.22)	5.70 (13.83)	2.90 (9.99)
CoH 92	25.60 (30.37)	68.80 (56.06)	39.20 (38.74)	31.50 (34.15)	5.30 (13.33)	2.40 (8.84)
Mean		68.77	39.75	31.60	5.65	2.50
Highly Susceptible						
CoH 108	34.30 (35.88)	69.80 (56.63)	39.00 (38.62)	31.60 (34.23)	5.30 (13.33)	2.10 (8.23)
CoH 70	35.90 (36.82)	67.50 (55.28)	38.20 (38.17)	31.60 (34.20)	4.90 (12.79)	1.70 (7.42)
CoJ 64	41.70 (40.22)	66.70 (54.77)	37.60 (3785)	31.40 (34.10)	4.60 (12.37)	1.60 (7.35)
Co 1148	47.00 (43.28)	66.70 (54.77)	37.80 (37.98)	31.00 (33.85)	4.70 (12.55)	2.20 (8.55)
Mean		67.67	38.15	31.40	4.87	1.90
SEm CD(P=0.05)	2.287 6.794	0.69 2.06	0.70 2.08	0.48 1.44	0.02 0.07	0.04 0.14
r(P=0.05)		-0.564	-0.599	-0.747	-0.861	-0.466

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

Cultivar/accession	Incidence (% dead hearts)*	NDF (%)	ADF (%)	Cellulose (%)	Lignin (%)	Silica (%)
	1	2	3	4	5	6
Least Susceptible						
Co 87263	8.10 (16.58)	70.10 (56.83)	42.50 (40.70)	33.30 (35.23)	6.70 (14.99)	2.50 (9.05)
CoS 767	11.30 (19.38)	71.50 (57.73)	43.40 (42.22)	34.00 (35.68)	6.90 (15.25)	2.50 (9.10)
Mean		70.80	42.95	33.65	6.80	2.50
Moderately Susceptible						
CoH 2	18.40 (25.43)	67.90 (55.49)	41.30 (39.99)	32.40 (34.70)	6.50 (14.79)	2.40 (8.85)
CoH 15	18.60 (25.55)	68.40 (55.82)	41.20 (39.85)	31.90 (34.40)	6.80 (15.17)	2.50 (9.07)
CoH 99	21.80 (27.83)	68.60 (55.91)	41.40 (40.06)	32.20 (34.56)	6.20 (14.45)	3.00 (10.05)
CoH 92	25.60 (30.37)	68.00 (55.53)	40.60 (39.58)	32.00 (34.47)	6.10 (14.28)	2.50 (9.10)
Mean		68.20	41.12	32.12	6.40	2.60
Highly Susceptible						
CoH 108	34.30 (35.88)	68.70 (55.98)	39.90 (38.90)	33.90 (34.40)	5.80 (13.95)	2.20 (8.89)
CoH 70	35.90 (36.82)	65.80 (55.24)	39.80 (39.13)	32.20 (34.59)	5.60 (13.70)	2.00 (8.22)
CoJ 64	41.70 (40.22)	66.40 (54.59)	39.10 (38.69)	31.80 (34.30)	5.41 (13.42)	1.90 (7.97)

Co 1148	47.00 (43.28)	66.40 (54.59)	39.70 (39.05)	31.90 (34.40)	5.50 (13.59)	2.30 (8.70)
Mean		66.82	39.62	31.95	5.50	2.10
SEm	2.287	0.64	.026	0.41	0.09	0.03
CD(P=0.05)	6.794	1.91	0.78	1.22	0.28	0.09
r(P=0.05)		-0.568	-0.764	-0.760	-0.707	-0375

Figures in parentheses are angle transformed values

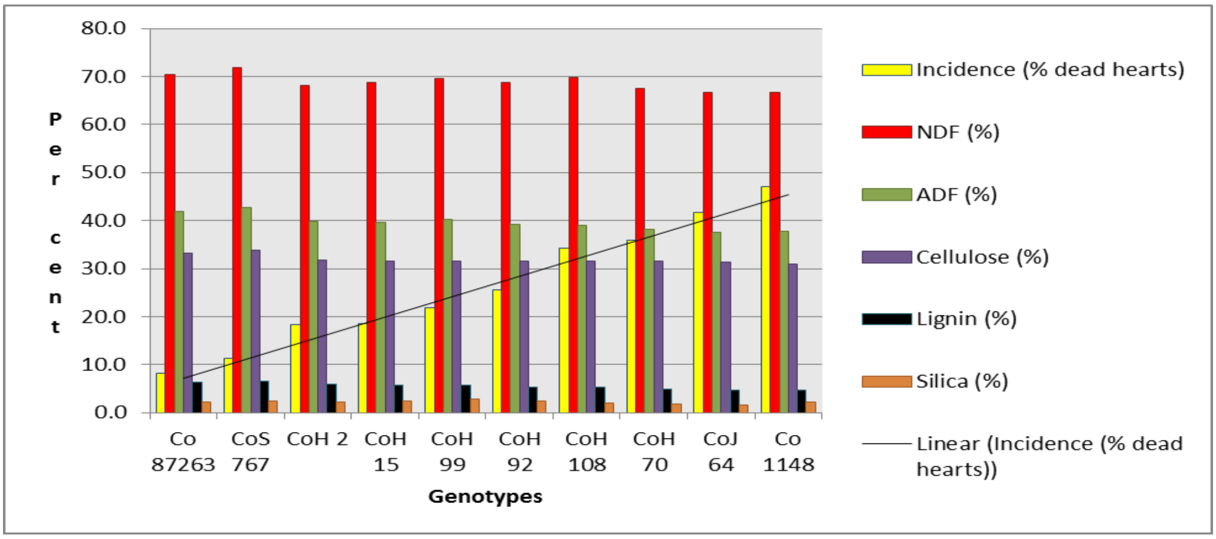

Fig 1: ADF, NDF, cellulose, lignin and silica contents of sugarcane genotypes at 45 days after planting

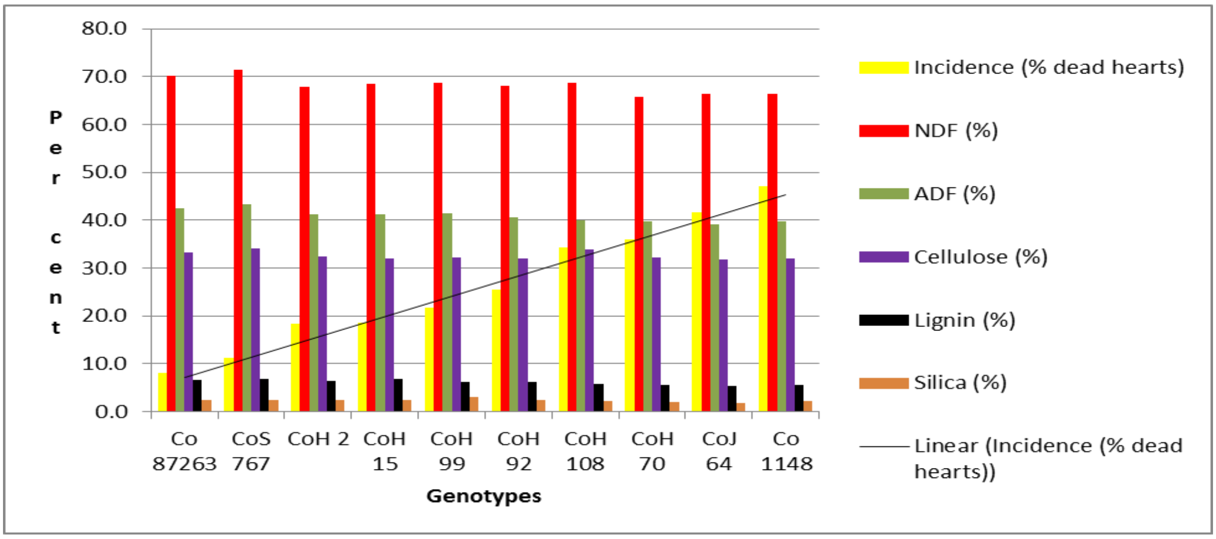

Fig 2: ADF, NDF, cellulose, lignin and silica contents of sugarcane genotypes at 60 days after planting

Fig 3: Dead heart in sugarcane

Conclusion

It is inferred from the present study that sugarcane varieties with certain plant biochemical like Neutral and acid detergent fibre (NDF, ADF), cellulose, lignin and silica present in resistant plant have an adverse effect on growth, survival and reproduction of insect. Predominantly these biochemicals bring about changes in the insect feeding behavior which then reduce the chances of pest survival, owing to starvation or semistavation coupled 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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