Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2021; 9(1): 834-839

Received: 06-10-2020
Accepted: 04-12-2020

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Bio-chemical basis of resistance to stem borer, *Chilo partellus* (Swinhoe) infesting forage sorghum, *Sorghum bicolour* (L.) Moench

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Abstract
The results on biochemical characters of resistance to stem borer revealed that varieties AFS-28 and SSG-59-3 found to be resistant exhibiting less mean per cent dead hearts i.e. 67.00 and 67.00 percent. The varieties AFS-32, MP Chari and COFS-29 found to be highly susceptible to stem borer incidence exhibiting cent per cent dead hearts on 44 days after emergence (3rd week of August). The correlation between infestation level and biochemical characters was significant except fat, hemicellulose and HCN. The percentage of cellulose, lignin, ADF, NDF, crude fibre, dry matter, ash, silica, phosphorus, calcium, total phenol and polyphenol were significantly highly negatively correlated with infestation level, indicating that fall in these factors caused increase in the pest population. However, percentage of moisture, organic matter, nitrogen and crude protein were highly significantly positively correlated with infestation level, indicating that rise in these factors caused increase in the pest population.

Keywords: Biochemical characters, resistance, stem borer, sorghum

Introduction
Sorghum [*Sorghum bicolor* (L.) Moench] is one of the important food, feed and fodder crops in the world. It is cultivated widely throughout tropical, subtropical and temperate region of the world. It is used for human consumption especially in rural areas. It has also great potential to supplement fodder resources in India because of its wide adaptation, rapid growth, high green and dry fodder yield with high ratooning and drought tolerance. The crop is grown in about 8.45 million hectares with annual grain production of 7.4 million tones in India. It ranks third next to rice and wheat both in area and production [2]. Green fodder is the cheapest source of feed for milch, beef and draft animals. Therefore, development of fodder resources of the country becomes a high priority national programme. Insect pests are one of the major limiting factors for low yield of sorghum. In India, nearly 32.1% of total crop produce was lost due to insect pests [5]. The sorghum crop is attacked by 150 species of insect pests during its lifespan [17]. Among different insects, stem borer is most important which causes dead hearts. Use of insecticides for the control of insect pests is not advisable on sorghum as a fodder crop due to hazardous effect of insecticidal residues on animals. Hence, it is highly essential to find out varieties which are resistant or tolerant to different insect pests. Therefore, it is required to find out resistant varieties of forage sorghum against stem borer so far as plant protection is concern. Susceptibility of sorghum to stem borer was positively correlated with percent moisture and nitrogen and negatively correlated with neutral detergent fibres, acid detergent fibres, cellulose, lignin, silica and total phenols content of the plant [11]. The correlations between leaf injuries due to *C. partellus* with these biochemical factors were positively correlated [16]. Plant characters such as biochemical associated with resistance to stem borer should be quantified and identified and such plant characters can be used as "marker traits" to screen and select for resistance to stem borer.

Materials and Methods
Biochemical characters of plants
To study the biochemical characters, different varieties of sorghum were sown on the field of Main Forage Research Station, AAU, Anand. For analyzing the biochemical characters, 500 g plant samples of each variety/genotype were taken at 44 days after emergence, dried in oven at 60-80 °C and different biochemical were analyzed by adopting following procedure.

Moisture content
Two g of dried sample was taken in a previously weighed crucible and put it in oven for drying at 60-80 °C for overnight. After oven drying it was removed and again weighed and moisture content was calculated by following formula [23].

Dry matter content
The moisture content was analyzed by above procedure and by using moisture content, the dry matter content was calculated by following formula [23].

Ash content
Two gram of dried sample was taken in a previously weighed crucible and put it in muffle furnace until 600°c temperature. After burning it was removed and placed in desiccators for cooling at room temperature and again weighed and ash content was calculated by following formula [23].

Organic matter content
The ash content was analyzed by above procedure and by using ash content, the organic matter content was calculated by following formula [23].

Nitrogen content
Estimation of total nitrogen content in green leaf and leaf-sheath samples, each weighing 40 mg, was carried out by macro-Kjeldahl's method following the procedures described by the Association of Official Agricultural Chemists [4].

Crude protein content
Estimation of crude protein content in green leaf and leaf-sheath samples were carried out by macro-Kjeldahl's method described by the Association of Official Agricultural Chemists [4].

HCN content
The procedure for HCN content was carried out by following method [23].

Fat content
The fat content was determined by the following procedure [1].

Crude fibre content
Crude fibre was determined by loss in weight by ignition [23].

Silica content
The silica content was determined by the following procedure [1].

Calcium content
The calcium content was determined by the following procedure [1].

Phosphorus content
The phosphorus content was determined by the following procedure [1].

NDF (Neutral Detergent Fibre) content
The NDF content was determined by the following procedure [23].

ADF (Acid Detergent Fibre) content
The ADF content was determined by the following procedure [23].

Cellulose, Lignin & Hemicellulose content
The Cellulose, Lignin & Hemicellulose content was determined by the following procedure [23].

Total phenolcontent
The total phenol content was determined by the method of Simon and Ross [21].

Polyphenol content
The polyphenol content was determined by Calorimetric method [20].

Results and Discussions
The screening of different varieties of sorghum against *C. partellus* was evaluated based on per cent dead hearts. Evaluation of biochemical factors *viz*., moisture, organic matter, fat, cellulose, hemicelluloses, lignin, nitrogen, crude protein, ADF, NDF, crude fibre, dry matter, ash, silica, phosphorus, calcium, HCN, total phenol and polyphenol responsible for resistance to *C. partellus*. The data obtained are presented in Table-1 to 3 and correlation coefficient values in Table-4.

Moisture
The amount of moisture was higher in highly susceptible varieties while it was lower in resistant varieties. The observations on percentage of moisture were recorded on 44 days after emergence. The average maximum and minimum percentage of moisture was observed in the varieties SSG-59-3 (7.07) and AFS-28 (6.87), respectively. In moderately resistant varieties, it was maximum in AFS-36 (7.05) and minimum in AFS-30 (6.82). The percentage of moisture was 7.02 in susceptible variety AFS-34. In highly susceptible varieties, the maximum percentage of moisture was noted in COFS-29 (8.12) and minimum in AFS-32 (7.96).Khurana and Verma [11] found that percent moisture was less in resistant varieties to *C. partellus*.

Organic matter
The amount of organic matter was higher in highly susceptible varieties while it was lower in resistant varieties. The data on percentage of organic matter on 44 days after emergence (Column-5 in Table-1) revealed that among resistant varieties, the maximum and minimum percentage of organic matter was noted in the varieties SSG-59-3 (80.26) and AFS-28 (80.03), respectively.

Fat
The amount of fat was higher in resistant and moderately varieties while it was lower in highly susceptible varieties. The observations on percentage of fat were recorded on 44 days after emergence. The data on percentage of fat on 44 days after emergence (Column-6 in Table-1) revealed that among resistant varieties, the maximum and minimum percentage of fat was found in the varieties SSG-59-3 (3.04) and AFS-28 (2.45), respectively.

Cellulose
The amount of cellulose was higher in resistant varieties while it was lower in highly susceptible varieties. The observations on percentage of cellulose were recorded on 44 days after emergence. So far average percentage of cellulose is concern (Column-7 in Table-1), the maximum percentage of cellulose was noted in AFS-28 (30.21) and minimum in

SSG-59-3 (29.69) among resistant varieties. The present finding is also in conformity with the finding of Khurana and Verma [11] who found that amount of cellulose was high in varieties resistant to stem borer and shoot fly in sorghum.

Hemicellulose

The amount of hemicellulose was higher in susceptible varieties while it was lower in resistant varieties. The observations on percentage of hemicellulose were recorded on 44 days after emergence. The data on percentage of hemicellulose on 44 days after emergence (Column-8 in Table-1) revealed that among resistant varieties, the maximum and minimum percentage of hemicellulose was noted in the varieties SSG-59-3 (29.41) and AFS-28 (28.91), respectively.

Lignin

The amount of lignin was higher in resistant varieties while it was lower in highly susceptible varieties. The observations on percentage of lignin were recorded on 44 days after emergence. The data on percentage of lignin on 44 days after emergence (Column-9 in Table-1) revealed that among resistant varieties, the maximum and minimum percentage of lignin was recorded in the varieties AFS-28 (6.07) and SSG-59-3 (6.05), respectively. Lignin can strengthen cell walls against digestion and therefore can be anti-nutritional for spotted stem borer **[6].**The present finding is also in conformity with the finding of Khurana and Verma [11]and who reported that amount of lignin was high in resistant varieties to stem borer and shoot fly in sorghum.

Nitrogen

The amount of nitrogen was higher in highly susceptible varieties while it was lower in resistant varieties. The observations on percentage of nitrogen were recorded on 44 days after emergence. The data on percentage of nitrogen on 44 days after emergence (Column-4 in Table-2) revealed that among resistant varieties, the equal percentage of nitrogen was found in both varieties AFS-28 and SSG-59-3 (1.47).According to Singh *et al.* [22], low concentrations of nitrogen in sorghum seedlings reduced the dead hearts formation by shoot fly. Arabjafari and Jalali [3]reported higher percentage of nitrogen in the susceptible variety to *C. partellus* in maize. Distinctly low leaf chlorophyll, carotenoid, nitrogen, crude protein and moisture content were noticed in resistant varieties as compared to susceptible ones [15].

Crude Protein

The amount of crude protein was higher in highly susceptible varieties while it was lower in resistant varieties. The observations on percentage of crude protein were recorded on 44 days after emergence. So far average percentage of crude protein is concern (Column-5 in Table-2), the maximum percentage of crude protein was recorded in AFS-28 (9.21) and minimum in SSG-59-3 (9.16) among resistant varieties. Praveen *et al.* [13] in a study on the biochemical changes during crop growth period of resistance and susceptible varieties of maize against stem borer, reported that increase in total soluble protein content in vegetative part of the plant may be responsible for the susceptibility. Similarly, findings were reported in maize infested by stem borer [10].Crude protein contains some 20 different amino acids, usually 10 (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) of them are essential in the diet, the others can be synthesized or derived from ten. Amino acids are required for growth and development of an insect [7].

Acid Detergent Fibre (ADF)

The amount of ADF was higher in resistant varieties while it was lower in highly susceptible varieties. The observations on percentage of ADF were recorded on 44 days after emergence. The data on percentage of ADF on 44 days after emergence (Column-6 in Table-2) revealed that among resistant varieties, the maximum and minimum percentage of ADF was recorded in the varieties AFS-28 (36.65) and SSG-59-3 (36.41), respectively. The present findings also fall in same line with results of Khurana and Verma [11]who reported high percentage of acid detergent fibres in resistant varieties to stem borer and shoot fly in sorghum.

Neutral Detergent Fibre (NDF)

The amount of NDF was higher in resistant varieties while it was lower in highly susceptible varieties. The observations on percentage of NDF were recorded on 44 days after emergence. The data on percentage of NDF on 44 days after emergence (Column-7 in Table-2) revealed that among resistant varieties, the maximum and minimum percentage of NDF was found in the varieties SSG-59-3 (65.82) and AFS-28 (65.56), respectively. The present findings are also in conformity with results of Khurana and Verma [11] who reported high percentage of neutral detergent fibres in resistant varieties to stem borer and shoot fly in sorghum.

Crude fibre

The amount of crude fibre was higher in resistant varieties while it was lower in highly susceptible varieties. The observations on percentage of crudefibre was recorded on 44 days after emergence. The data on percentage of crude fibre on 44 days after emergence (Column-8 in Table-2) revealed that among resistant varieties, the maximum and minimum percentage of crude fibre was noted in the varieties SSG-59-3 (26.29) and AFS-28 (25.69), respectively. Previous findings also confirm this as fiber composed of cellulose, hemicellulose and lignin are the primary plant cell wall components and shows resistance to stem borers [18].

Dry matter

The amount of dry matter was higher in resistant varieties while it was lower in highly susceptible varieties. The observations on percentage of dry matter were recorded on 44 days after emergence. The maximum percentage of dry matter was recorded in AFS-28 (93.13) (Column-9 in Table-2) and minimum in SSG-59-3 (92.93) among resistant varieties.

Ash

The amount of ash was higher in resistant varieties while it was lower in highly susceptible varieties. The observations on percentage of ash were recorded on 44 days after emergence. The data on percentage of ash on 44 days after emergence (Column-4 in Table-3) revealed that among resistant varieties, the maximum and minimum percentage of ash was found in the varieties AFS-28 (19.97) and SSG-59-3 (19.74), respectively.

Silica

The amount of silica was higher in resistant varieties as compared to susceptible and highly susceptible varieties. The

observations on percentage of silica were recorded on 44 days after emergence. The data on percentage of silica on 44 days after emergence (Column-5 in Table-3) revealed that among resistant varieties, the maximum and minimum percentage of silica was recorded in the varieties AFS-28 (4.83) and SSG-59-3 (4.74), respectively. higher amount of Silica in stem tissues of the resistant variety to *C. partellus* in maize [3].

Phosphorus

The amount of phosphorus was higher in resistant varieties as compared to susceptible and highly susceptible varieties. The observations on percentage of phosphorus were recorded on 44 days after emergence. The data on percentage of phosphorus on 44 days after emergence (Column-6 in Table-3) revealed that among resistant varieties, the maximum and minimum percentage of phosphorus was observed in the varieties AFS-28 (0.74) and minimum SSG-59-3 (0.64). Arabjafari and Jalali [3] reported that higher percentage of phosphorus in stem tissues of variety resistant to *C. partellus* in maize. The present findings are also in conformity with this the findings.

Calcium

The amount of calcium was higher in resistant varieties as compared to susceptible and highly susceptible varieties. The observations on percentage of calcium were recorded on 44 days after emergence. The maximum percentage of calcium was recorded in SSG-59-3 (0.86) (Column-7 in Table-3) and minimum in AFS-28 (0.82) among resistant varieties. The amount of calcium might make the tissues tough to chew for larvae of stem borer. Hence, the varieties with high amount of calcium are resistant to attack of larvae of stem borer.

HCN

The amount of HCN was higher in highly susceptible varieties as compared to resistant varieties. The observations on ppm of HCN were recorded on 44 days after emergence. The data on ppm of HCN on 44 days after emergence (Column-8 in Table-3) revealed that among resistant varieties, the maximum and minimum ppm of HCN was recorded in the varieties AFS-28 (49.85) and SSG-59-3 (29.22), respectively.

Total phenols

The amount of total phenols was higher in resistant varieties as compared to susceptible and highly susceptible varieties. The observations on total phenols (mg/g) were recorded on 44 days after emergence. The data on total phenols (mg/g) on 44 days after emergence (Column-9 in Table-3) revealed that among resistant varieties, the maximum and minimum total phenols (mg/g) were found in the varieties AFS-28 (9.50) and SSG-59-3 (8.59), respectively. Khurana and Verma [11] reported high amount of total phenols in varieties resistant to stem borer and shoot fly in sorghum. The highest total phenol content was observed in resistant variety NAC-6004 and was lowest in the susceptible variety CM-300 both in leaves and stem [13]. Higher concentrations of total phenols found in the resistant group could be one of the factors contributing towards tolerance with antibiotic effect against the attacking insects. Higher content of total phenols in resistant varieties might have contributed to defense mechanism of plant again insect pests [19] acting as antifeedants and repellants. Moreover, these biochemical factors such as phenols and sugars also play an important role in plant defense mechanism to *C. partellus* [6] as they strengthen cell walls against digestion and therefore can be anti-nutritional for spotted stem borer [18]. The present findings are also in conformity with the findings of above workers.

Polyphenols

The amount of polyphenols was higher in resistant varieties as compared to susceptible and highly susceptible varieties. The observations on poly phenols (mg/g) were recorded on 44 days after emergence. The data on polyphenols (mg/g) on 44 days after emergence (Column-10 in Table-3) revealed that among resistant varieties, the maximum and minimum polyphenols (mg/g) were found in the varieties SSG-59-3 (0.58) and AFS-28 (0.54), respectively. Kabre and Ghorpade [9] reported higher amount of polyphenol in resistant varieties to stem borer in maize. The present findings are also in conformity with the findings of above workers.

Correlation coefficient

The varieties evaluated in field conditions for their susceptibility /resistance to *C. partellus* were further studied to know the factor (biochemical) responsible for imparting susceptibility/resistance to *C. partellus*. The original data on infestation levels (per cent dead hearts) and biochemical characters viz., moisture, organic matter, fat, cellulose, hemicelluloses, lignin, nitrogen, crude protein, ADF, NDF, crude fibre, dry matter, ash, silica, phosphorus, calcium, HCN, total phenol, polyphenol recorded in different varieties are presented in table-1 to 3. The data on infestation of stem borer were correlated with biochemical parameters. The correlation coefficient values (r) are also presented in table-4. The correlation between infestation level and biochemical characters was significant except fat, hemicellulose and HCN. The amount of cellulose, lignin, ADF, NDF, crude fibre, dry matter, ash, silica, phosphorus, calcium, total phenol and polyphenol were significantly highly negatively correlated with infestation level, indicating that decrease in amount of these factors caused an increase in the pest infestation. The amount of moisture, organic matter, nitrogen and crude protein were highly significantly positively correlated with infestation, indicating that increase in these factors caused an increase in the pest population. The research work on correlation between dead hearts due to *C. partellus* infestation and biochemical parameters is scanty. Khurana and Verma [11] reported that susceptibility of sorghum to *A. soccata* was positively correlated with phosphorus and negatively correlated with the content of total phenols. The present finding is also in conformity with the finding of Ramnath and Reddy [14] and Mate *et al.* [12] who reported positive correlation between susceptibility to shoot fly infestation and nitrogen content of the plant. Rao and Panwar [16] reported that nitrogen, crude protein and moisture content were positively correlated with leaf injury due to *C. partellus*. The phenolic acids *viz*., ferulic acid and p-coumaric acid, and the nutritional biochemical factors such as protein, oil, chlorophyll-b, total chlorophyll and total carotenoid showed significant negative correlations, while total sugars and starch had positive correlation with pupal period of *C. partellus* [8].

Table 1: Mean percentage of different biochemical constituents of 44 days old sorghum plants

Sr. No.	Varieties	Mean Percentage of dead hearts by *C. partellus*	Mean percentage of					
			Moisture	Organic matter	Fat	Cellulose	Hemicellulose	Lignin
Resistant varieties								
1	AFS-28	67	6.87	80.03	2.45	30.21	28.91	6.07
2	SSG-59-3	67	7.07	80.26	3.04	29.69	29.41	6.05
Moderately resistant varieties								
3	AFS-26	71	6.83	80.31	3.16	30.20	28.62	5.91
4	AFS-30	73	6.82	81.05	2.52	30.00	28.67	5.89
5	AFS-36	81	7.05	83.28	2.76	24.98	29.39	4.26
Susceptible varieties								
6	AFS-34	84	7.02	83.22	2.49	25.15	30.72	4.20
Highly Susceptible varieties								
7	AFS-32	100	7.96	85.70	2.65	19.47	27.86	2.74
8	MP Chari	100	8.02	85.31	2.59	19.01	29.57	2.83
9	COFS-29	100	8.12	85.21	2.41	18.73	28.63	2.91

Table 2: Mean percentage of different biochemical constituents of 44 days old sorghum Plants

Sr. No.	Varieties	Mean Percentage of dead hearts by *C. partellus*	Mean percentage of					
			Nitrogen	Crude protein	ADF	NDF	Crude fibre	Dry matter
Resistant varieties								
1	AFS-28	67	1.47	9.21	36.65	65.56	25.69	93.13
2	SSG-59-3	67	1.47	9.16	36.41	65.82	26.29	92.93
Moderately resistant varieties								
3	AFS-26	71	1.49	9.28	36.67	65.28	25.86	93.17
4	AFS-30	73	1.49	9.33	37.24	65.91	25.73	93.18
5	AFS-36	81	1.67	10.41	32.59	61.98	23.81	92.95
Susceptible varieties								
6	AFS-34	84	1.61	10.08	31.87	62.60	24.17	92.98
Highly Susceptible varieties								
7	AFS-32	100	1.99	12.42	29.67	57.53	22.37	92.04
8	MP Chari	100	2.07	12.97	28.87	58.44	22.82	91.98
9	COFS-29	100	2.06	12.90	29.44	58.07	22.64	91.88

Table 3: Mean percentage of different biochemical constituents of 44 days old sorghum Plants

Sr. No.	Varieties	Mean Percentage of dead hearts by *C. partellus*	Mean percentage of				HCN (ppm)	Total phenols (Mg/g)	Poly phenols (Mg/g)
			Ash	Silica	Phosphorus	Calcium			
Resistant varieties									
1	AFS-28	67	19.97	4.83	0.74	0.82	49.85	9.50	0.54
2	SSG-59-3	67	19.74	4.74	0.64	0.86	29.22	8.59	0.58
Moderately resistant varieties									
3	AFS-26	71	19.69	4.31	0.66	0.84	37.48	8.97	0.53
4	AFS-30	73	18.95	4.63	0.65	0.81	50.60	9.39	0.59
5	AFS-36	81	16.72	3.58	0.32	0.58	57.21	7.61	0.37
Susceptible varieties									
6	AFS-34	84	16.78	3.76	0.31	0.58	19.73	7.33	0.38
Highly Susceptible varieties									
7	AFS-32	100	14.30	2.87	0.14	0.25	61.56	5.75	0.25
8	MP Chari	100	14.69	2.74	0.18	0.31	52.39	5.14	0.26
9	COFS-29	100	14.79	2.70	0.17	0.33	58.63	5.40	0.24

Table 4: Correlation between different biochemical constituents and dead hearts caused by *C. partellus*

Biochemical constituents	Dead hearts caused by *C. Partellus*
Moisture	0.919**
Organic matter	0.988**
Fat	- 0.440
Cellulose	- 0.986**
Hemicellulose	- 0.107
Lignin	- 0.988**
Nitrogen	0.973**
Crude protein	0.973**
ADF	- 0.968**
NDF	- 0.980**
Crude fibre	- 0.973**
Dry matter	- 0.919**
Ash	- 0.988**
Silica	- 0.982**
Phosphorus	- 0.962**
Calcium	- 0.986**
HCN	0.486
Total phenols	- 0.970**
Polyphenol	- 0.962**
$r_{0.05}$ (7 df)	0.754
$r_{0.01}$ (7 df)	0.875

* Significant (At 5 %), ** Significant (At 1 %)

Conclusion

The biochemical characters of resistance to stem borer revealed that varieties AFS-28 and SSG-59-3 found to be resistant exhibiting less mean per cent dead hearts Whereas, the varieties AFS-26, AFS-30 and AFS-36 appeared to be moderately resistant. The variety AFS-34 found to be susceptible whereas varieties AFS-32, MP Chari and COFS-29 found to be highly susceptible to stem borer. The amount of cellulose, lignin, ADF, NDF, crude fibre, dry matter, ash, silica, phosphorus, calcium, total phenol and polyphenol were significantly more in resistant and moderately resistant varieties than in the susceptible and highly susceptible. The information about these biochemical characters also will aid the evaluation of breeding lines so as to further increase (in cultivated crops) the levels of resistance to stem borer.

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