Asian Journal of Pharmacy and Life Science ISSN 2231 – 4423
Vol. 1 (4), Oct-Dec, 2011

Biochemical changes in the *Sorghum bicolor* leaves during leaf sugary disease and after neem leaf extract treatment

Gurunath Jadhav*, A. H. Rajasab
Mycology and Plant Pathology Laboratory
Department of Post-Graduate Studies and Research in Botany
Gulbarga University, Gulbarga-585106
Karnataka, India

Abstract
The biochemical changes in leaf sugary disease / disorder (LSD) affected, healthy leaf and Neem Leaf Extract (NLE) was reported in the present investigation. Protein, total reducing sugar, total sugar, total chlorophyll, total proline and total phenol contents were estimated in LSD affected; healthy leaf and NLE treated sorghum bilijola variety. In LSD affected leaf, the protein, total reducing sugar, total sugar and total chlorophyll content was decreased to 64.52%, 58.54%, 58.34% and 79.37% respectively. Whereas, the total content of phenol and proline in LSD affected leaf has shown four fold increase when compared to healthy plant.

Key words: *Sorghum leaf sugary disease, Protein, Total chlorophyll, Phenol, Proline reducing sugar, and Sugar content estimation*
Corresponding Author's Email: gurukj@rediffmail.com
Received: 15/10/11 Accepted: 31/11/11

INTRODUCTION

Sorghum bicolor (L.) Moench is the fifth most important cereal crop after wheat, rice, maize, and barley in terms of production. The total world annual sorghum production is over 60 million tons from a cultivated area of 46 million ha. *Rabi* sorghum suffers from leaf injury, resulting in exudation, which crystallizes after the evaporation of water. The exudates contain 52.15% sucrose, 4.2% glucose, 2.7% maltose and 1.0% free amino acids (AICSIP, 1975). The secretion encourages the development of black sooty mould, which affects the photosynthesis of the plants and in severe cases, ear emergence is inhibited. Annual loss of 10 to 15% was reported (Chavan *et al.,* 1959) in LSD affected field. There is no report of physiological changes associated with biochemical changes in the leaf sugary disease. Hence, in the present investigation, biochemical changes in leaf sugary disease/disorder (LSD) affected leaf compared to healthy leaf and NLE treated leaf was studied.

MATERIALS AND METHODS

Protein was estimated (Lowry's *et al.,* 1951) method. Phenol was estimated (Bray and Throne, 1964) method. Proline was estimated (Bates *et al.,* 1973) method. Total chlorophyll content was estimated (Arnon's, 1949) method. Total sugars were estimated by Anthrone method (Morris, 1948). Reducing sugar was estimated (Miller's, 1972) method.

RESULTS

Estimation of protein

Protein content in the Sorghum leaf variety bilijola was 0.62 to 0.82 mg/g on 15^{th} and 45^{th} day of the treatment. On 15^{th} day of treatment (27^{th} day of emergence) leaf sugary disease was observed in which protein content was decreased to 0.22 mg/g (reduction of 64.52%). In the 10%NLE treated plant

 ISSN 2231 – 4423

protein content was 0.45 mg/g, which was 51.2% more than protein content in leaf sugary disease (LSD) affected plant. Similarly in 20 and 30% NLE treated plants the proteins content has increased slightly to 0.47 mg/g and 0.50 mg/g respectively, with percent increase of 53.2% and 56% when compared to protein content of leaf sugary disease (LSD) affected. This trend continues even at 30th day and 45th days observations. This clearly indicates Neem leaf extract of 10, 20 and 30% have decreased the leaf sugary disease (LSD) by inhibiting aphids (Table-1).

Estimation of reducing sugar

The total reducing sugar in the healthy *Sorghum bicolor* variety bilijola was 0.41 mg/g in the leaves of leaf sugary disease (LSD) it has decreased to 0.17 mg/g with the percent decrease of 58.54%. When compared to leaf sugary disease (LSD) in the NLE treated plants total reducing sugar as increased gradually. On the 15th day of observation, the total reducing sugar was 0.18.0.31 and 0.32 mg/g with the percent increase of 5.6, 45.17 and 46.88% respectively. This trend is same in the 30th day and 45th days. This clearly indicates that because of LSD the reducing sugar in the LSD plants decreases, but due to NLE treatment less LSD and less aphid population was observed, that increases reducing sugar content in the NLE treated plants (Table-2).

Total Sugar content

Total sugar content in the *Sorghum bicolor* variety bilijola was ranged between 0.6 mg/g to 0.89 mg/g between 15th days to 45th days of observations. On the 15th days of observation (27 days of emergence), the total sugar in the healthy plant was 0.6 mg/g but the LSD plants showed reduced sugar contents of 0.25 mg/g with 58.34% decrease. In NLE treated plants the total sugar was raised gradually to 0.32 mg/g 0.38 mg/g and 0.41 mg/g at 10, 20 and 30% NLE treated plants respectively. This increase was gradually with 21.88, 34.22 and 39.05% increase respectively. This trend continues even at 30th day and 45th days observations respectively (Table-3).

Total Chlorophyll content

The total chlorophyll content of the leaf is an important factor for normal growth and development of the plant, in the healthy plants the total chlorophyll content was 6.88 mg/100g in the leaves of *Sorghum bicolor* variety bilijola, but when plant was affected by LSD the total chlorophyll content decreases to 1.42 mg/100g with 79.37% inhibition. On 15th day of observation the total chlorophyll content was more with 4.5 mg/100g when compared in the LSD plants with 68.45% more than LSD plants. In 20 and 30% NLE treated plants Chlorophyll content was increased to 5.74 mg/100g and 6.36 mg/100g respectively, with 75.27 and 77.68% increased, when compared to LSD plants. This trend continues even in the 30th day and 45th days of observations respectively (Table - 4).

Total Proline content

Proline content in the Sorghum leaf variety bilijola was 1.70, 1.27ug/100mg on 15th to 45th days of the treatment. On 15th day of treatment (27th day after emergence) leaf sugary disease was observed to this stage proline content was 6.80 mg/g which was about five fold increase in the 10% NLE treated plant, Proline content was 1.66 mg/g which was 28% less than proline content when compared to LSD plant. Similarly in 20% and 30% NLE treated plants the proline content has decreased slightly to 1.50 mg/g and 1.34 mg/g respectively, with percent decrease of 22.05 and 19.70%. When compared to proline content in LSD. This trend continues even at 30th day and 45th days observations. This clearly indicates Neem leaf extracts of 10, 20 and 30% have decreased the LSD by inhibiting aphid (Table-5).

Estimation of phenol

Phenol content in the *Sorghum bicolor* variety bilijola was ranged between 1.58 mg/g to 2.40 mg/g between 15th day to 45th days of observations. On the 15th day

 ISSN 2231 – 4423

of observation (27th days of emergence) the total phenol in the LSD plant was 6.26mg/g, but the healthy plants showed reduced phenol contents of 1.58 mg/g with four-fold decrease. In NLE treated plants the phenol was decreased gradually, to 1.40 mg/g, 1.32 mg/g and 1.22 mg/g at 10, 20 and 30% NLE treated plants respectively. This decrease was gradually with 22.36%, 21.08% and 19.48% decreased respectively. This trend continues even in 30th day and 45th days treatment respectively. This clearly indicates NLE of 10, 20 and 30% have decreased the LSD by inhibiting aphids (Table-6).

DISCUSSION

The Physiology and the Biochemistry of the Sorghum leaf showing leaf sugary disease (LSD) has not so for being understood well. So, in this direction Protein, Reducing sugar, Total Sugar, Chlorophyll, Proline and Phenols contents, were estimated in healthy, LSD affected and NLE treated leaves shows with 10, 20 and 30% NLE at 15, 30 and 45 days of intervals. There was a decrease 64.52% of protein content that was observed in the LSD affected leaves and a general phenomenon that occurs in the host during pathogenesis and physiological disorder.

The results indicate that total phenol content increases four times in the LSD leaf, when compared to healthy plant leaf. Increase in the phenolic content may be attributed to the action of enzymes catalyzing various biosynthesis processes (Davis *et al.,* 1953) suggested enzymes may act on the native conjugated phenolic causing an increase of phenolic substances in diseased plant parts. The phenolic content in the leaf of LSD was high which reduced the aphid (*Melanaphis sacchari*) population about 20 times less. Similar results were observed (Mote and Shahane, 1988) with *Delphacid* and Aphid infestation on *Sorghum* leaf of different varieties. Phenolic compounds are the most widely distributed secondary metabolites. Ubiquitously present in the plant kingdom. Among the cereals, Sorghum has the highest content of phenolic compounds, reaching up to 6% (w/w) in some varieties (Deshpande *et al.,* 1986; Beta *et al.,* 1999; Doka *et al.,* 2004; Awika and Rooney 2004 and Dicko *et al.,* 2005).

CONCLUSION

In the present study phenolic content in the healthy plant ranges between 1.58-2.4 mg/g. whereas, LSD affected leaves showed increased concentrations of phenolic compounds ranging from 6.26 to 9.4 mg/g dry weight.

ACKNOWLEDGMENT

I thanks to Gulbarga University, Gulbarga for providing RGNF Senior research fellowship.

Table-1: Protein content (mg/g) of healthy, leaf sugary disease (LSD) affected and NLE treated leaves

Sl. No.	Treatments (%)	Protein content (mg/g)		
		15-day	30-day	45-day
01	NLE 10	0.45 ± 0.025	0.47 ± 0.054	0.50 ± 0.054
02	NLE 20	0.47 ± 0.053	0.48 ± 0.025	0.51 ± 0.048
03	NLE 30	0.50 ± 0.042	0.51 ± 0.033	0.53 ± 0.060
04	CWS	0.27 ± 0.051	0.22 ±0.050	0.23 ± 0.063
05	LSD	0.22 ± 0.042	0.19 ± 0.044	0.19 ± 0.039
06	HPL	0.62 ± 0.047	0.73 ± 0.033	0.82 ± 0.032

Data represents an average of 10 replicates indicates ± SE

 ISSN 2231 – 4423

Table-2: Total Reducing Sugar content (mg/g) of healthy, leaf sugary disease (LSD) affected and NLE treated leaves

Sl. No.	Treatments (%)	Total Reducing Sugar content (mg/g)		
		15-day	30-day	45-day
01	NLE 10	0.18 ± 0.016	0.20 ± 0.055	0.22 ± 0.070
02	NLE 20	0.31 ± 0.09	0.34 ± 0.091	0.36 ± 0.074
03	NLE 30	0.32 ± 0.089	0.35 ± 0.076	0.37 ± 0.074
04	CWS	0.18 ± 0.016	0.20 ± 0.025	0.22 ± 0.070
05	LSD	0.17 ± 0.047	0.19 ± 0.047	0.21 ± 0.083
06	HPL	0.41 ± 0.023	0.45 ± 0.094	0.49 ± 0.048

Data represents an average of 10 replicates indicates ± SE

Table-3: Total Sugar content (mg/g) of healthy, leaf sugary disease (LSD) affected and NLE treated leaves

Sl. No.	Treatments (%)	Total Sugar content (mg/g)		
		15- day	30-day	45-day
01	NLE 10	0.32 ± 0.089	0.39 ± 0.020	0.55 ± 0.048
02	NLE 20	0.32 ± 0.089	0.42 ± 0.038	0.67 ± 0.083
03	NLE 30	0.41 ± 0.075	0.42 ± 0.050	0.65 ± 0.083
04	CWS	0.26 ± 0.091	0.24 ± 0.047	0.27 ± 0.032
05	LSD	0.25 ± 0.055	0.22 ± 0.083	0.26 ±0.078
06	HPL	0.60 ± 0.015	0.58 ± 0.030	0.89 ± 0.083

Data represents an average of 10 replicates indicates ± SE

Table-4: Total Chlorophyll content (mg/100g) of healthy, Leaf sugary disease (LSD) affected and NLE treated leaves

Sl. No.	Treatments (%)	Total Chlorophyll content (mg/100g)		
		15- day	30-day	45-day
01	NLE 10	4.50 ± 0.64	4.26 ± 0.86	4.51 ± 0.45
02	NLE 20	5.74 ± 0.39	5.28 ± 0.30	5.26 ± 0.48
03	NLE 30	6.36 ± 0.09	6.18 ± 0.15	6.30 ±0.35
04	CWS	2.37 ± 0.36	2.20 ± 0.16	2.22 ± 0.47
05	LSD	1.42 ± 0.53	1.34 ± 0.18	1.33 ± 0.96
06	HPL	6.88 ± 0.80	6.32 ± 0.25	6.23 ± 0.81

Data represents an average of 10 replicates indicates ± SE

 ISSN 2231 – 4423

Table-5: Total Proline content (mg/g) of healthy, Leaf sugary disease (LSD) affected and NLE treated leaves

Sl. No.	Treatments (%)	Total Proline content (mg/g)		
		15- day	30-day	45-day
01	NLE 10	1.66 ± 0.05	1.76 ± 0.04	1.76 ± 0.09
02	NLE 20	1.50 ± 0.03	1.62 ± 0.07	1.70 ± 0.47
03	NLE 30	1.34 ± 0.05	1.58 ± 0.89	1.64 ± 0.44
04	CWS	4.50 ± 0.02	5.60 ± 0.00	4.60 ± 0.44
05	LSD	6.80 ± 0.05	6.00 ± 0.16	5.60 ± 0.44
06	HPL	1.70 ± 0.02	1.21 ± 0.09	1.27 ± 0.47

Data represents an average of 10 replicates indicates ± SE

Table-6: Total Phenol content (mg/g) of healthy, Leaf sugary disease (LSD) affected and NLE treated leaves

Sl. No.	Treatments (%)	Total Phenol content (mg/g)		
		15- day	30-day	45-day
01	NLE 10	1.14 ± 0.09	1.42 ± 0.60	1.56 ± 0.20
02	NLE 20	1.32 ± 0.60	1.26 ± 0.09	1.48 ± 0.34
03	NLE 30	1.22 ± 0.81	1.40 ± 0.94	1.38 ± 0.27
04	CWS	3.78 ± 0.71	5.48 ± 0.83	5.98 ± 0.83
05	LSD	6.26 ± 0.09	7.78 ± 0.08	9.40 ± 0.07
06	HPL	1.58 ± 0.08	2.22 ± 0.18	2.40 ± 0.15

Data represents an average of 10 replicates indicates ± SE

REFERENCES

1. Awika. J. M and Rooney, L. W. Sorghum phytochemicals and their potential aspects on human health. *Phytochemistry.* 2004; **65:** 1199-1221.

2. Beta T., Rooney L. W., Marovatsanga, L. T., Taylor, J. R. N. Phenolic compounds and kernel characteristics of Zimbabwean sorghums. *Jl. Sci. Food Agric.* 1999; **79:** 1003-1010.

3. Chung T., Wong T. Y., Wei C. I., Huang, Y. W. and Lin, Y. Tannins and human health: A review. *Crit. Rev. Food Sci. Nutr.* 1998; **38:** 421-464.

4. Davis D., Waggoner P. E. and Diamond A. E. *Nature* (London) 1953; **172:** 929.

5. Deshpande S. S., Cheryan M., Salunkhe D. K. Tannin analysis of food products. CRC. Crit. Rev. *Food, Sci. Nutr*. 1986; **24:** 401-449.

6. Dicko M. H., Gruppen H., Barro C., Van Berkel W. J. H., Voragen. A. G. J. Impact of phenolics and related enzymes in Sorghum varieties for the resistance and susceptibility to biotic and abiotic stresses. *Jl. Chem. Ecol.* 2005; **31:** 2671-2688.

7. Doka O., Bicanic D. D., Dicko M. H., Slingerland M. A. Photoacoustic approach to direct determination of the total phenolic content in red- Sorghum flours. *Jl. Agric. Food Chem.* 2004; **52:** 2133-2136.

8. Krueger C. G., Vestling M. A., Reed J.D. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of heteropolyflavan-3-ols and glucosylated heteropolyflavans in sorghum (Sorghum bicolor (L.) Moench. *Jl. Agric. Food Chem.* 2003; **51:** 538-543.

9. Mote V. N. and Shahane A. K. Studies on cultivar reaction of Sorghum to delphacid, aphid and leaf sugary exudation. *Sorghum Newsletter*. 1988; **31:** 60.