Indian J. Plant Physiol., Vol. 11, No. 4, (N.S.) pp. 385-390 (Oct.-Dec., 2006)

EFFECT OF SENNA STEM TREATMENTS ON GROWTH, NET PHOTOSYNTHESIS, NITROGEN METABOLISM AND YIELD OF PEARL MILLET

B.K. GARG*, H.A. KHAN AND Z.D. KAVIA

Central Arid Zone Research Institute, Jodhpur 342 003

Received on 7 Jan., 2005, Revised on 19 Dec. 2006

SUMMARY

Effects of senna stems @ 2.5 and 5.0 t ha^{-1} and their extracts in chloroform and water were studied on pearl millet [*Pennisetum glaucum* (L..) Br. Cv. HHB-67] under rainfed condition. Application of senna stems and their extracts significantly increased leaf area, grain yield and dry matter production of pearl millet. Maximum favourable effects on plant growth and grain yield were observed with the application of water extract of senna stems. Leaf metabolites (viz. total chlorophyll, soluble protein, free amino acids), nitrate reductase activity and net photosynthetic rate at vegetative and flowering stages were significantly improved with the application of senna stems and their extracts in water and chloroform. Water extract of senna stems had maximum beneficial effects on leaf metabolites, nitrate reductace activity and photosynthetic efficiency. The results indicate a possibility of presence of growth promoting substance in senna stem because such dramatic increase within a short time can not be attributed to senna residue effects only. The occurrence of triacontanol in senna stems alongwith other nutrients and unknown factors might have contributed towards their positive effects on growth and metabolism of pearl millet.

Key words : **Net photosynthesis, nitrogen metabolism, pearl millet, senna stem, senna extract**

INTRODUCTION

Senna *(Cassia angustifolia*) is an important commercial medicinal crop. Initially confined to Tamil Nadu, its cultivation has spread to Maharashtra, Gujarat and Rajasthan. The plant is highly drought hardy and is cultivated on marginal lands. Senna is valued for its cathartic properties as its leaves and pods contain anthraquinone glycosides - sennoside A and sennoside B - used in the pharmaceutical industry for preparing laxatives (Singh *et al.* 1998, Tripathi 1999). However, senna stems, which are considered a waste material so far, have also been found by some farmers of Rajasthan villages to be useful for crop growth and yield when used as soil application. Despite several reports on the favourable effects of various crop residues on soil properties, plant growth and yield (Prasad and Power 1991, Aggarwal *et al.* 1997) the use of senna stems as residue is nowhere mentioned. Garg *et al.* (2003) were the first to report beneficial effects of senna stems on growth, yield and nitrogen metabolism of wheat and suggest a possibility of the presence of growth promoting substance triacontanol in senna stems. The effects of triacontanol in enhancing plant growth and physiological processes, including photosynthetic efficiency, are well known (Ries 1985, Souza *et al.* 1999, Shrivastava *et al.* 2001). These observations prompted the present study to explore the effects of senna stems and their extracts in water and chloroform on growth, photosynthesis and nitrogen metabolism of pearl millet under field conditions.

*Corresponding author, E-mail: bkgarg@cazri.res.in

MATERIALS AND METHODS

The present investigation was conducted in the field, in microplots (3 x 2 m^2) with pearl millet [*Pennisetum glaucum* (L.) R. Br. cv. HHB 67] during 2003 *kharif* season. The experimental soil was loamy sand soil (Typic Camborthids) having 7.1% clay, 5.6% silt, 63.1% fine sand and 24.1% coarse sand and having 0.28% organic carbon and 0.023% total nitrogen. The soil contained 80 kg ha^{-1} available N, 12 kg ha^{-1}available P and 120 kg ha^{-1} available K. Senna stems were obtained from the farmer's field and following nine treatments were employed adopting randomized block design (RBD) with four replications. T_1-control, T_2-soil application of senna stems @ 2.5 t ha^{-1}, T_3-soil application of senna stems @ 5.0 t ha^{-1}, T_4-chloroform extract of senna stems 2.5 t ha^{-1}, T_5-chloroform extract of 5.0 t ha^{-1} senna stems, T_6-water extract of 2.5 t ha^{-1} senna stems, T_7-water extract of 5.0 t ha^{-1} senna stems, T_8-residue left after chloroform extraction from T_5 and T_9-residue left after water extraction from T_7.

For preparation of extracts of senna stems in chloroform, 1.5 or 3.0 kg senna stems (material equivalent to 2.5 or 5.0 t ha^{-1} for T_4 and T_5 treatments) were extracted in pure chloroform in a Soxhlet apparatus for 48 hours and the extract was made to a volume of 250 ml. The identity of triacontanol (CH_3 $(CH_2)_{28}$ CHOH) in the above extract was confirmed by its infrared spectroscopic analysis as well as by co-TLC with an authentic sample of triacontanol. Afterwards the extract was added to 2 kg of dry soil collected from experimental plots separately for each replicate, which was allowed to dry completely so that all the chloroform evaporated. After removing the chloroform, the dry soil containing the triacontanol was mixed thoroughly into the experimental plots. The residue left after chloroform extraction in T_5 treatment (5.0 t ha^{-1} senna stems) was added to another plots designated for T_8 treatment.

For preparation of water extracts, required quantities of senna stems (1.5 or 3.0 kg for T_6 and T_7 treatments) were immersed in 20 litre tap water in glazed pots for 48 hours with occasional stirring and then stems were removed. The crude extract so prepared was applied uniformly to experimental plots earmarked for T_6 and T_7 treatments. The residue left from T_7 treatment (5.0 t ha^{-1}) was used for T_9 treatment. Senna stems, their extracts in water and chloroform and residue were added to the respective plots 2 days before sowing. Sowing was done on 8th July 2003 and all plots received a basal dose of 40 kg ha^{-1} N through urea and 40 kg ha^{-1} P_2O_5 through single super phosphate. The experiment was conducted under rainfed conditions.

Observations were recorded on plant growth in terms of plant height, number of leaves and leaf area (using LICOR-3000 leaf area meter) at vegetative (25 DAS) and flowering (50 DAS) stages of growth. Data were based on 3 plants from each of the four replicates. Net photosynthetic rates were measured using LICOR 6200 portable photosynthesis system in two uppermost fully expanded leaves of intact plants in the field between 10.00-11.30 a.m. on four replicates under each treatment. At the same time levels of total chlorophyll (Arnon 1949), soluble protein (Lowry *et al.* 1951) free amino acids (Yemm and Cocking 1955) and nitrate reductase activity (Jaworski 1971) were analysed, in triplicate, from two uppermost fully expanded leaves of representative plants under each treatment. Plant performance was adjudged from final above ground dry matter (biomass) and grain yield. Significance of the data was assessed through analyses of variance adopting RBD.

RESULTS AND DISCUSSION

Soil application of senna stems and their extracts in water and chloroform significiantly increased the performance of pearl millet crop (Table 1). The grain yield increased by 23.2, 51.5 and 59.6% with application of senna stems, chloroform extract and water extract of senna stems, respectively (at 5 t ha^{-1} level) as compared to control. The increase in total biomass production was highest with water extract (46.2% over control), followed by chloroform extract (37.1%) and senna stems (19.7%). Similar positive effects were observed on stover yield and harvest index with a significant increase with chloroform and water extracts. Residues left after water extraction had no significant effect on grain yield or dry matter producion, whereas the residue left after chloroform extraction showed a significant effect. The yield improvement of pearl millet by application of senna stems or their extracts indicates that some growth promoting substance may be present in senna stems causing such

Table 1. Influence of senna stems, their extracts and residue on total biomass, grain and stover yields and harvest index of pearl millet

Treatments	Grain yield (kg ha^{-1})	Total biomass (kg ha^{-1})	Stover yield (kg ha^{-1})	Harvest index (%)
T_1- Control	932	3356	2424	27.8
T_2- Senna stems (2.5 t ha^{-1})	1032	3648	2616	28.3
T_3- Senna stems (5.0 t ha^{-1})	1148*	4016*	2868*	28.5
T_4- Chloroform Ext. (2.5 t ha^{-1})	1192*	4148*	2956*	28.7
T_5- Chloroform Ext. (5.0 t ha^{-1})	1452*	4600*	3188*	30.6
T_6- Water Ext. (2.5 t ha^{-1})	1232*	4380*	3148*	28.1
T_7- Water Ext. (5.0 t ha^{-1})	1488*	4908*	3420*	30.3
T_8- Residue of Chl. Ext. (5.0 t ha^{-1})	1092*	3748*	2686*	28.3
T_9- Residue of water Ext. (5.0 t ha^{-1})	976	3460	2484	28.2
LSD (p = 0.05)	142	357	247	-

* Significant over control

promotive effects in a very short time which may not be attributed to residiue effects alone. The presence of triacontanol in senna stems (Garg *et al.* 2003) and the significant contribution of triacontanol in yield enhancement have been reported in several studies (Shrivastava *et al.* 2001, Mukherjee *et al.* 2001). Occurrence of nutrients and other growth promoting substance in senna stems may not be ruled out besides some contribution due to residue effects (Prasad and Power 1991, Aggarwal *et al.* 1997). In this regard, Garg *et al.* (2003) reported that senna stems contain appreciable amounts of nitrogen (1.26%), zinc (90 µg g^{-1}dw) and iron (353 µg g^{-1} dw) and has a C:N ratio of 33.3. Thus both hormonal and nutritional constituents of senna stems contribute towards the increased growth and yield of pearl millet.

Senna-induced enhancement in plant growth was associated with a significant increase in photosynthetic rate. Application of senna stems and their extracts in chloroform and water significantly increased leaf area, net photosynthetic rate as well as chlorophyll concentration at both vegetative and flowering stages (Table 2). The increase in leaf area was highest with the application of water extract at both the stages. At the vegetative stage, water extract (5 t ha^{-1} level) enhanced leaf area by 76.6% over the control, while at the flowering stage the enhancement was of the order of 34.9%. Likewise, net photosynthetic rate increased the most by water extract, followed by chloroform extract and senna stems at both the growth stages. The effects due to residue left after chloroform or water extracts were not significant on net photosynthetic rate although leaf area was increased significantly. The concentration of chlorophyll also increased by soil application of senna stems or their extracts and maximum favourable effect was observed at higher concentration (5 t ha^{-1}) in all cases. Water extract of senna stems showed highest beneficial effect, followed by chloroform extract and senna stems at both the growth stages. It appears that favourable effects of senna stems and their extracts are due to the presence of some growth promoting substance and triacontanol may be one of these. The significant role of triacontanol in increasing leaf area, net photosynthesis and chlorophyll content is well documented (Souza *et al.* 1999, Borowski *et al.* 2000, Kumarvelu and Souerche, 2001, Garg *et al.*, 2003). However, the observed promotive effects may be due to a combined action of triacontanol with some unknown factors present in senna stems.

Application of senna stems either as such or in the form of water and chloroform extracts significantly enhanced nitrate reductase (NR) activity and contents

Table 2. Influence of senna stems, their extracts and residue on leaf area, net photosynthetic rate and chlorophyll content of pearl millet at vegetative and flowering stages

Treatments	Leaf area (cm^2 $plant^{-1}$)		Net photosynthesis (μmol $m^{-2}s^{-1}$)		Total chlorophyll (mg g^{-1} d w)	
	Vegetative	Flowering	Vegetative	Flowering	Vegetative	Flowering
T_1- Control	229.5	349.9	13.92	20.45	9.01	5.55
T_2- Senna stems (2.5 t ha^{-1})	257.6	376.8	15.02	23.18*	10.29*	5.86
T_3- Senna stems (5.0 t ha^{-1})	375.8*	433.8*	17.14*	25.30*	11.30*	6.18*
T_4- Chloroform Ext. (2.5 t ha^{-1})	306.5*	404.7*	14.85	22.90*	11.11*	5.76
T_5- Chloroform Ext. (5.0 t ha^{-1})	398.0*	445.1*	17.69*	25.15*	11.51*	6.36*
T_6- Water Ext. (2.5 t ha^{-1})	281.7*	396.6*	15.04	23.38*	10.27*	5.93
T_7- Water Ext. (5.0 t ha^{-1})	405.3*	472.2*	17.75*	23.87*	11.55*	6.69*
T_8- Residue of Chl. Ext. (5.0 t ha^{-1})	276.6*	384.5	15.12	21.89	10.49*	5.69
T_9- Residue of water Ext. (5.0 t ha^{-1})	239.3	379.2	13.91	20.88	10.07	5.92
LSD (p = 0.05)	30.2	42.3	1.53	2.43	1.08	0.46

* Significant over control

of soluble protein and free amino acids in the pearl millet leaves at both the growth stages (Table 3). The enhancement in NR activity was particularly important as NR is the key enzyme for nitrate reduction in higher plants (Sinha and Nicholas 1981). Thus our results indicate a favourable influence of senna stems on nitrogen metabolism also. In this regard the marked increase in NR activity by water extract (91.6% over control), senna stems (69.8% over control) and chloroform extract (57.9%) at the vegetative stage is noteworthy. Such effects at the flowering stage were lesser in magnitude, though significant in all cases, and higher at 5 t ha^{-1} level. However, the effects due to residue left from chloroform and water extracts were far less or insignificant. The increase in contents of soluble protein and free amino acids with application of various forms of senna stems further indicates the presence of some growth promotive substances in senna stems. There are reports that NR activity is enhanced by triacontanol application in green gram seedlings (Kumarvelu *et al.* 2000) and wheat leaves (Garg *et al.* 2003). Further studies may elucidate the various factors responsible for affecting nitrogen metabolism by senna stems and their extracts.

This study indicates that soil application of senna stems or their extracts in water and chloroform to pearl millet crop before sowing could significantly improve plant growth and grain yield. The maximum favourable effects were obtained at 5 t ha^{-1} level and water extract of senna stems was most promotive. The beneficial effects of senna stems and extracts were mediated through enhanced photosynthetic efficiency and more efficient nitrogen metabolism. As residues left after chloroform and water extracts were less effective it has been speculated that senna stems contain some growth promoting substances (such as triacontanol and others); this needs to be confirmed.

Table 3. Influence of senna stems, their extracts and residue on nitrate reductase activity and contents of soluble protein and free amino acids of pearl millet at vegetative and flowering stages.

Treatments	Nitrate reductase activity ($\mu g\ NO_2 g^{-1}\ d\ w\ h^{-1}$)		Soluble protein (mg g^{-1} d w)		Free amino acids (mg g^{-1} d w)	
	Vegetative	Flowering	Vegetative	Flowering	Vegetative	Flowering
T_1- Control	188.5	150.7	63.7	33.3	15.92	14.34
T_2- Senna stems (2.5 t ha^{-1})	255.9*	177.2*	73.1	33.7	21.26*	14.64
T_3- Senna stems (5.0 t ha^{-1})	320.2*	202.5*	78.0*	40.9*	25.54*	15.88*
T_4- Chloroform Ext. (2.5 t ha^{-1})	232.7*	158.8	71.3	33.4	25.42*	14.50
T_5- Chloroform Ext. (5.0 t ha^{-1})	297.2*	192.4*	81.8*	37.1*	29.46*	15.72*
T_6- Water Ext. (2.5 t ha^{-1})	290.9*	159.1	82.7*	34.3	25.96*	14.74
T_7- Water Ext. (5.0 t ha^{-1})	351.2*	208.9*	91.6*	39.4*	29.23*	15.76*
T_8- Residue of Chl. Ext. (5.0 t ha^{-1})	259.0*	164.9	67.0	31.0	20.87*	14.01
T_9- Residue of water Ext. (5.0 t ha^{-1})	241.6*	154.5	72.0	34.4	18.23	14.18
LSD (p = 0.05)	40.4	19.8	10.1	3.7	3.31	0.91

* Significant over control

ACKNOWLEDGEMENTS

The authors thank the Director, CAZRI, Jodhpur for providing facilities. Technical help rendered by Mr. A.R. Sudi and Mr. O.P. Char is duly acknowledged.

REFERENCES

Aggarwal, R.K., Praveen Kumar and Power, J.F. (1997). Use of crop residue and manure to conserve water and enhance nutrient availability and pearl millet yield in arid tropical region. *Soil Tillage Res.***41:** 43-51.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris. Plant Physiol.* **24:** 1-15.

Borowski, E., Blamowski, Z.K. and Michalek, W. (2000). Effects of Tomatex/Triacontanol on chlorophyll fluorescence and tomato (*Lycopersicon esculentum)* yields. *Acta Physiol. Plant.* **22:** 271-274.

Garg, B.K., Khan, H.A. and Kavia, Z.D. (2003). Effect of senna stems and nitrogen fertilization on growth, yield, net photosynthesis and nitrogen metabolism of wheat. Paper presented at National Seminar on "Physiological Interventions on Improved Crop Production and Quality" held at RARS, Tirupati, Dec. 12-14, 2003. Abstr. No. S_4O_5 p. 112.

Kumaravelu, G. and Soureche, R. (2001). Triacontanol- its effect on germination and seedling growth of black gram, cultivar T-9. *Adv. Plant. Sci.* **14:** 143-148.

Kumaravelu, G., Livingstone, V.D. and Ramanujam, M.P. (2000). Triacontanol induced changes in the growth, photosynsthetic pigments, cell metabolites and yield of green gram. *Biol. Plant.* **43:** 287-290.

Jaworski, E. (1971). Nitrate reductase assay in intact plant tissue *Biochem. Biophys. Res. Commun.* **43:** 1274-1279.

Lowry, O.H. Rosenbrough, N. Z., Farr, A.L. and Randall, R.J. (1951). Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193:** 265-275.

Mukherjee, P., Bandi, S. and De, G. C. (2001). Studies on bio-efficiency of different triacontanol products on growth and yield of groundnut. *J. Interacademicia* **5:** 150-155.

Prasad, R. and Power, J.F. (1991). Crop residue management. *Adv. Soil. Sci.***15:** 205-251.

Ries, S. (1985). Regulation of plant growth by triacontanol. *CRC Crit. Rev. Plant Sci.* **2:** 239-285.

Shrivastava, T., Namadeo, K.N., Kumar, M.M., Dwivedi, R.K. Tiwari, R.K. and Shrivastava, T. (2001). Effect of plant growth regulators on growth, yield and nutrient uptake by chickpea (*Cicer arietinum* L.) *Crop Res.* (Hisar) **21 :** 301-307.

Singh, S.P., Raj, K.L., Patra, D.D., Singh, D. and Kumar, S. (1998). Cultivation of the senna (*Cassia angustifolia)* in India. Farm Bulletin No 008, pp. 1-9. Central Institute of Medicinal and Aromatic Plants, Lucknow.

Sinha, S.K. and Nicholas, D.J. (1981). Nitrate reductase. In : L.G. Paleg and D. Aspinall (eds.), The Physiology and Biochemistry of Drought Resistance in Plants, pp. 145-169. Academic Press, New York.

Souza, G.F., Venkataraman, D. and Awathi, M.G. (1999). Effect of triacontanol on physiological attributes of coffee seedlings. *J. Coffee Res.* **27**: 103-109.

Tripathi, Y.C. (1999). *Cassia angustifolia,* a versatile medicinal crop. *Inter. Tree Crop J.* **10:** 121-129.

Yemm, E.W. and Cocking, E.C. (1955). Determination of amino acids with ninhydrin. *Analyst.* **80:** 209-230.