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Changes in Photosynthesis, Enzyme Activities and Production of Anthraquinone and Sennoside Content of Coffee Senna (*Senna occidentalis* L.) by Triacontanol

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ABSTRACT

Coffee senna plants grown in soil-containing pots were sprayed with five concentrations of TRIA [10^{-0} (control), 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M] at 15-day intervals. TRIA is a well known potent plant growth-promoting substance for many agricultural and horticultural crops. Keeping the importance of this medicinal plant in mind, a hypothesis was designed to determine whether foliar sprays of TRIA could augment crop productivity, photosynthesis, activities of enzymes, and the production of anthraquinone and sennoside content of coffee senna. The plant fresh and dry weights, total chlorophyll and carotenoid content, nitrate reductase activity, carbonic anhydrase activity, and leaf -N, -P, -K and -Ca contents were analyzed at 120, 270 and 300 days after sowing (DAS). Net photosynthetic rate, transpiration rate and stomatal conductance were measured only at 270 DAS. The seed-protein content and anthraquinone and sennoside contents were analyzed at harvest (330 DAS). Depending on the observed data, we conclude that a foliar spray of 10^{-6} M TRIA significantly stimulated most of the studied attributes. A spray of 10^{-6} M TRIA increased seed-yield and seed-protein content by 44.92 and 13.40%, respectively when compared to untreated plants. TRIA also stimulated the production of anthraquinone and sennoside contents compared to control plants.

Keywords: Carbonic anhydrase, nitrate reductase, nutraceuticals, TRIA

INTRODUCTION

Coffee senna (*Senna occidentalis* L.), commonly known as 'Badi Kasondi' (Fabaceae), is grown throughout tropical and subtropical countries of the world including India, for its roots, flowers and seeds which have medicinal properties. It is a decongestant and is used for treatment of cough, whooping cough, convulsions, and heart disease (The Wealth of India 1992).

Anthraquinones (**Fig. 1**) are an important group of natural products occurring in higher plants as glycosides. They are found in a large number of plant families, including the Fabaceae. The leaves and seeds of coffee senna contain anthraquinone glycosides which have analgesic, antibacterial, anti-hepatotoxic, antifungal, anti-inflammatory, antiseptic, laxative and purgative properties (The Wealth of India 1992; Kirtikar and Basu 1995; Morris 1997, 1999). The plant parts (leaves and seeds) contain anticancer properties. It is also used in the treatment of skin fungal diseases (Thomson 1987, 1996). Furthermore, the seeds have been used as a substitute for coffee (The Wealth of India 1992; Morris 1999). The leaves and pods possess a group of anthracene derivatives collectively known as sennosides (**Fig. 1**), which form an important source of organic laxatives. There is worldwide demand for its use in pharmacopoeial preparations and in modern as well as traditional systems of medicine (The Wealth of India 1992; Kirtikar and Basu 1995).

Today, agronomists are trying to devise additional ways to improve yield limits of existing demand of medicinal plants. The most feasible and successfully adopted technique is the foliar application of diluted aqueous solutions of plant growth regulators (PGRs) for optimization of genetic potential of a crop. Out of a variety of PGRs, triacontanol (TRIA) is a long chain primary alcohol ($C_{30}H_{61}OH$) known to be a potent plant-growth promoting substance for many agricultural and horticultural crops (Ries 1985, 1991; Naeem *et al.* 2009).

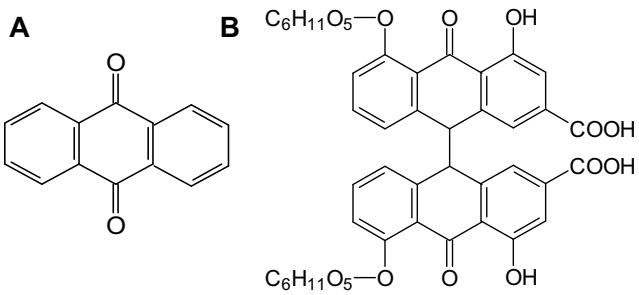

Fig. 1 Structural formulae of anthraquinone (**A**) and sennoside (**B**).

Taking in account of the demand of this medicinal plant, the present study aimed to evaluate whether the foliar spray of TRIA could enhance the crop productivity, physiological activities and quality attributes of coffee senna.

MATERIALS AND METHODS

Plant material and growth condition

Healthy seeds of coffee senna were received from the USDA-ARS, Plant Genetic Resources Conservation Unit, Griffin, GA, USA. Healthy seeds of uniform size were selected and their viability was tested. Thereafter, the seeds were surface-sterilized with 95% ethyl alcohol for 5 min and then washed thoroughly with distilled water before sowing. The seeds were sown in 25-cm diameter pots at a depth of 2 cm in the soil. Prior to sowing, the experimental pots were filled with 5.0 kg of a homogenous mixture containing soil and farmyard manure (5: 1). The soil was maintained at proper

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Original Research Paper

moisture to ensure better germination of the seeds. Physico-chemical characteristics of the soil were: texture-sandy loam, pH (1:2) - 8.0, E.C. (1:2) -0.49 m mhos cm^{-1}, available N, P and K – 96.85, 7.82 and 149.5 mg kg^{-1} soil, respectively. The soil samples were tested at the Government Soil Testing Laboratory, Quarsi Farm, Aligarh. A uniform basal level of P (10 mg per kg soil) and Ca (120 mg per kg soil) was applied to the soil.

Experimental setup

The pot experiment was performed in natural conditions of the greenhouse in the Department of Botany, A.M.U., Aligarh (27° 53′ N latitude, 78° 51′ E longitude, and 187.45 m altitude). The experiment was conducted using a simple randomized block design. Treatments consisted of 5 concentrations of TRIA, viz. 10^{-0} (control), 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M applied as a foliar spray in 15-day intervals. Each treatment was replicated three times and each replicate had three plants. The plants were sufficiently watered as and when needed.

Growth and yield analyses

At 120 (vegetative stage), 270 (flowering stage) and 300 (pod-filling stage) days after sowing (DAS), three plants from each treatment were uprooted carefully and washed with tap water to remove all adhering foreign particles and were dried using blotting papers. Then, the plant fresh weight was recorded. The plants were dried at 80°C for 24 h using a hot air oven, and the plant dry weights were recorded. For the yield attributes, nine plants from each treatment were collected at harvest (330 DAS). The pods were threshed and cleaned. The number of pods per plant and number of seeds per pod were recorded. 100-seed weight and seed-yield per plant was calculated accordingly using an electronic balance.

The fresh leaves were used for the analyses of various physiological and biochemical attributes except for leaf -N, -P, -K and -Ca contents.

Determination of net photosynthetic rate, transpiration rate and stomatal conductance

Net photosynthetic rate, transpiration rate and stomatal conductance were measured on sunny days at 11:00 am on youngest fully expanded leaves of coffee senna using IRGA (Infra Red Gas Analyzer, LICOR 6200 Portable Photosynthesis System, Lincoln, Nebraska, USA). Before recording the measurement, the IRGA was calibrated and zero was adjusted approximately every 30 min during the measurement period. The atmospheric conditions during measurement were photosynthetically active radiation (PAR) = 1016 ± 6 l µmol m^{-2} s^{-1}, relative humidity = 43 ± 3%, atmospheric temperature = 40 ± 1°C and atmospheric CO_2 = 360 µmol mol^{-1}. The ratio of atmospheric CO_2 to intercellular CO_2 concentration was constant. Each leaf was enclosed in a 1-l gas exchange chamber for 60 sec. All the attributes measured by the IRGA were recorded three times for each treatment. Photosynthesis was measured at 270 DAS.

Estimation of total chlorophyll and carotenoid contents

Total chlorophyll (Chl) and carotenoid content in fresh leaves were estimated by the method of MacKinney (1941) and MacLachlan and Zalik (1963), respectively. One hundred mg of fresh tissue from interveinal leaf areas was ground using a mortar and pestle containing 80% acetone. The optical density (OD) of the solution was recorded at 645 and 663 nm for Chl estimation and at 480 and 510 nm for carotenoids content estimation using a spectrophotometer (Spectronic 20D, Milton Roy, USA).

Determination of nitrate reductase (NR) activity

Nitrate reductase (E.C. 1.6.6.1) activity in the leaf was determined by the intact tissue assay method of Jaworski (1971), which is based on the reduction of nitrate to nitrite as per the following biochemical reaction:

$$NO_3^- + NADH + H^+ \xrightarrow{NR} NO_2^- + NAD^+ + H_2O$$

Chopped leaf pieces (200 mg) were incubated for 2 h at 30°C. Each vial contained 2.5 mL of 0.1 M phosphate buffer, 0.5 mL of 0.2 M potassium nitrate, and 2.5 mL of 5% isopropanol. After incubation, 1% sulphanilamide and 0.02% *N*-(1-naphthyl) ethylenediamine dihydrochloride (NED-HCL) were added. The nitrite formed subsequently was colorometrically determined at 540 nm after azocoupling with sulphanilamide and NED-HCL. The NR activity was expressed as nM NO_2^- g^{-1} FW h^{-1}.

Determination of carbonic anhydrase (CA) activity

Carbonic anhydrase (E.C. 4.2.1.1) activity was measured in fresh leaves, using the method as described by Dwivedi and Randhawa (1974). Two hundred mg of fresh leaf pieces were weighed and transferred to Petri plates. The leaf pieces were dipped in 10 mL of 0.2 M cystein hydrochloride solution for 20 minutes at 4°C. To each test tube, 4 mL of 0.2 M sodium bicarbonate solution and 0.2 -mL of 0.022% bromothymol blue were added. The reaction mixture was titrated against 0.05 N HCl using methyl red as indicator. The enzyme was expressed as µM CO_2 kg^{-1} leaf FW s^{-1}.

Estimation of leaf nutrients

Leaf samples from each treatment were digested for the estimation of leaf -N, -P, -K and -Ca content. The leaves were dried in a hot air oven at 80°C for 24 h. Dried leaves were powdered using the mortar and pestle and the powder was passed through a 72 mesh. The sieved leaf-powder was used for N, P, K and Ca content. One hundred mg of oven-dried leaf powder was carefully transferred to a digestion tube where 2 mL of AR (analytical reagent) grade concentrated sulphuric acid was added also. This solution was heated on a temperature controlled assembly at 80°C for about 2 h and then cooled for about 15 min at room temperature. Afterwards 0.5 mL of 30% hydrogen peroxide (H_2O_2) was added to the solution. The addition of H_2O_2 followed by heating was repeated until the contents of the tube became colourless. The prepared aliquot (peroxide-digested material) was used to estimate per cent N, P, K and Ca in the leaves on the dry weight basis.

Estimation of nitrogen, phosphorus, potassium and calcium contents

Leaf-nitrogen content was estimated according to method of Lindner (1944) with slight modification by Novozamsky *et al.* (1983). The leaf-dried powder was digested in H_2SO_4 in a digestion tube. A 10 mL aliquot (peroxide-digested material) was poured into a 50 mL volumetric flask where 2 mL of a 2.5 N sodium hydroxide and 1 mL of 10% sodium silicate solutions were added to neutralize the acid excess and prevent turbidity. A 5 mL aliquot of this solution was poured into a 10 mL graduated test tube and a 0.5 mL Nessler's reagent was added. The absorbance of the solution was recorded at 525 nm, using a spectrophotometer.

The method of Fiske and Subba Row (1925) with a slight modification by Rorison *et al*. (1993) was adapted to estimate the leaf-phosphorus content in the digested material. The same aliquot (peroxide-digested material) was used to determine the leaf-phosphorus content. A 5 mL aliquot was poured into a 10 mL graduated test tube where 1 mL of molybdic acid (2.5%) was added, followed by addition of 0.4 ml 1-amino-2-naphthol-4-sulphonic acid. When the colour became blue, the volume was increased to 10 ml with the addition of double distilled water (DDW). The absorbance of the solution was recorded at 620 nm using the spectrophotometer.

Potassium and calcium contents in the leaves were analyzed flame-photometrically. Both contents were estimated in the same aliquot with the help of emission spectra using specific filters. In the flame-photometer (Model, C150, AIMIL, India), the solution (peroxide-digested material) was discharged through an atomizer in the form of a fine mist into a chamber, where it is drawn into a flame. Combustion of the elements produces light of a particular wavelength [λ max for K= 767 nm (violet)]. The light produced was passed through the appropriate filters to impinge upon a photoelectric cell that activates a galvanometer. Both leaf-potas-

sium and -calcium content were estimated in the same aliquot and the readings were recorded with the help of emission spectra using specific filters in a flame-photometer.

Estimation of seed-protein content

The protein content in seeds was estimated by the method of Lowry *et al.* (1951). The seed powder (50 mg) was transferred to a mortar and 5% cold trichloroacetic acid (TCA) was added. In addition, 0.5 mL of Folin phenol reagent was added rapidly with immediate mixing. The seed powder containing solution was maintained at room temperature for thirty minutes. Extracted protein was recorded at 660 nm using the spectrophotometer. The observed readings were compared with a calibration curve obtained by using a known dilution of standard egg albumin solution. The per cent seed-protein content was calculated on the dry weight basis.

Estimation of total anthraquinone glycosides content

Total anthraquinone glycosides content in seeds were analyzed using the spectrophotometer as described in Standard of ASEAN Herbal Medicine (ASEAN Countries 1993). Seed powder (1.2 g) was refluxed with 30 mL H_2O, centrifuged for 10 minutes and 0.1 mL of 2 M HCL was added and then extracted three times with 15 mL chloroform. The chloroform layer was discarded, and the aqueous layer was collected. Afterwards 0.10 g of sodium carbonate was added and the content was shaken thoroughly for 3 min. Aglycone fraction was extracted with ether resulting in the formation of two layers (aqueous layer and ether layer). The ether layer was evaporated and the residue was taken, dissolved in 10 mL of 0.5% (w/v) magnesium acetate in MeOH. Consequently, a pink colour developed. The OD of this solution developed and was recorded at 515 nm and expressed as per cent on the dry weight basis.

Estimation of total sennoside content

Total sennoside content in pods was estimated by the method of Habib and EI-Sebakhy (1980). Dried powder was extracted three times with 20, 20 and 10 mL DDW, and the extractions were maintained in a boiling water bath for 15 min. Ten mL of 10% ferric chloride solution was added, and incubated in boiling water bath at 80°C for 20 min, followed by the addition of 0.1 mL of ether. Ether was evaporated by keeping it in hot water. Thereafter, 10 mL of 1 N NaOH was added for developing the red colour. Then, the OD was measured at 525 nm. Total sennoside content was calculated using a standard curve of sennoside and expressed in per cent on the dry weight basis.

Statistical analysis

The data were statistically analysed using analysis of variance (ANOVA) by SPSS (ver. 17; SPSS Inc., Chicago, IL, USA). The treatment means were separated by Duncan's multiple range test ($P < 0.05$). Data were presented as mean ± SE.

RESULTS AND DISCUSSION

Growth and yield attributes

The effect of TRIA spray was significant on plant fresh and dry weights of coffee senna at 120, 270 and 300 DAS. TRIA at 10^{-6} M was the most effective concentration in increasing the values of all growth attributes over their respective controls at all growth stages (**Table 1**). TRIA enhanced the plant fresh and dry weights by 46.5 and 50.4% at 300 DAS when compared to control plants (**Table 1**). However, 10^{-5} M TRIA caused adverse effects on all growth attributes at 120, 270 and 300 DAS (**Table 1**). Previously, the growth-promoting effects of TRIA on various attributes, including fresh and dry weights, has been reported by Misra and Srivastava (1991), Kumaravelu *et al.* (2000), Muthuchelian *et al.* (2003), Giridhar *et al.* (2005), Naeem and Khan (2005), Chaudhary *et al.* (2006), Khan *et al.* (2006), Sharma *et al.* (2006), Khan *et al.* (2007) and Naeem *et al.* (2009) in various medicinal plants. A significant improvement in growth attributes could presumably be ascribed to the well known effect of exogenously applied TRIA on elongation of internodes through cell division and cell extensibility (Taiz and Zeiger 2006).

It was observed that TRIA significantly enhanced seed-yield in comparison to the un-treated plants. The application of TRIA (10^{-6} M) resulted in maximum seed-yield (44.9% increase over control) and increased number of pods per plant (41.2%). However, the effect of TRIA on number of seeds per pod and 100-seed weight was non-significant (**Table 2**). In the present study, the improved seed-yield of the plants could be due to enhanced rates of photosynthesis, increased uptake of nutrients, improved translocation of photosynthates and other metabolites to the reproductive organs. Furthermore, among the yield attributes, significant increase in number of pods also might have played a pivotal role in increasing seed-yield (**Table 2**). These results corroborates the findings of Ries and Houtz (1983), Ivanov and Angelov (1997), Borowski *et al*. (2000), Kumaravelu *et al*. (2000), Chaudhary *et al*. (2006), Khan *et al.* (2006), Sharma *et al*. (2006), Khan *et al*. (2007) and Naeem *et al*. (2009) regarding various crops.

Physiological and biochemical attributes

The data presented in **Figs. 2-7**, indicates that spray of TRIA improved most of the physiological and biochemical attributes significantly at all the growth stages. However, TRIA did not increase carotenoid content at 300 DAS and

Table 1 Effect of five concentrations of foliar spray of TRIA (10^{-0}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M) on growth attributes of coffee senna (*Senna occidentalis* L.) at 120, 270 and 300 DAS. Means within a column followed by the same letter are not significantly different ($p \leq 0.05$). Means of three replicates ± SE.

Attributes	DAS	TRIA concentrations (M)				
		10^{-0}	10^{-8}	10^{-7}	10^{-6}	10^{-5}
Fresh weight per plant	120	17.90 ± 0.69 d	18.95 ± 0.55 bc	20.63 ± 0.73 b	25.90 ± 0.64 a	23.31 ± 0.53 b
	270	105.9 ± 1.56 e	110.4 ± 1.09 d	124.9 ± 1.05 c	159.9 ± 1.56 a	155.8 ± 0.86 b
	300	190.4 ± 1.30 e	206.5 ± 1.51 d	221.4 ± 1.36 c	278.9 ± 1.38 a	273.2 ± 1.79 b
Dry weight per plant	120	3.52 ± 0.13 c	4.20 ± 0.13 b	4.46 ± 0.12b c	5.03 ± 0.12 a	4.6 ± .12 b
	270	19.36 ± 0.41 d	21.47 ± 0.61 c	25.10 ± 0.52 b	28.15 ± 0.79 a	26.15 ± 0.66 b
	300	34.82 ± 0.68 e	40.72 ± 0.57 d	44.84 ± 0.61 c	52.38 ± 0.70 a	50.20 ± 0.55 b

Table 2 Effect of five concentrations of foliar spray of TRIA (10^{-0}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M) on yield attributes of coffee senna (*Senna occidentalis* L.) at 330 DAS. Means within a column followed by the same letter are not significantly different ($p \leq 0.05$). Means of three replicates ± SE.

Attributes	TRIA concentrations (M)				
	10^{-0}	10^{-8}	10^{-7}	10^{-6}	10^{-5}
Number of pods per plant	51.0 ± 1.2 d	55.0 ± 1.3 d	59.0 ± 1.3 c	72.0 ± 1.2 a	67.0 ± 1.2 b
Number of seeds per pod	45.0 ± 0.17 a	45.0 ± 0.17 a	45.3 ± 0.20 a	45.3 ± 0.20 a	45.0 ± 0.17 a
100-seed weight	1.84 ± 0.02 a	1.80 ± 0.01 a	1.84 ± 0.02 a	1.82 ± 0.01 a	1.80 ± 0.01 a
Seed-yield per plant	39.76 ± 0.21 e	45.12 ± 0.22 d	47.18 ± 0.24 c	57.62 ± 0.22 a	56.50 ± 0.23 b

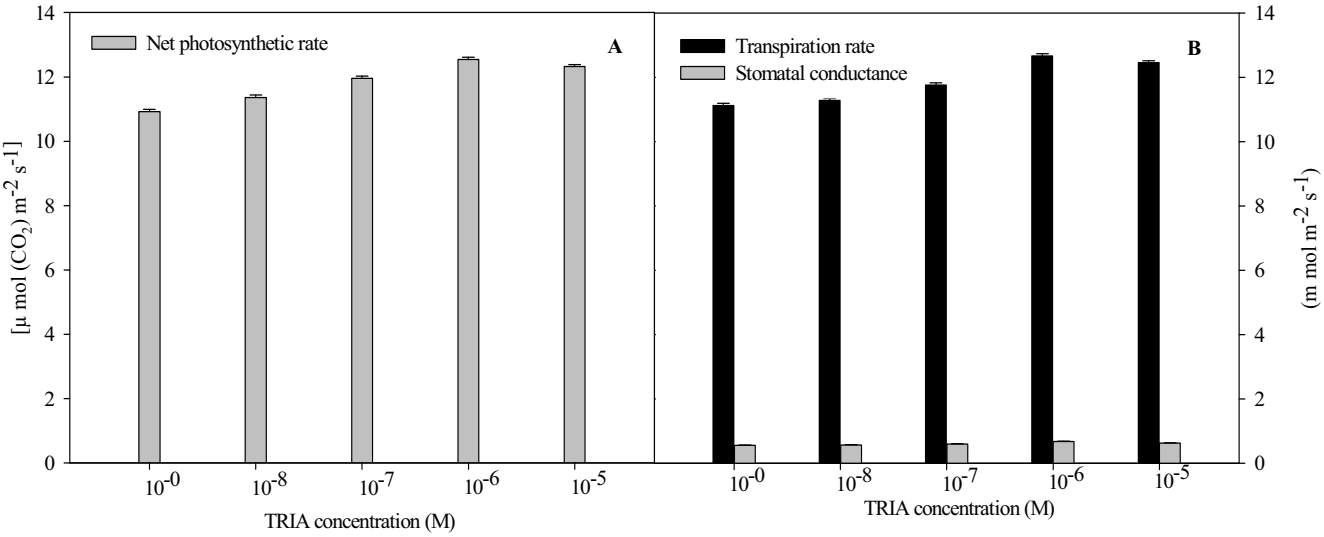

Fig. 2 Effect of five concentrations of TRIA (10^{-0}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M) on net photosynthetic rate (**A**), transpiration rate and stomatal conductance (**B**) of coffee senna studied at 270 DAS (Means of three replicates). Error bars (-) show SE.

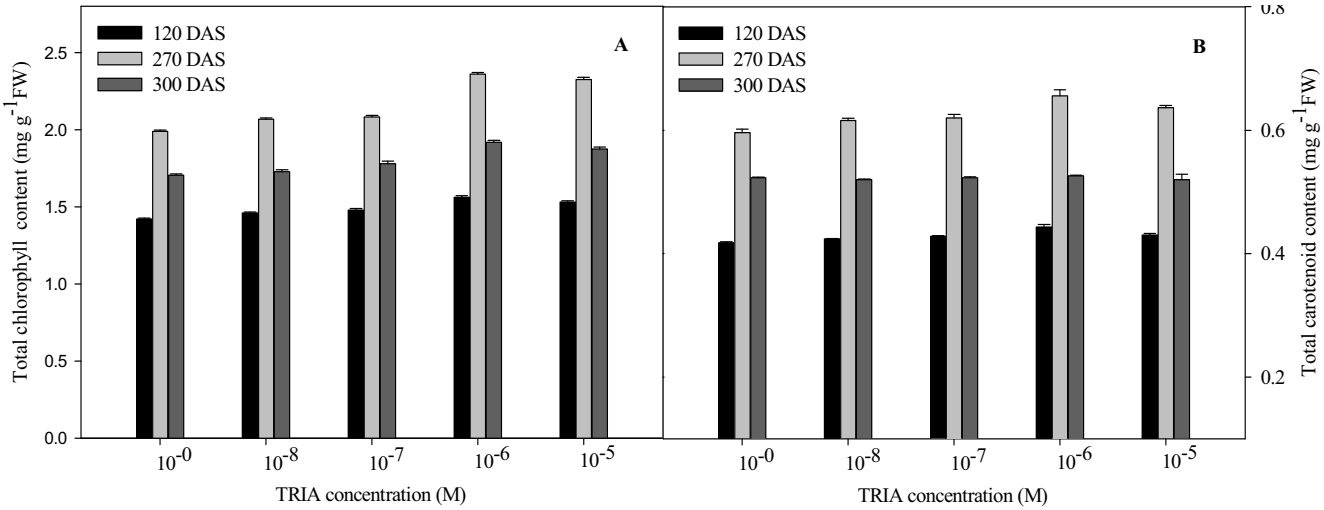

Fig. 3 Effect of five concentrations of TRIA (10^{-0}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M) on total Chl (**A**) and carotenoid content (**B**) of coffee senna studied at 120, 270 and 300 DAS (Means of three replicates). Error bars (-) show SE.

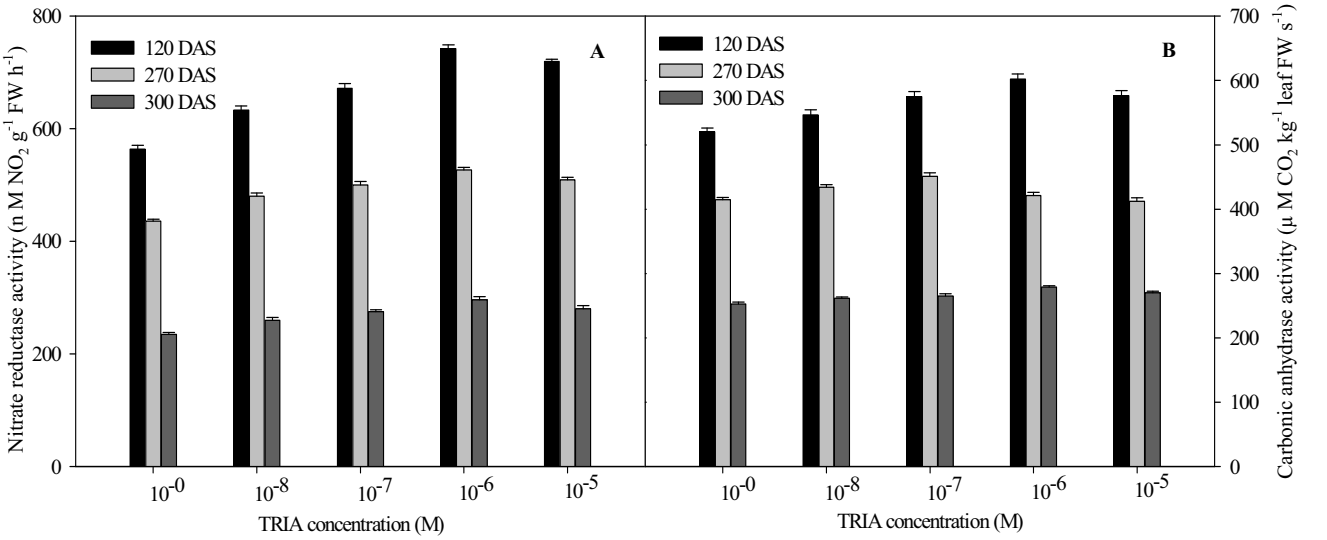

Fig. 4 Effect of five concentrations of TRIA (10^{-0}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M) on nitrate reductase activity (**A**) and carbonic anhydrase activity (**B**) of coffee senna studied at 120, 270 and 300 DAS (Means of three replicates). Error bars (-) show SE.

result was found non-significant (**Fig. 3**). TRIA at 10^{-6} M accelerated the rate of photosynthesis, transpiration and stomatal conductance by 14.7, 13.8 and 19.6% at 270 DAS compared to non sprayed TRIA plants (**Fig. 2**). A higher transpiration rate was noted in TRIA treated plants could be expected as transpiration is known to depend on stomatal

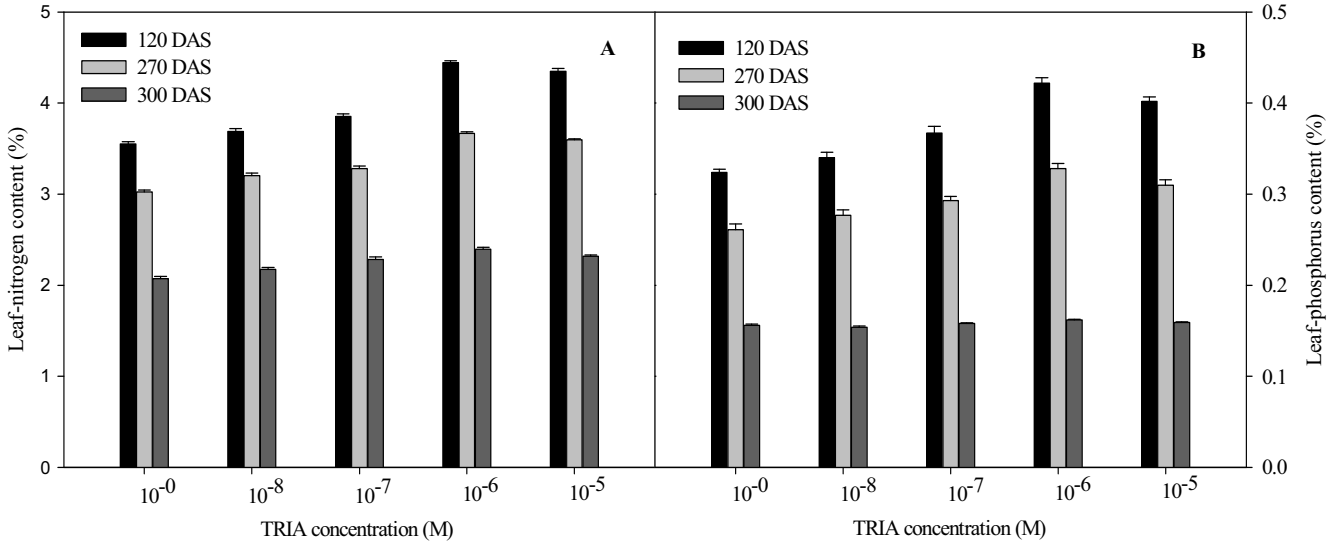

Fig. 5. Effect of five concentrations of TRIA (10^{-0}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M) on leaf-nitrogen and -phosphorus content (**A, B**) of coffee senna studied at 120, 270 and 300 DAS (Means of three replicates). Error bars (-) show SE.

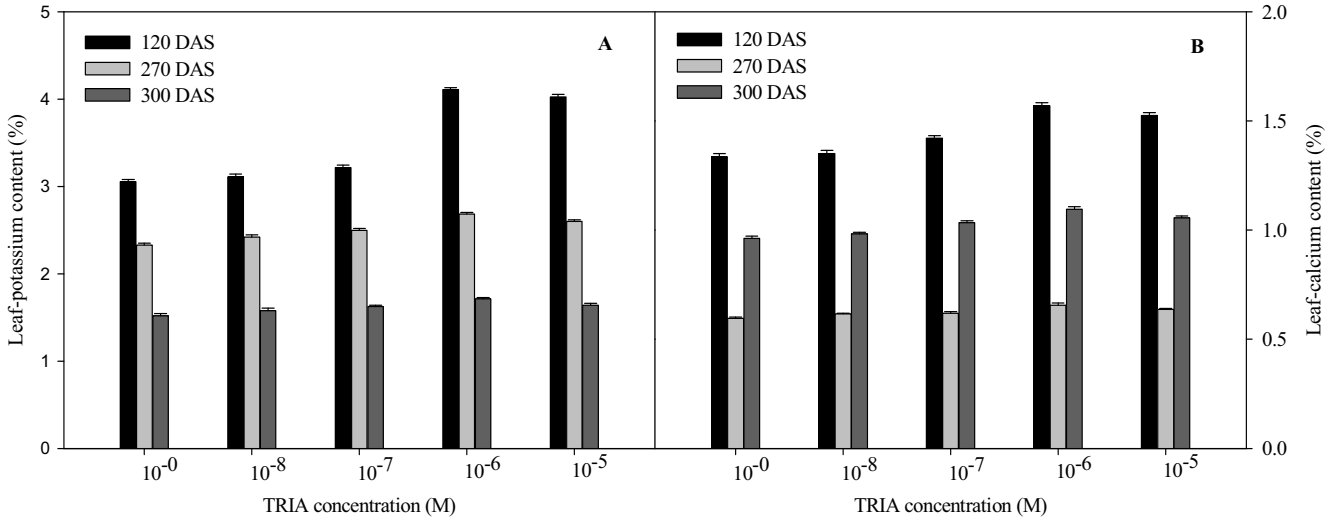

Fig. 6 Effect of five concentrations of TRIA (10^{-0}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M) on leaf -potassium and -calcium content (**A, B**) of coffee senna studied at 120, 270 and 300 DAS (Means of three replicates). Error bars (-) show SE.

conductance (Jarvis and Davies 1998). Since the stomatal conductance exhibited considerable improvement in the TRIA sprayed plants, a significant increase in the rate of transpiration was quite obvious. Improvement in photosynthesis has already been reported as an important response to TRIA, which in turn could be associated with increased chlorophyll content in leaves (Ivanov and Angelov 1997; Naeem *et al.* 2009). Earlier studies have demonstrated the increased rate of CO_2 fixation and photosynthesis in the plants by the application of TRIA (Srivastava and Sharma 1990; Misra and Srivastava 1991; Ivanov and Angelov 1997; Kumaravelu *et al*. 2000; Chen *et al*. 2003; Muthuchelian *et al.* 2003; Naeem *et al*. 2009).

The application of TRIA enhanced photosynthetic pigments significantly. The spray of 10^{-6} M TRIA caused a significant increase in the total chlorophyll and carotenoid content by 9.92 and 5.98%, respectively (**Fig. 3**). However, the values decreased for all the physiological and biochemical attributes when TRIA concentrations higher than 10^{-6} M were used. The increased content of the pigments in TRIA treated plants might be attributed to the increase in the number and size of chloroplasts as revealed by Ivano and Angelov (1997), Chen *et al.* (2003) and Muthuchelian *et al.* (2003).

The TRIA concentration of 10^{-6} M proved optimum for NR activity and an increase of 31.7% over the control was observed at 120 DAS (**Fig. 4**). A spray of 10^{-6} M TRIA concentration caused a significant increase in NR activity at 120, 270 and 300 DAS, whereas, 10^{-5} M TRIA showed negative response as compared with 10^{-6} M concentration of TRIA. The significant effect of TRIA on NR activity has also been reported by Ries and Houtz (1983), Srivastava and Sharma (1990), Misra and Srivastava (1991), Kumaravelu *et al.* (2000) and Muthuchelian *et al.* (2003) and Naeem *et al*. (2009). Furthermore, TRIA concentration (10^{-6} M) significantly increased the leaf-N concentration (**Fig. 5**) and may have increased the capacity of leaves to assimilate more amounts of nitrogen with nitrate reductase activity.

Application of 10^{-6} M TRIA exhibited highest CA activity in the leaves among all studied treatments (**Fig. 4**). TRIA increased the stomatal conductance (**Fig. 2**) that might have facilitated the diffusion of carbon dioxide into the stomata. In turn, the CO_2 might have been acted upon by CA. Finally, the CO_2 could be reduced by ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) in the chloroplast stroma. A probable reason for the enhancement of CA activity due to TRIA application might be the *de novo* synthesis of CA, which involves translation/transcription of the genes associated (Okabe *et al.* 1980).

TRIA application was effective for increasing leaf -N, -P, -K and -Ca content at most of the growth stages of the plant. The TRIA at 10^{-6} M elevated the leaf-N, -P, -K and Ca

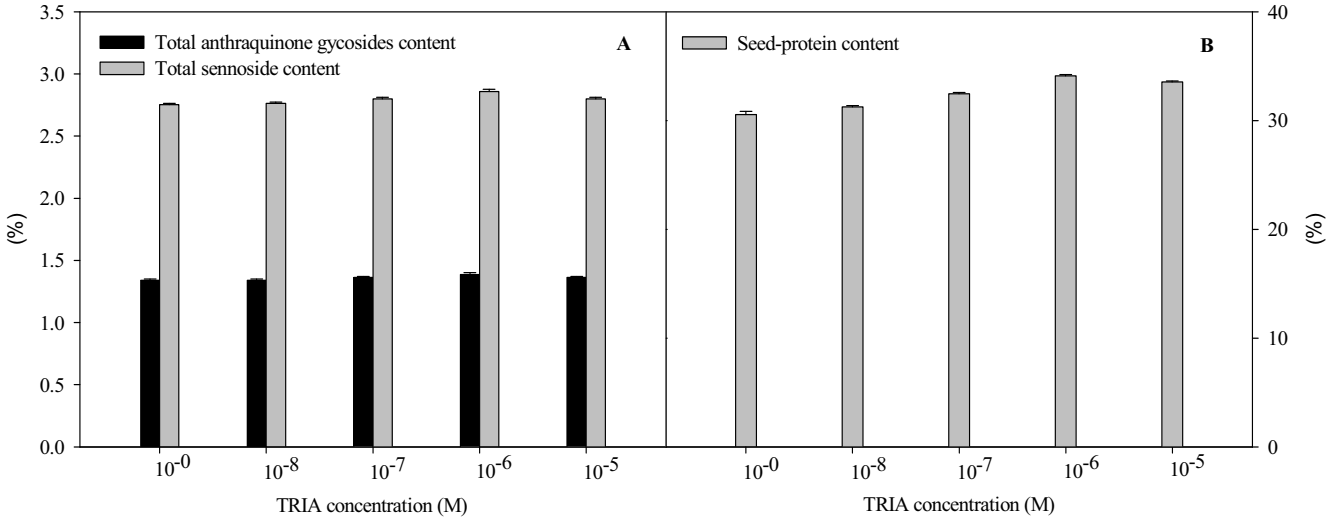

Fig. 7 Effect of five concentrations of TRIA (10^{-0}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M) on total anthraquinone glycosides content and sennoside content (**A**), and on seed-protein content (**B**) of coffee senna studied at 300 DAS (Means of three replicates). Error bars (-) show SE.

content by 25.1, 30.2, 34.6 and 17.4% at 120 DAS, respectively (**Fig. 5, 6**). The Control plants possessed the minimum values at all respective growth stages. A higher content of leaf -N, -P, -K and -Ca content in TRIA treated plants could be attributed to the higher metabolic activity and increased dry matter production that might have resulted in enhanced water and nutrient uptake from soil (Sharma *et al.* 2002), followed by smooth translocation of photosynthates and other metabolites to the sinks that might have contributed to the improved yield of TRIA treated plants.

Furthermore, TRIA mediates activation of a number of membrane bound enzymes (Ries and Houtz 1983; Savithiry *et al.* 1992). The stimulation of these enzymes leads to dephosphorylation of L (+) forms of AMP, ADP and ATP, resulting in the formation of 9-β-L(+) adenosine, which triggers a cascade of events leading to rapid physiological responses (Ries *et al*. 1990; Ries 1991). The present findings are in accordance with TRIA effects regarding plant nutrient elements (Knowles and Ries 1981; Ries and Houtz 1983; Kumaravelu *et al*. 2000; Chaudhary *et al.* 2006; Khan *et al*. 2006, Khan *et al.* 2007, Naeem *et al.* 2009).

Quality attributes

TRIA spray appreciably increased seed-protein content compared to the control. The TRIA concentration at 10^{-6} M proved the best and increased the seed-protein content by 16.4% as compared to control (**Fig. 7**). The increase in protein content by TRIA application might be ascribed to increased nitrogen content in leaves that might have promoted amino acid synthesis leading to the improved protein content in the seed. These results coincide with those obtained by Knowles and Ries (1981), Ries and Houtz (1983), Kumaravelu *et al.* (2000), Muthuchelian *et al.* (2003) and Naeem *et al.* (2009).

TRIA spray was also effective in increasing anthraquinone content as compared to the control plants. The TRIA concentration (10^{-6} M) proved the best and improves the production of anthraquinone glycosides and sennoside contents (**Fig. 7**). Anthraquinone compounds in plants occur as glycosides with one or more sugar molecules. They act as stimulant cathartics and increase the tone of the smooth muscle in the wall of the large intestine. They also participate in the processes of metabolism, respiration, division of cells, oxidative phosphorylation, complexation with DNA and RNA, and perhaps, in other physiological processes of vital importance. In higher plants, anthraquinones are present in oxidized, reduced, glycoside and condensed forms. Furthermore, anthraquinones are used as dyes, pigments, analytical reagents and chemical means for plant protection (Thomson 1987, 1996; Muzychkina 1998).

On the other hand, sennosides form an important source of organic laxatives. It appears that an increase in sennoside content was dependent on TRIA triggering certain enzymes which are involved in biosynthesis of these compounds. An increase in the total sennoside content indicates a diversion of primary metabolites to secondary metabolites (Bilia *et al.* 1992). The previous findings suggested that application of various PGRs improved the sennoside content (Bhatia *et al.* 1980).

In conclusion, application of TRIA improved significantly crop productivity, physiological activities and quality attributes of coffee senna at the 10^{-6} M concentration. Thus, 10^{-6} M TRIA concentration, as a foliar spray, might be recommended for maximizing the productivity and quality attributes (anthraquinone and sennoside content) of this medicinal herb under field conditions.

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