

ROLE OF MICRONUTRIENTS FOR IMPROVING YIELD AND SENNOSIDE CONTENTS IN TRADITIONAL MEDICINAL PLANT *CASSIA ANGUSTIFOLIA* VAHL.

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ABSTRACT

The uses of micronutrients have emerged as an important tool in improving agricultural production. The study was conducted to compare the effects of three micronutrients on the yield parameters and sennoside (a and b) contents in leaves and pods of medicinal plant *Cassia angustifolia*. The micronutrients solutions of CuSO₄, FeSO₄, ZnSO₄ (500, 1000, 1500 mg/l) and commercial micronutrient mixture, Miczink, (0.50, 0.75, 1 ml/l) were applied foliarly to the seedlings of 45 DAS. The treatment was continued upto flowering stage at the interval of 15 days. DW spread plants were considered as control for treatments of micronutrients. Performances of different micronutrients in *C. angustifolia* indicated that all the micronutrients used have caused statistically significant increase in yield attributes and sennoside contents over control. Amongst the different treatments of micronutrients FeSO₄, ZnSO₄ (1500 mg/l) and Miczink (1 ml/l), had shown maximum positive influence on yield parameters in *C. angustifolia*. While for enhancing sennoside a and b contents from leaves and pods, CuSO₄ (1000 and 1500 mg/l) emerged best treatment over control.

KEY WORDS: *Cassia angustifolia*, Foliar spray, HPLC, Micronutrients, Sennoside, Yield Attributes.

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INTRODUCTION

Different strategies like, use of dormancy breaking agrochemicals, proper irrigation and fertilizers, hybrid seeds, micronutrients etc. are generally used to achieve vigorous growth and to enhance the flowering, fruiting, yield and production of commercially important secondary metabolites. Amongst these, use of micronutrients is proven and widely used technique for different crops including medicinal plant to achieve these objectives.

The term micronutrients include those of the elements such as As, Cd, Co, Cu, Fe, Hg, Mn, Ni, Se, Zn etc which constitute about 1% of the total elemental content of the soil are called trace elements. Trace elements (micronutrients that have been studied most extensively and that are essential for the nutrition of higher plants i.e. B, Cu, Fe, Mn, Mo and Zn. The use of micronutrients have emerged as an important tool in improving agricultural production and to help in removing many of the barriers imposed by heredity and/or environmental stress. Hence the study was conducted to compare the effects of three micronutrients on the yield parameters and sennoside (a and b) contents in leaves and pods of

medicinal plant *Cassia angustifolia* under natural condition.

Cassia angustifolia (Family: Caesalpiniaceae) (Fig. 1.a), popularly known as senna, is a valuable plant drug in ayurvedic and modern system of medicine for the treatment of constipation and is used to cure a large number of intestinal diseases¹. Sennoside A and B (Fig. 2 b.) were the two anthraquinone glycosides that are responsible for purgative action of senna. Senna leaves and pods used in the preparation of Cathartic and Purgative drug. Senna is valued in medicine for its cathartic properties, especially in habitual constipation. The drug which induces the defecation is called cathartic drugs. A number of terms are used to designate the cathartic drugs. The commonly employed words are Laxative, Cathartic and Purgative. Sennoside is mild and safe cathartic among the anthraquinone group. However, the performance of this plant under foliar application of micronutrients and the effect of these micronutrients on its yield attribute and sennoside production have not been studied well. The objective of this research was to evaluate the effect of foliar application of micronutrients on yield parameters and sennoside contents of senna

MATERIAL AND METHODS

Plant Material

Authentic seeds of *Cassia angustifolia* Vahl were obtained from National Research Centre for Medicinal and Aromatic Plants, Boriavi, Gujarat.

Field Experimental

Field culture experiments were conducted in the research field of Department of Botany, University of Pune, from October 2008 to May 2009 to determine the effect of foliar application of micronutrients on the, yield parameters and secondary metabolites content i.e. sennoside a and b in leaves and pods of senna. All experiments were conducted in a complete randomized design. The pre-soaked seeds of senna were sown in ridges and furrows cover with thin layer of soil. The distance between two plants was 30 cm and the distance between two rows was 40 cm. Each treatment had 3 replications and each replication had 10 seedlings. Light irrigation was immediately given after seed sowing. Standard inter cultivation practices were used throughout the experiment. Temperatures during the experiment were in the range of 28-30°C during day and 19-21°C at night.

Micronutrients Treatments

The plants were sprayed four times at 45, 60, 75 and 90 days after sowing with freshly prepared solutions of CuSO₄, FeSO₄ and ZnSO₄ (500, 1000, 1500 mg/l) Miczink, (0.50, 0.75, 1 ml/l) using 1.5, 2.5, 3.5 and 4.5 liter of solution respectively for each experimental plot. All the chemicals used were of AR grade. The treatment was continued up to flowering stage (90DAS) at the interval of 15 days. DW spread plants were considered as control.

Yield Parameters Analysis

Yield attributes like number of flowers per plant, number of pods per plant, pods fresh and dry weight, 100 seed weight were measured. Pod formation began at 90 DAS and therefore sampling of pods was done at 105 and 120 DAS. The fresh and dry weight of the whole plant was measured at post flowering stage (120DAS). For this randomly selected healthy plants were uprooted from each treatment and control at the stages mentioned above. After cleaning the roots fresh weight of ten plants was measured immediately with the help of electronic top pan balance and expressed in gram per plant and from this average fresh weight of each plant was determined. This plant after taking the fresh weight was kept in oven at 40°C still the constant weight was obtained. The constant weight was recorded as dry weight of each plant.

For analysis of sennoside a and b contents ten randomly selected third leaf from top of each plants from control

and treatment was selected at 60 DAS and green pods at 120 DAS while, kept in oven at 60°C for 48 h and used for HPLC analysis.

HPLC Analysis of Sennoside Contents

HPLC analysis was done at 60DAS from leaves and 120DAS from pods. All the chemicals used were of AR grade. Methanol and water were obtained from Merck (Mumbai). The standard for the experiment was obtained from Mehta Pharmaceuticals Pvt. Ltd. (Mumbai). The dried leaves (1.0 gm.) were finely powered and extracted with hexane (3 X 25 mL). The hexane extract was discarded and 25 ml of methanol:water (70:30, v/v) was added to mark, the suspension left overnight at room temperature (25°C) and then extracted with the methanol:water mixture (2 X 25 mL). The extract was made up to 100mL with methanol: water and 10 µL samples were subjected to HPLC analysis. The extraction of pods was performed exactly as described above for leaf sample. The solution containing known concentration of range 10-100 µg/ml of sennoside was prepared in methanol, and used as standard for HPLC analysis.

HPLC analysis was performed using a Water modular system consisting of two model 501 pumps, an automated gradient controller, a model U6K injector, an in-line solvent degasser, a model 996 photodiode array detector and Millennium 2010 chromatography management software. A Symmetry C₁₈ column (150 X 406mm) was used for analysis, and spectral acquisition was performed at 285nm after scanning the standards. The solvent system consist of (A) methanol: water: acetic acid (80:20:0.1v/v/v; pH 4.0). The flow rate was maintained at 0.6ml/min for the first 20 min, while at 30 min it was 1.0ml/min. The HPLC data including height and area of the sennoside peak at particular retention time, of standard sennoside sample (with known concentration) was recorded. This data of standard sennoside sample was compared with the data obtained from plant samples and content of sennoside in the respective plant samples was calculated.

Statistical Analysis

The data was presented as arithmetic means of three replicates ±standard deviation. The significance of the mean differences was explored through one-way-ANOVA statistics followed by DMRT (Duncan's multiple range test) at p=0.05 as a post hoc test. SPSS for Windows ver. 11.5 and Microsoft Excel 2003 were used to carry out statistical analyses and graphical data presentations.

RESULTS AND DISCUSSION

Number Of Flowers

The influence of different micronutrients on various yield parameters in *Cassia angustifolia* clearly indicated that the yield parameters had shown positive increase over control. The increase in number of flowers per plant was maximum in FeSO₄ 1500mg/l (43.26%) recorded in Fig2. The next better treatment was ZnSO₄ 1500mg/l (39.24%) followed by Miczink 1ml/l (34.21%). However the treatment of CuSO₄ 1500mg/l was comparatively less effective (22.50%). Total number of flowers present on a plant is one of the important yields contributing character in various plants. The correlation between number of flowers per plant and economic yield has been well established.

Number Of Pods

The impact of various micronutrients on number of pods per plant recorded in Fig 2, illustrated that all the treatments had favoured the positive increase in number of pods per plant over control. The percentage increase was ranging from 14.29% to 40.57%. The impact of FeSO₄ (1500mg/l) was highly significant, as it has caused maximum increase in number of pods per plant by 40.57%. Amongst the remaining treatments ZnSO₄ (1500mg/g), Miczink (1ml/l) and CuSO₄ (1500mg/l) were following the previous one. They have caused 38.11%, 28.37% and 20.19% increase in number of pods per plant over control. Application of Zn significantly increased the number of mature pods per in *Phaseolus vulgaris*^{2,3}.

Pods Fresh Weight

The results on pods fresh weight per plant recorded in Fig 3 revealed that all micronutrients had positively influenced the pods fresh weight per plant over control. The percentage increase over control was in the range of 9.24% to 37.26%. The maximum increase in pods fresh weight was 37.26% in ZnSO₄ 1500mg/l. The next better treatment was FeSO₄ 1500mg/l, which had caused 35.24%, increase in pod fresh weight. The Miczink 1ml/l and CuSO₄ 1500mg/l had caused 33.21% and 16.23% increase over control respectively.

Pods Dry Weight

The impact of various micronutrients on dry weight of pods per plant recorded in Fig 3 revealed that all micronutrients used in the present studies had increased the fresh as well as dry weight of pods per plant. However the increase in yield was more significant in dry weight of pods. The increase in dry weight during field experiment was 30.49% in ZnSO₄ 1500mg/l. It was the highest increase over control and other treatments. While FeSO₄ 1500 mg/l has caused 28.41% increase in pod dry weight and emerged as second better treatment.

Even the performance of Miczink (1ml/l) was very promising over control, because it has also caused the positive increase in pods dry weight by 27.52% over control followed by CuSO₄(1500mg/l) 11.63%.

Dry Weight Of Seeds

The influence of various micronutrients on 100 seed's dry weight presented in Fig 4 revealed that the 100 seeds dry weight was positively increased by all the treatments of micronutrients over the control. The percentage increase in dry weight of seeds was in the range of 15.39% to 32.53%. The treatment of ZnSO₄ (1500mg/l) emerged as the best treatment for enhancing dry weight of seeds (32.53%). It was followed by FeSO₄ 1500 mg/l (29.56%), Miczink 1ml/l (26.53%), CuSO₄ 1500mg/l (21.34%). These results were similar with the findings of Zaiter *et al*⁴ and Elballal *et al*³, who concluded that trace elements increased 1000-seed weight in snap beans. Application of Fe and Zn resulted in higher yield of seed in *Pimpinella anisum*⁵.

Fresh Weight Per Plant

The effects of micronutrients treatment on fresh weight per plant for field grown plants of *Cassia angustifolia* are presented in Fig 5. From the results it was clearly seen that all the micronutrients used in the present investigation had significantly and positively caused the enhancement in fresh biomass of treated plants over control. The highest increase in fresh weight per plant was recorded in ZnSO₄ 1500 mg/l. (30%). The next best treatment was Miczink 1 ml/l (26.2 %). The remaining two treatments i.e., FeSO₄ and CuSO₄ with 1500mg/l registered 22.6 % and 11.3% increase over control respectively. Higher supplies of Zn are beneficial for higher biomass production in medicinal herb *Cineraria maritime*⁶ while Zn application increased biomass production in *Jasminum grandiflorum*⁷. Foliar spray of Fe increased growth and yield of *Ambrosia maritima*⁸ plants.

Dry Weight Per Plant

The results on dry weight per plant influenced by various micronutrients treatments during field experiments in *Cassia angustifolia* presented in Fig 5 revealed that dry weight per plant was increased in the range of 3.3% to 19.7% in field experiment. ZnSO₄ 1500mg/l was more effective than other treatments and control for causing the accumulation of dry matter (19.7%); it was followed by Miczink 1ml/l (15.3%) and FeSO₄ 1500mg/l (13.9%). The treatment of CuSO₄ (1500mg/l) was less effective as compared to other treatments (7.3%). Application of micronutrient resulted in a considerable increase in all yield attributes of snap bean^{4,9}. While application of Fe significantly increased the yield of pigeonpea compared to control^{10,11}.

Sennoside Contents

From the figure 5 and 6; it is seen that all the micronutrients treatments resulted in to increased sennoside content as compared to control. Amongst all the treatments, CuSO₄ with 1500mg/l (26.59% and 29.14%) had a pronounced effect on the sennoside content from leaves and pods respectively which was followed by CuSO₄ at 1000mg/l (21.58% and 23.58%) and 500mg/l (14.26 and 17.26%). Next effective treatment for stimulate the sennoside content was FeSO₄ 1500mg/l which caused increase by 8.02% and 13.26% in leaves and pods respectively. The treatments of Miczink and ZnSO₄ were also quite effective over control for enhancing the sennoside content (Figure No.6 and 7).

The overall results on various yield parameters such as number of flowers per plant, number of pods per plant, fresh and dry weight of pods and even dry weight of seeds were positively influenced by the treatments of micronutrients such as CuSO₄, FeSO₄, ZnSO₄ and Miczink. Amongst the different treatments of micronutrients, FeSO₄, ZnSO₄ and Miczink had shown maximum positive influence on yield parameters in *Cassia angustifolia*. While for enhancing sennoside content from leaves and pods, CuSO₄ with all three concentrations emerged best treatments over other micronutrients and control.

Fe treatment increased the gentiopicroside content by 4.0% in *Gentiana manshurica*¹². While Cu was to be more effective than Zn for enhancing the yield of lepidine content in *Lepidium sativum*¹³. Application of Cu and Fe increased menthol yield by 44 and 35% respectively in *Japanese mint*¹⁴.

The comparative account regarding the performance of different micronutrients in *C. angustifolia* indicated that all the micronutrients used have caused statistically significant increase in yield and sennoside content over control, but the effect of CuSO₄ and FeSO₄ was more significant and hence use of these micronutrients can be recommended for commercial purpose after studying the cost benefit ratio. All the results on yield parameters and sennoside content were showing positive and significant correlations.

Increased yield due to micronutrients application may be attributed to enhanced photosynthetic activity and increased production and accumulation of carbohydrates and favourable effect on vegetative growth and retention of flowers and fruits, which increased number of fruits per plant besides increasing the size¹⁵.

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Figure 1.a *Cassia angustifolia* field grown plant.

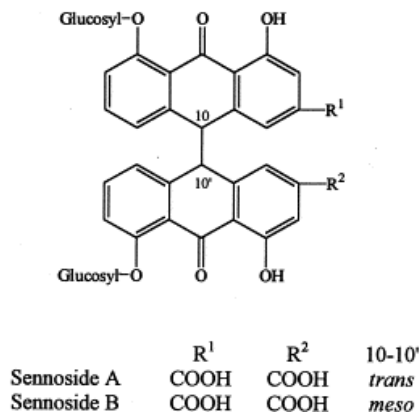


Figure 1. b sennoside structure

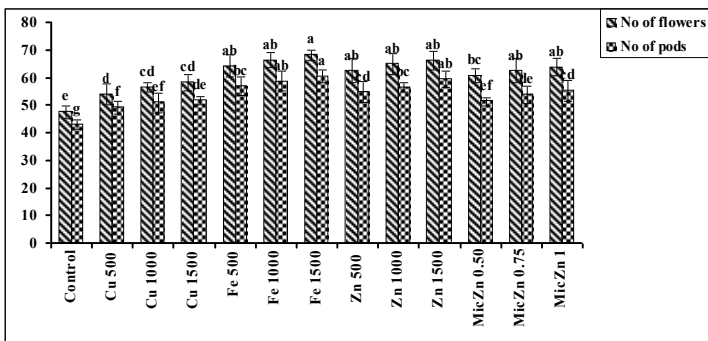


Fig.2. Effect of micronutrients on number of flowers and pods per plant of *Cassia angustifolia*

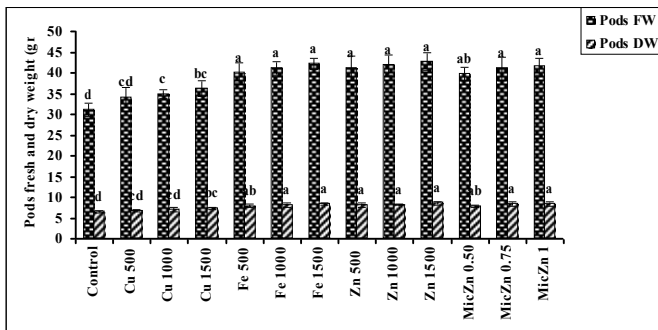


Fig. 3. Effect of micronutrients on pods fresh and dry weight per plant of *Cassia angustifolia*

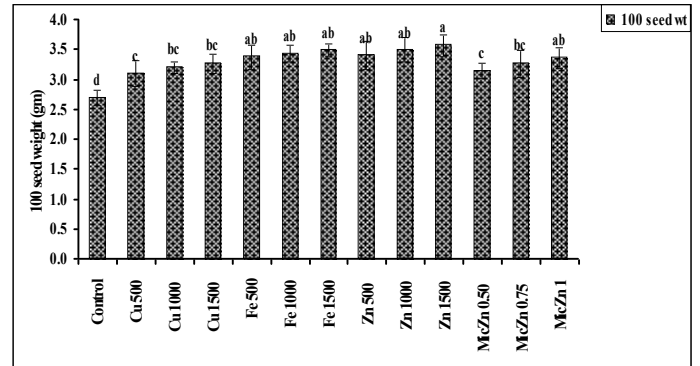


Fig.4 Effect of micronutrients on 100 seed weight of *Cassia angustifolia*

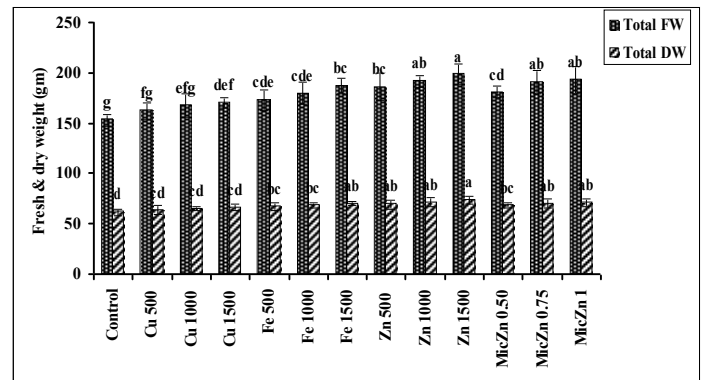


Fig.5 Effect of micronutrients on total fresh and dry weight of *Cassia angustifolia*

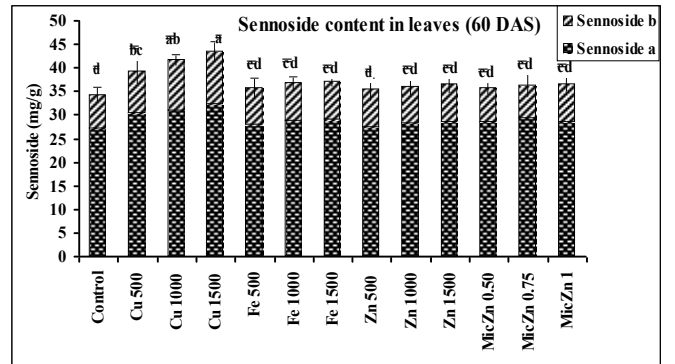


Fig.6 Effect of micronutrients on sennoside content a and b from leaves (60 DAS) of *Cassia angustifolia*

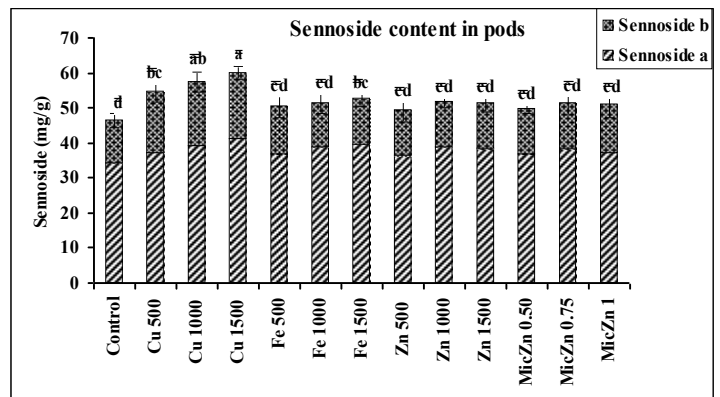


Fig. 7 Effect of micronutrients on sennoside content a and b in pods of *Cassia angustifolia*

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