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Effect of Zinc and Boron Application on Leaf Area, Photosynthetic Pigments, Stomatal Number and Yield of Cashew

Lakshmipathi*, J.D. Adiga, D. Kalaivanan, B.M. Muralidhara and P. Preethi

ICAR-Directorate of Cashew Research, Puttur-574 202, Karnataka, India

**Corresponding author*

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A B S T R A C T

An experiment was conducted to study the effect of exogenous application of boron and zinc at different concentrations at three important growth stages (flushing, flowering and fruiting) on leaf area, chlorophyll content, carotenoids, stomatal count and yield of cashew var. Bhaskara. Irrespective of growth stages, foliar application of zinc sulphate (0.5%) + borax (0.1%) was found to be superior in all the parameters. Out of six treatments, the highest chlorophyll content (chl a, chl b and chl t), carotenoids and leaf area were recorded in trees sprayed with zinc sulphate (0.5%) + borax (0.1%). At flushing stage, spraying with zinc sulphate (0.5%) + borax (0.1%) resulted in highest stomatal number (31.05) whereas unsprayed (control) trees recorded least stomatal number (12.18). Thus, leaf area, chlorophyll content, carotenoids and stomatal count were increased in trees sprayed with above combination of micronutrients than unsprayed trees. Spraying of zinc sulphate (0.5%) + borax (0.1%) recorded highest nut yield (10.80 kg/tree), whereas unsprayed trees recorded least nut yield (3.20 kg/tree). This study demonstrated the potential of zinc sulphate (0.5%) + borax (0.1%) in improving various biochemical parameters *viz.*, chlorophyll 'a', chlorophyll 'b', total chlorophyll, carotenoids and leaf area in cashew which are important determinants in increasing cashew nut production.

Introduction

Cashew (*Anacardium occidentale* L.) belongs to the family Anacardiaceae and is native to Brazil. It was introduced into India by Portuguese travellers in the 16th century for afforestation and soil conservation. Cashew is grown in India, Brazil, Vietnam, Tanzania, Mozambique, Indonesia, Sri Lanka and other tropical Asian and African Countries. India was the first country to exploit the international trade of cashew kernels in the early part of 20th Century (Chavan and Raut, 2013). It earns valuable foreign exchange for the country and is therefore regarded as a "gold mine" (Adiga and Kalaivanan, 2013). Tree nuts are globally consumed for their desirable sensory and nutritional attributes. Among the dry fruits, cashew nuts are very popular due to their characteristic odour and taste. Cashew nuts are a good source of proteins (20%), carbohydrates (23%) and fats (45%) (Bhattacharjee *et al.*, 2003). The global production of cashew is around 37, 13, 467 MT from a total of 60, 37, 313 hectares (FAO, 2014). India is the second largest producer of raw cashew nut in the world. India produces about 0.78 million MT of cashew from an area of 1.04 million hectares with a productivity of 0.75 MT/ha (DCCD, 2017).

Pigments in general and chlorophyll in particular are most important for green plants to effectively harvest solar radiation and to convert it into chemical energy. Chlorophyll 'a' plays an important primary role in the photosynthetic process. The status of chlorophyll pigments in the leaf tissue is thus a major determinant of overall photosynthetic efficiency of the plants. The photosynthetic efficiency directly influences the growth, development and yield of the crops. The carotenoids, are organic pigments and act as passive light filters that would reduce light intercepted by chlorophyll (Williams *et al.*, 2003) and thereby provide protection from reactive oxygen species (ROS) (Steyn *et al.*, 2002). Prolonged flowering, poor production of hermaphrodite flowers, low fruit set and premature fruit drop are some major problems plaguing cashew cultivation across the country (Bhat *et al.*, 2010). Cashew productivity can be improved through adoption of appropriate nutrient management regime. Micronutrients deficiency in soil and plants is a worldwide nutritional problem and very severe in many countries (Alloway, 2008; Mousavi *et al.*, 2007). Plants vary in their demand for micronutrients, as these are involved in almost all physiological functions. Some of these elements are redox-active and are cofactors in many enzymes. They have enzyme-activating functions and play structural role in stabilizing proteins (Hänsch and Mendel, 2009).

Numerous nutritional trials, especially those involving the major plant nutrients have been undertaken in India as well as in other tropical countries. However, the influence of micronutrients *viz*., zinc and boron on cashew productivity has been poorly explored. Foliar application of micronutrients can be resorted for improving growth, photosynthesis, flowering and fruiting in cashew. Hence, it is worthwhile to find out the effect of foliar application of zinc and boron on the concentration of pigments like chlorophyll 'a' & 'b' during the plant growth as it is vital for photosynthesis. Hence, an investigation was taken up to study the influence of foliar application of zinc and boron on leaf area, photosynthetic pigments and stomatal density in cashew.

Materials and Methods

The experiment was conducted at ICAR - Directorate of Cashew Research, Puttur, Karnataka. The experimental site situated in a cashew growing belt, has typical lateritic soils of the west coast, located 87 m above mean sea level with latitude of 12.77° N and longitude of 75.22° E. The climate is hot and humid throughout the year with an average annual rainfall of 3,500 mm, distributed mainly from June to September. The mean annual temperature was 27.6 ^{o}C and mean maximum and minimum temperature were 36 ^{o}C and 20 ^{o}C, respectively. The study was carried out on 10 years old plantation (variety Bhaskara) by adopting Randomized Block Design (RBD) with 6 treatments and 4 replications. The details of the treatments were control (T_1), borax (0.1%) (T_2), borax (0.2%) (T_3), zinc sulphate (0.5%) (T_4), zinc sulphate (0.5%) + borax (0.1%) (T_5), zinc sulphate (0.5%) + borax (0.2%) (T_6). The micronutrients were sprayed during flushing, flowering and fruiting stage using foot pump paddle sprayer covering the entire canopy. Leaf area was measured using CI-202 Portable Laser Area Meter.

Leaf chlorophyll content

Chlorophyll 'a', chlorophyll 'b' and total chlorophyll content of the leaves were measured following the method of Arnon

(1949) and carotenoids by Goodwin's method (1954). One gram leaf sample was taken and chlorophyll was extracted in 80 per cent acetone by grinding in clean mortar. The resulting green liquid was filtered through whatman No. 1 filter paper. The grinding and filtration were repeated 3-4 times for each sample with fresh aliquots of 80 per cent acetone for ensuring the complete extraction of chlorophyll. The final volume was made to 100 ml with 80 per cent acetone. The absorbance of chlorophyll was recorded with spectrophotometer (Spectronic 20) at 645 and 663 nm against 80 per cent acetone solvent as blank. The entire procedure was carried out in a dark room to avoid the loss of chlorophyll with direct contact of light. Chlorophyll 'a' and chlorophyll 'b' contents were calculated. In contrast to the chlorophylls, which absorb light in two regions of the visible spectrum, the carotenoids exhibit intense absorption only in 350-500 nm.

Calculations

Arnon's equation to convert absorbance measurements to mg Chl g^{-1} leaf tissue is given below.

Chl a (mg g^{-1}) = [(12.7 × A663) - (2.6 × A645)] × ml acetone / mg leaf tissue

Chl b (mg g^{-1}) = [(22.9 × A645) - (4.68 × A663)] × ml acetone / mg leaf tissue

Total Chl = Chl a + Chl b.

C_{x+c} = 1000 A470 – 1.90 Chl a – 63.14 Chl b/214, (x = xanthophylls and c = carotenes)

Stomatal frequency

Stomata counts were recorded from the mature leaves according to the methods described by Beakbane and Mujumder (1975). The stomatal density was recorded at 10 x 100 magnification field. On each leaf film, three microscopic fields were examined and average was worked out.

Statistical analysis

Data generated from the experimental plots were analyzed using SAS 9.3 version of the statistical package (SAS Institute Inc, 2011). Analysis of variance (ANOVA) was performed using SAS PROC ANOVA procedure. Means were separated using Fisher's protected least significant difference (LSD) test at a probability level of $p<0.05$.

Results and Discussion

Leaf chlorophyll content

Chlorophyll a

Foliar application of micronutrients (Zn and B) significantly affected chlorophyll content (Table 1). At flushing stage, spraying of $ZnSO_4$ (0.5%) + borax (0.1%) recorded highest chlorophyll 'a' (1.58) content followed by borax (0.1%) (1.51), $ZnSO_4$ (0.5%) (1.44), $ZnSO_4$ (0.5%) + borax (0.2%) (1.37) and borax (0.2%) (1.01), while unsprayed trees (control) recorded least chlorophyll value (0.73).

Application of $ZnSO_4$ (0.5%) + borax (0.1%) recorded highest chlorophyll 'a' (1.44) content and unsprayed trees (control) recorded least value (0.64) at flowering stage. At fruiting stage also, spraying of $ZnSO_4$ (0.5%) + borax (0.1%) resulted in highest chlorophyll 'a' (1.25) while, unsprayed trees (control) recorded least value (0.37). The chlorophyll content increased gradually with leaf expansion in cashew due to spraying of Zn and B (Balasimha, 1991). Zinc is important for the formation and activity of chlorophyll and in the functioning of several enzymes and the growth hormone (auxin).

Chlorophyll b

At flushing stage, spraying of $ZnSO_4$ (0.5%) + borax (0.1%) resulted in highest Chlorophyll 'b' (0.54) followed by borax (0.1%) (0.50), $ZnSO_4$ (0.5%) (0.49), $ZnSO_4$ (0.5%) + borax (0.2%) (0.46), borax (0.2%) (0.34), while unsprayed trees (control) recorded least value (0.24) (Table 1). At flowering stage, spraying of $ZnSO_4$ (0.5%) + borax (0.1%) resulted in highest Chlorophyll 'b' (0.52) while unsprayed trees (control) recorded least value (0.21). At fruiting stage, spraying of $ZnSO_4$ (0.5%) + borax (0.1%) resulted in highest Chlorophyll 'a' (0.97) while, unsprayed trees (control) recorded least value (0.14).

Total chlorophyll

Effect of foliar application of micronutrients on total chlorophyll of cashew is presented in Table 1. At flushing stage, spraying of $ZnSO_4$ (0.5%) + borax (0.1%) resulted in highest total chlorophyll (2.12) while, unsprayed trees (control) recorded least value (0.97). At flowering stage, spraying of $ZnSO_4$ (0.5%) + borax (0.1%) resulted in highest total chlorophyll (2.78) while unsprayed trees (control) recorded least value (1.17). At fruiting stage, spraying of $ZnSO_4$ (0.5%) + borax (0.1%) resulted in highest total chlorophyll (2.21) while unsprayed trees (control) recorded least value (0.51).The chlorophyll content increased gradually with leaf expansion due to foliar spray of micronutrients (Balasimha, 1991 and Palanisamy *et al.,* 1993).

Chlorophyll a/b

Chlorophyll a/b ratio was not significantly influenced by foliar application of micronutrients at flushing and flowering stage (Table 1). However, at fruiting stage the highest chlorophyll a/b ratio (2.79) was observed in unsprayed trees (control) and lowest chlorophyll a/b ratio (1.27) was observed in trees sprayed with $ZnSO_4$ (0.5%) + borax (0.1%). Micronutrient deficiencies affect carbohydrate pools and photosynthesis. Reduction in chlorophyll content leads to chlorosis in leaves, ultimately affecting the chloroplast system and photosynthesis (Balakrishnan *et al.*, 2000).

Carotenoids

With respect to different micronutrients, spraying of $ZnSO_4$ (0.5%) + borax (0.1%) resulted in highest carotenoids (0.54) followed by borax (0.1%) (0.48), $ZnSO_4$ (0.5%) (0.41), $ZnSO_4$ (0.5%) + borax (0.2%) (0.32), borax (0.2%) (0.29), while, it was least in control (0.16) (Table 2). At flowering stage also, spraying of $ZnSO_4$ (0.5%) + borax (0.1%) recorded highest carotenoids (0.55) whereas unsprayed (control) trees recorded least (0.12) carotenoids. Highest (0.44) and lowest (0.10) carotenoids content was recorded with spraying of $ZnSO_4$ (0.5%) + borax (0.1%) and control (unsprayed trees) during fruiting stage, respectively.

In the present study chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoids were significantly improved by the foliar application of Zn and B. These attributes increased with application of $ZnSO_4$ @ 0.5%. Similar increase in Chl a, b, total and carotenoids was observed by Nahed *et al.,* (2007) in *Salvia farinacea* by the foliar application of Zn. Results are also in line with Massoud *et al.,* (2005) for pea plants and Farahat *et al.,* (2007) and Wenrong *et al.,* (2008) for *Cupressus sempervirens* observed that Zn deficiency resulted decline in leaf Chl content. Zinc application enhances the rate of photochemical reductions and Chl content in cucumber (Kazemi, 2013). In case of B the highest values for these attributes were recorded in plants sprayed with 0.1% Borax and decreased at 0.2% level of Borax.

Table.1 Effect of micronutrients on chlorophyll content in cashew variety Bhaskara

Treatment	Chl a mg/g			Chl b mg/g			Chl t mg/g			Chl a/b		
	Flush	Flow	Fruit	Flush	Flow	Fruit	Flush	Flow	Fruit	Flush	Flow	Fruit
Control	0.73^{E}	0.64^{E}	0.37^{D}	0.24^{D}	0.21^{D}	0.14^{F}	0.97^{E}	1.17^{E}	0.51^{F}	3.14	3.00	2.79^{A}
Borax (0.1%)	1.51^{B}	1.41^{AB}	1.22^{A}	0.50^{AB}	0.50^{A}	0.86^{B}	2.00^{B}	2.66^{AB}	2.08^{B}	3.06	2.83	1.38^{DE}
Borax (0.2%)	1.01^{D}	0.92^{D}	0.61^{C}	0.34^{C}	0.32^{C}	0.29^{E}	1.35^{D}	1.69^{D}	0.90^{E}	3.06	2.87	2.16^{BC}
$ZnSO_4$ (0.5%)	1.44^{BC}	1.37^{BC}	1.20^{A}	0.49^{AB}	0.39^{BC}	0.64^{C}	1.92^{BC}	2.59^{B}	1.81^{C}	3.04	3.60	1.89^{CD}
$ZnSO_4$ (0.5%) + borax (0.1%)	1.58^{A}	1.44^{A}	1.25^{A}	0.54^{A}	0.52^{A}	0.97^{A}	2.12^{A}	2.78^{A}	2.21^{A}	2.97	2.76	1.27^{E}
$ZnSO_4$ (0.5%) + borax (0.2%)	1.37^{C}	1.32^{C}	1.04^{B}	0.46^{B}	0.41^{B}	0.39^{D}	1.83^{C}	2.44^{C}	1.43^{D}	2.98	3.20	2.69^{AB}
General mean	1.27	1.18	0.95	0.43	0.39	0.55	1.70	2.22	1.49	3.04	3.04	2.03
SE(d)	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.07	0.04	0.23	0.26	0.25
LSD (5%)	0.07	0.07	0.08	0.07	0.07	0.07	0.09	0.15	0.10	NS	NS	0.54

Table.2 Effect of micronutrients on leaf area, carotenoids and stomatal numbers in cashew

Treatment	Leaf area (cm^{2})			Carotenoids mg/g			Stomatal number		
	Flush	Flow	Fruit	Flush	Flow	Fruit	Flush	Flow	Fruit
Control	68.83^{F}	66.78^{F}	64.58^{F}	0.16^{D}	0.12^{E}	0.10^{E}	17.80^{D}	15.50^{E}	12.10^{E}
Borax (0.1%)	144.43^{B}	144.00^{B}	139.78^{B}	0.48^{A}	0.43^{B}	0.37^{B}	25.78^{B}	25.78^{B}	21.08^{B}
Borax (0.2%)	98.45^{E}	97.38^{E}	96.05^{E}	0.29^{C}	0.27^{D}	0.20^{D}	19.78^{CD}	18.53^{D}	14.25^{DE}
$ZnSO_4$ (0.5%)	129.85^{C}	127.95^{C}	125.13^{C}	0.41^{B}	0.35^{C}	0.34^{B}	22.10^{C}	20.70^{C}	17.50^{C}
$ZnSO_4$ (0.5%) + borax (0.1%)	156.10^{A}	153.83^{A}	151.65^{A}	0.54^{A}	0.55^{A}	0.44^{A}	31.05^{A}	28.33^{A}	24.75^{A}
$ZnSO_4$ (0.5%) + borax (0.2%)	106.62^{D}	106.35^{D}	104.53^{D}	0.32^{C}	0.26^{D}	0.26^{C}	20.77^{C}	18.88^{CD}	16.50^{CD}
General mean	117.38	116.05	113.62	0.37	0.33	0.28	22.88	21.28	17.70
SE(d)	2.55	1.87	2.23	0.03	0.02	0.02	1.35	0.99	1.40
LSD (5%)	5.43	3.98	4.75	0.06	0.06	0.04	2.89	2.12	2.99

Table.3 Effect of micronutrients on nut yield of cashew (*Anacardium occidentale* L.) var. Bhaskara

Treatment	Nut yield kg/tree
Control	3.20^{F}
Borax (0.1%)	9.60^{B}
Borax (0.2%)	6.40^{E}
$ZnSO_4$ (0.5%)	8.20^{C}
$ZnSO_4$ (0.5%) + borax (0.1%)	10.80^{A}
$ZnSO_4$ (0.5%) + borax (0.2%)	7.60^{D}
General Mean	7.63
SE(d)	0.072
LSD at 5%	0.154

The reduction in chlorophyll and carotenoids at higher level of B may be due to its toxic effect or production of reactive oxygen species (ROS) which hinders the biosynthesis of these pigments or it binds SH group chloroplast and destroys its structure and function and decreases chlorophyll biosynthesis (Hou *et al.,* 2007). Higher concentrations of B resulted in a pronounced reduction in the photo-reduction activities of PSII. The physiological analysis of photosynthetic pigments like Chl a, b, total and carotenoids were significantly increased by application of micronutrients due to enhancement in secondary metabolites (Shitole and Dhumal, 2012).

Leaf area

Plant leaf area is an important determinant of light interception and consequently of transpiration, photosynthesis as well as plant productivity. Significant difference was observed in leaf area with respect to foliar application of micronutrients in cashew (Table 2). Among the treatments, spraying of $ZnSO_4$ (0.5%) + borax (0.1%) at flushing stage resulted in highest leaf area (156.10 cm^2) followed by borax (0.1%) (144.43 cm^2), $ZnSO_4$ (0.5%) (129.85 cm^2), $ZnSO_4$ (0.5%) + borax (0.2%) (106.62 cm^2), borax (0.2%) (98.45 cm^2) and control (68.83 cm^2). At flowering stage, the leaf area was found to be maximum (153.83 cm^2) in $ZnSO_4$ (0.5%) + borax (0.1%) spray and minimum (66.78 cm^2) in unsprayed (control) trees. Foliar application of $ZnSO_4$ (0.5%) + borax (0.1%) during fruiting stage recorded significantly higher leaf area (151.65 cm^2) over other treatments. Significant increase in leaf area could be due to increased metabolic activity by increased supply of nutrients (Goudriaan and Van Laar, 1994; Wahdan *et al.*, 2011). Role of micro nutrients in increasing leaf area can be ascribed to their influence on cell division and cell elongation. Higher leaf area values recorded with $ZnSO_4$ (0.5%) + borax (0.1%) may also be due to increased concentration of photosynthates in the shoot (Nunez *et al.*, 1998; Zoffoli *et al.*, 2009 and Zahoor *et al.*, 2011) as reported in grape (*Vitis vinifera* L.).

Stomatal number

The stomata are apertures in the epidermis, each bounded by two guard cells. Their main function is to allow gases such as carbon dioxide, water vapors and oxygen to move rapidly into and out of the leaf. Stomatal density can vary within leaves, plants, and individuals of a single species. Data on stomatal number as influenced by foliar application of micronutrients is presented in

Table 2. The maximum number of stomata under the leaves was recorded with spraying of $ZnSO_4$ (0.5%) + borax (0.1%) (31.05) followed by borax (0.1%) (25.78) and $ZnSO_4$ (0.5%) (22.10). Minimum number of stomata was found in control (17.80). The remaining treatments had intermediate number of stomata. Application of $ZnSO_4$ (0.5%) + borax (0.1%) at flowering stage recorded the highest stomatal number (28.33) and control recorded the least (15.50) number of stomata. At flowering stage, spraying of $ZnSO_4$ (0.5%) + borax (0.1%) resulted in highest stomatal number (24.75) whereas unsprayed (control) trees recorded least (12.10) stomatal number. This was due to the fact that nutrients are readily absorbed by leaves and translocated within the plant when they are dissolved in water and sprayed on them resulting in higher leaf area and stomatal number. Higher leaf area as a result of micronutrient spray might have been the chief cause for increased number of stomata in micronutrient sprayed trees (Farshid Aref, 2011).

Nut yield

Foliar application of micronutrients played a significant role in increasing nut yield of cashew. The highest nut yield (10.80 kg/tree) was recorded with spraying of zinc sulphate (0.5%) + borax (0.1%) which was significantly superior over other treatments (Table 3). The lowest yield of 3.2 kg/tree was recorded with control (unsprayed trees). Nut yield depends on the synthesis and accumulation of photosynthates and their distribution among various plant parts. The synthesis, accumulation and translocation of photosynthates depend upon efficient photosynthetic structure as well as the extent of translocation into sink and also on plant growth and development during early stages of crop growth. This may be attributed to fulfillment of the demand of the crop by higher assimilation and translocation of photosynthates from source (leaves) to sink (nut), through supply of required nutrients by foliar spray of micronutrients. These results are in corroboration with the findings of Tariq *et al.,* (2014). Razzaq *et al.,* (2013) reported that foliar application of Zn enhanced productivity with better fruit quality in 'Kinnow' mandarin because Zn plays an active role in biosynthesis of auxins (Alloway, 2008). Boron application increases fruit set and yield in several fruit and nut trees, including almond, Italian prune, olive, and sour cherry (Slavko *et al.,* 2001). Increased stomatal number due to application of zinc sulphate (0.5%) + borax (0.1%) increases inflow of carbon dioxide into the mesophyll tissue resulting more photosynthates, latter partitioned towards nut resulted in more nut yield (Aliyu *et al.,* 2011).

In the present study, foliar application of zinc sulphate @ 0.5% and borax @ 0.1% was found to improve the leaf area, photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids) and stomatal density in cashew and also effective in enhancing the nut yield of cashew.

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