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Morphological and biochemical response of sunflower (*Helianthus annus* L.) to different doses of soil boron application

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Abstract
A pot culture experiment was conducted to explore the effect of boron on morphological, physiological and biochemical changes of sunflower (*Helianthus annus* L.) in laterite soil during zadi, 2018 at AICRP on Micronutrients, department of Soil Science and Agricultural Chemistry, OUAT, Bhubaneswar. The experiment was laid out in Factorial Completely Randomised block design with two factors (5 Soil types and 5 boron doses) and with 25 treatments replicated twice. It was observed that the growth increased significantly with the increase in boron dose. The uptake of calcium and magnesium as well as the carbohydrate and protein content within the plant was enhanced with the application of boron.

Keywords: Micronutrient, biochemical response, physiological repose, yield

1. Introduction

Indian mustard (*Brassica juncea* L.) is an important *Rabi* oilseed crop extensively grown as Sunflower being a major oil seed crop, plays an important role in Indian agriculture, industries and trade. While the vibrant sunflower is recognised for its beauty, it is also an important source of food. The development of high oil content sunflower in former Soviet Union has stimulated worldwide interest in the commercial exploitation of this crop. Sunflower being most sensitive crop to boron is most suitable crop for boron studies in plants. Studies have found that boron deficiency inhibits cell division and hence retards growth in sunflower (Deperon *et al*., 2007) [11]. The high yield and multiple uses of oil for both industrial and domestic purposes have induced the enterprising cultivators of these countries to take up its cultivation as a highly remunerative crop. A study was conducted to identify the morphological, biochemical and physiological responses in sunflower grown in acid soil to different doses of boron and establishing the critical limit of boron for the different soil types.

2. Material and Methods

2.1 Morphological parameters: The total number of leaves, leaf area per plant (cm^2), were measured in graphical method. After uprooting, the plants were oven dried at 90°C for the measuring the dry weight of leaves, stems and roots. Leaf Area Ratio and Specific Leaf Area was calculated by using the formula by Radford, 1967 [13].

2.2 Biochemical Analysis

Carbohydrate of leaf samples was determined by Yoshida *et al*. (2005) [14] procedure. Protein content of leaf and seed samples was determined by Lowry *et al*., (1951) [15] method. Calcium and magnesium analysis was done by extraction method using tripe acid titration method using EDTA. Estimation of boron was done by procedure given by Jackon *et al*., (1973) [1] using Azomethine -H colorimetric method.

2.3 Experimental Details: Factorial Completely Randomized Block Design was adopted for this experiment with 25 treatments (5 doses of boron and 5 types of soil) with 2 replications each. Black polybags were used for the experiment having an approximate 5 kg soil. To each polybag recommended NPK in 60:80:80 ratio was given. Boron was applied in the form of Borate to soil in 5 doses of 0 kg boron/ha, 0.5 kg boron/ha, 1.0 kg boron/ha, 1.5 kg boron/ha and 2 kg boron/ha.

Table 1: Initial properties of soil

Treatments	Soil pH	Soil EC (dSm-1)	Soil OC (%)	Soil boron (ppm)
S1	5.19	0.28	0.458	0.08
S2	4.47	0.36	0.725	0.62
S3	4.81	0.26	0.305	3.21
S4	5.03	0.27	0.362	0.21
S5	5.18	0.33	0.515	0.16

3. Results and Discussion

Periodical observations under each treatment with respect to the all attributes were taken at an interval of 15 days commencing from 30 days after sowing. The investigation revealed that growth increased significantly with the increase in boron dose from 0.5 kg boron/ha to 2 kg boron/ha in all soil types except in soil type 2 and soil type 3 which showed increase in growth up to boron dose of 1.5 kg boron/ha and 1kg boron/ha, respectively, and thereafter it declined.

In soil type 1 with boron dose 2 kg/ha maximum height at 75 DAS (134 cm) was recorded. Chlorophyll content (SPAD reading 12.83), leaf dry weight (3.9 g), head dry weight (4.8 g) was obtained in soil type 1 at boron dose 2 kg/ha. Leaf area per plant was highest at 45 DAS at boron dose 1 kg/ha (771.5 cm^2), The highest total dry weight was observed at 75 DAS.

Soil type 4 with boron dose 2 kg/ha recorded highest LAR (61.25 cm^2/g). The specific leaf area was observed highest at 45 DAS in soil type 5 at boron dose 2 kg/ha (220.3 cm2/g). Both LAR and SLA declined after 45 DAS.

The carbohydrate and protein content increased over control up to 9.98 % and 13.60 % respectively. The calcium content and magnesium in leaves content increased up to 31% and 13.4% respectively, over control at 45 DAS. The total boron concentration at 45 DAS and leaf boron concentration at 75 DAS was maximum in soil type 3 at boron dose 2 kg/ha i.e., 120.2 ppm and 90.10 ppm respectively.

Table 2: Effect of boron and soil types on plant height (cm) of sunflower at 45 DAS

	S1	S2	S3	S4	S5	Mean
B0	69.5	58.5	61	61	59.5	61.9
B0.5	86.5	61	78.5	65.5	68	71.9
B1.0	86.5	66	79	71	75.5	75.6
B1.5	87	83	74	74	75.5	78.7
B2.0	95.55	82.5	71	75	77.5	80.31
Mean	85.01	70.2	72.7	69.3	71.2	
	B	S	B*S			
SEM±	1.554	1.554	3.475			
CD(0.05)	4.536	4.536	10.142			

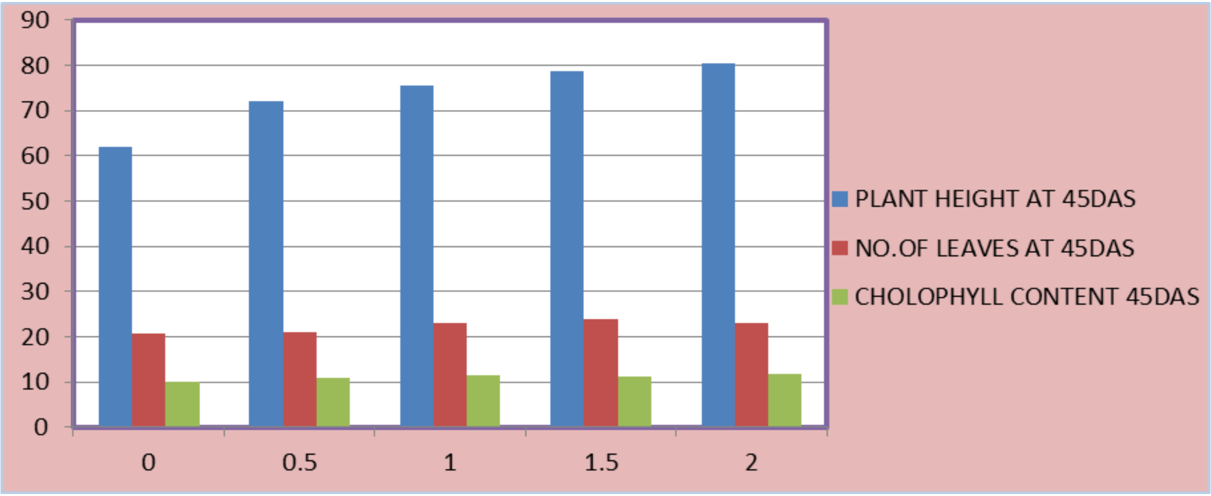

Fig 1: Relation of plant height, number of leaves and chlorophyll content with boron doses

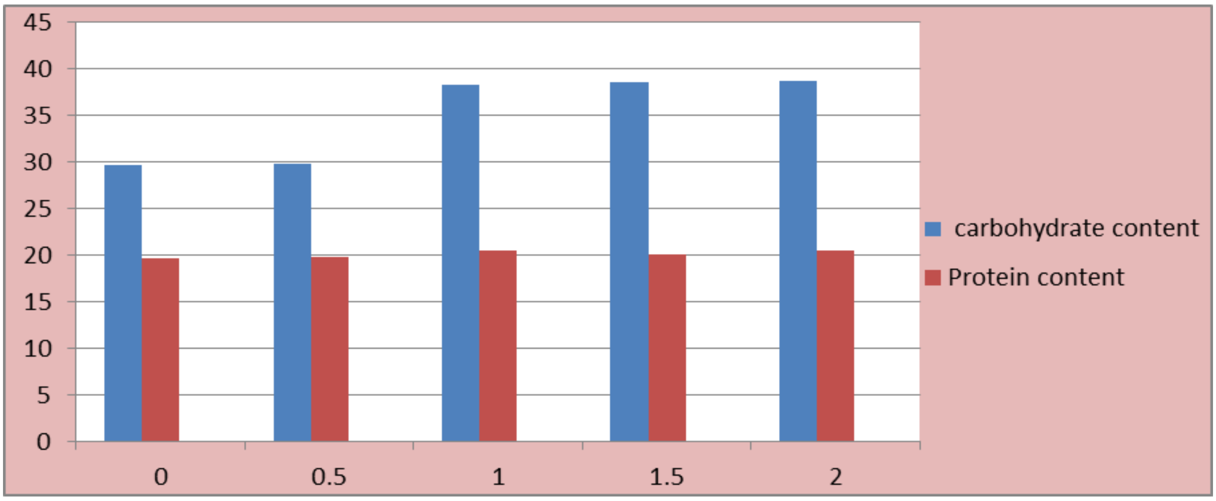

Fig 2: Relation of carbohydrate content and protein content with different boron doses

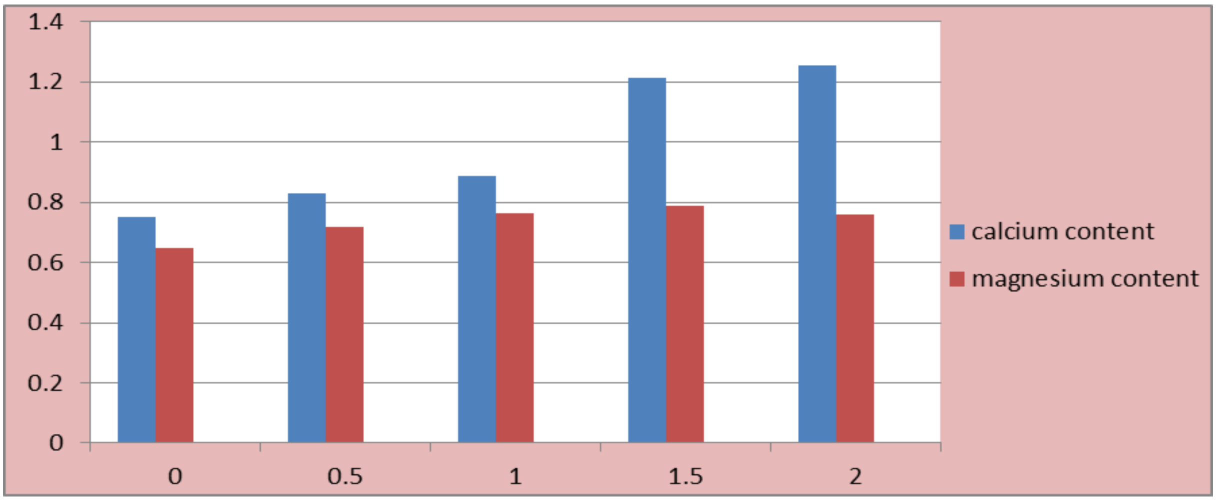

Fig 3: Relation of calcium content and magnesium content with different boron doses

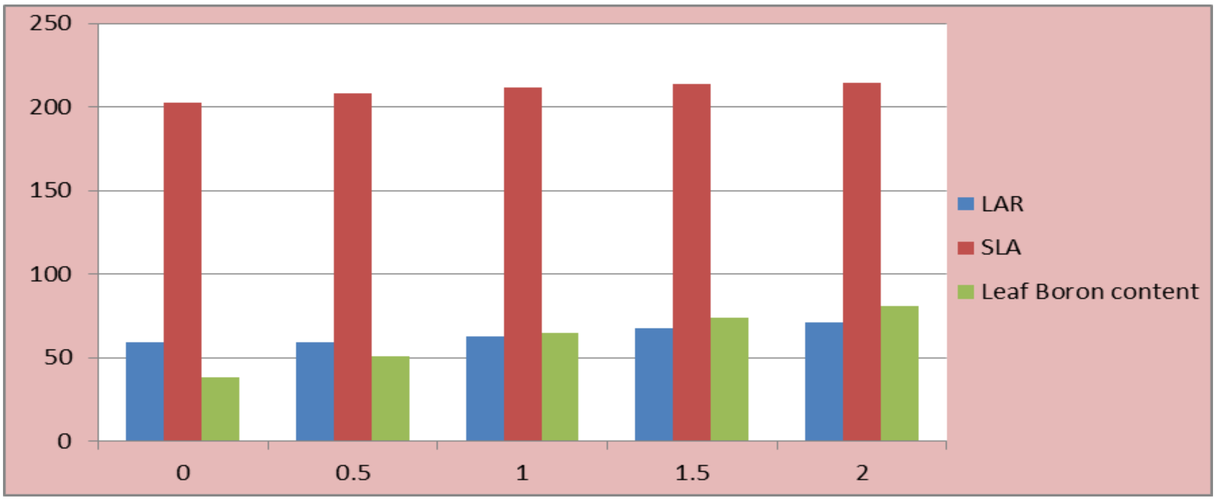

Fig 4: Relation of LAR, SLA and leaf Boron content with different boron doses

3.1 Morphological and Physiological response to boron application

Plant height of the cultivar as influenced by the different soil type and boron doses revealed that maximum increase in plant height was observed in boron dose 2 kg/ha at all the stages of growth. These observations suggested that plant height increased with increasing boron doses. These findings were supported by Asad *et al*., 2003 [2]. However, the soil having low content of boron responded better to boron application.

Vegetative growth of the plant is determined by the total number of leaves per plant and total leaf area. Response of sunflower to different doses of boron on leaf number and leaf area at 30 DAS reflects the fact that, different boron doses did not show any remarkable effect either on leaf number or leaf area per plant; probably it is too early for expression of the effects of boron on sunflower. After 15 days the leaf number as well as leaf area increased considerably in the treatments, which indicates that boron has a positive correlation with increase in leaf area of plant. Increased leaf area provides an improved leaf exposure to light and greater light penetration into the canopy, thereby creating favourable conditions for photosynthesis (Amery *et al*., 2011) [7]. This indicates the requirement of boron for optimum leaf growth. Leaf number remains unaltered in the different soil types at all the stages of growth but leaf area increased considerably in comparison to control. Higher leaf area than the control in treatments of B 1.5 kg/ha and B 2 kg/ha at all the stages of growth was observed. This increase in growth might be associated with boron role in increasing photosynthetic activity thus resulting in more plant height and number of leaves per plant; hence more leaf area was obtained in those treatments with optimum boron dose. Such an inference was earlier reported by Sharma *et al*., 2000 [9]. At later stages of growth there was a decline in leaf area due to less number of leaves and senescence.

Dry weight of leaf, stem, root as well as total dry weight of the plants are the measures of net gain of assimilates in plants. A marked increase in dry weight in boron treated plants except in soil type 2 boron dose 1kg/ha and soil type 3 boron dose 1.5 kg/ha and 2 kg/ha. It is already reported that boron application increased main stem length, volume and dry weight of the roots above ground biomass leaves and photosynthesis rate of soybean, (Peng *et al*., 2003) [8]. Total dry weight of the plant increased significantly with the increase in boron dose. The increase in dry weight of root is significant form more absorbtion of water and minerals and hence more growth (Josten *et al*., 1999) [12] There was increase in total dry weight (g) with increase in boron dose except for Soil type 2 and Soil type 3. The findings are in agreement with that of Asad et al., (2003) [2]. Higher growth indices such as LAR (148 cm^2/g at 45 DAS) and SLA (62.54 cm^2/g at 45 DAS) as obtained at boron dose 1.5 kg/ha in soil type 2 which was very clearly marked at 45 DAS. There was a progressive increase in leaf area and hence in the LAR and SLA with increase in boron dose. Boron has positive correlation with increase in leaf area of plant. Increased leaf area provides an improved leaf exposure to light and greater light penetration into the canopy, thereby creating favourable conditions for photosynthesis, which was in agreement with the present investigation. Amery *et al.*, 2011 [7] reported that application of borax resulted in higher light interception rate, leaf area index.

3.2 Biochemical changes in response to soil boron application

The chlorophyll content significantly increased due to application of boron in all treatments. The increase in chlorophyll content was supported by the positive influence of boron in activity of enzymes involved in photosynthesis, Hill reaction and net photosynthesis of sunflower. Chlorophyll content measured in SPAD was highest at 45 DAS and lowest at 30 DAS. Highest chlorophyll content was recorded at 45 DAS and lowest chlorophyll content was recorded at 75 DAS. The decrease in chlorophyll content at 75 DAS is due to senescence.

Soil type 1 with boron dose 2 kg/ha at 45 DAS recorded highest chlorophyll content of sunflower reading (12.82). There was a sharp decline in chlorophyll content in soil type 3 at boron dose 1.0 kg/ha due to the toxic impact of excessive boron at this dose containing higher native boron in soil. Higher concentration of boron had inhibitory effect on chlorophyll synthesis because of reduction in the absorption and translocation of calcium and magnesium at high boron concentration. These results agree with those of (Reid, 2007) [16] who reported that at higher boron doses, net photosynthesis and chlorophyll content decreased in sunflower.

As the level of boron dose increased, the carbohydrate content of stem at 45 DAS increased. This may presume due to active role of boron in sugar metabolism leading to accumulation of total sugar and starch. This is confirmed by Dordas, 2006 [5] who reported that boron application enhanced sunflower growth because of its significant role in carbohydrates transport, permeability of cell membranes, lignin biosynthesis, meristematic tissues growth, and avoiding leakage of plasma membrane solutes. According to Eion Scott's article on 'Effect of supra-optimal boron levels on respiration and carbohydrate metabolism of Helianthus annuus boron act as protector to sugars from polymerization and hence they are more available for translocation.

The protein content in sunflower leaves analysed under different treatments revealed increased boron dose showed a concomitant increase in boron content. Application of Boron caused a synergistic effect on Nitrogen uptake, enhanced nitrogen metabolism, amino acids which is directly linked with accumulation of amino acids, RNA, and protein synthesis and hence the protein content. Application of boron significantly improved the protein content in leaves at 45 DAS. Among the five soil types the protein content was higher in soil type two. Soil type 2 with boron dose 1.5 kg/ha recorded highest protein content (21.74%). This might be due to the fact that soil boron has a phenomenal impact on the protein synthesis in plants leading to higher protein content in sunflower seeds as well as leaves, Soil type 2 has higher boron concentration and hence responded better than other soil types. But it declined beyond boron dose of 1.5 kg/ha because at higher concentration there is denaturation of proteins and decreased nitrogen uptake. The low protein content in control may be due to impairment in the activity of boron dependent RNA polymerase.

The calcium and magnesium content in the leaves showed significant variation among different soil types having different boron concentration. Sathya, 2013 [10] made a similar observation where boron helped in the uptake of other nutrients. Soil type 1 at boron dose 2 kg boron/ha showed the maximum calcium and magnesium content.

3.3 Boron concentration in plant tissue: The concentration was highest in soil type 3 followed by soil type 2 in leaves, in seeds as well as the total boron concentration this. This was because these two soil types had higher soil boron content and organic matter which helped in the boron absorption. This is in accordance to previous studies that organic matter affects the availability of soil B is and it has a positive correlation between levels of soil organic matter and the amount of hot water-soluble boron. Similar observation was also made by Mandal *et al*., 2004 and Sharma *et al*., 2006 [17, 18].

In soil type 3 boron dose 2 kg/ha showed significantly higher concentration of total boron (120.2 ppm), seed boron (100.3 ppm), leaf boron (90.10 ppm). The boron concentration in plant tissue did not show a linear response with total dry matter and seed yield in soil type 3 and soil type 2. Growth declined beyond the boron dose of 1.5 kg/ha in soil type 2 and 1 kg/ha in soil type 3. This may be due to the high native boron content at this dose in these soil types. This is evident from the study of Bhattacharya *et al*., 2015 who suggested that increasing levels of soil B application beyond optimum level in soil reduced crop productivity.

4. Summary and Conclusion

The investigation revealed that growth increased significantly with the increase in boron dose from 0.5 kg boron/ha to 2 kg boron/ha in all soil types except in soil type 2 and soil type 3 which showed increase in growth up to boron dose of 1.5 kg boron/ha and 1 kg boron/ha, respectively, and thereafter it declined. There was progressive uptake of calcium and magnesium with the application of boron. It was also concluded that the carbohydrate and protein content were also significantly affected with the varied boron doses.

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