Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(2): 3515-3518
Received: 24-01-2018
Accepted: 25-02-2018

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Morpho-physiological and biochemical studies on foliar application of zinc, iron and boron in maize (*Zea mays* L.)

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Abstract
A pot experiment was conducted in the farm of Institute of Agricultural Sciences, Banaras Hindu University to study the effect of foliar spray micronutrient (zinc, iron and boron) on some morpho-physiological and biochemical parameters of maize. The experiment was conducted in completely randomized design (CRD), containing three replications with the following treatments: T0 control (not received spray), T1, T2 (Zinc as form $ZnSO_4$ [0.5%, 1% resp.]), T3, T4 (Fe as form $FeSO_4$) at 0.5% and 1% respectively. Treatments T5, T6 boron (B as form boric acid) at 0.3% and 0.6% respectively. Treatments were sprayed on plant leaves at the vegetative growth stage 30 DAS and 40 DAS. The experimental results revealed that morpho-physiological parameters like plant height (cm), number of leaves, leaf area (cm^2) as well as total dry matter per plant (g) was significantly increased through application of zinc and iron as compared with the control treatment. The biochemical traits also shown significant increment in chlorophyll content, sugar, starch and protein content (mg g^{-1}FW) as foliar spray of different micronutrients (Zn, Fe and B) compared with the control treatment. The study revealed that the foliar applications of iron and zinc significantly increases some biochemical characteristics like chlorophyll content and sugar (mg g^{-1}FW) respectively while starch content shown significant enhancement by effect boron foliar spray as compared to control treatment.

Keywords: boron; foliar spray; iron; micronutrients; maize (*Zea mays* L.); zinc

Introduction
Maize (*Zea mays* L.) is the most important coarse grains and is an important staple food in many countries of the world. Besides, being intensively used as human food and animal feed, it is a source of a large number of diversified industrial products including corn oil, corn starch, corn oil, baby corns, popcorns, alcoholic beverages, food sweeteners etc which is of great economic importance. The importance of maize can be understood by the fact that although there are large differences in maize yields, Maize is grown throughout the world. The maize being a day neutral plant can be grown round the year with rich soil types of having good drainage but ideal soil is sandy loam that stays moist.
The entire maize growing area in India is divided in five major zones Northern Hill Zone, North West Plain Zone, North East Plain Zone, Peninsular Zone and Central West Zone for valuable evaluation of the maize breeding materials and experimental cultivars (Anonymous, 2016) [6]. In India, the maize production has increased in Bihar, Himachal Pradesh, Jammu & Kashmir, Madhya Pradesh, Tamil Nadu and West Bengal (Anonymous, 2016) [6].
Maize can suffer from a number of micronutrient deficiencies like Zinc, Iron and Boron among these zinc deficiency is perhaps the most widespread problem. Zinc one of main limiting factor for the growth and yielding. Zinc deficiency results in annual yield reduction up to 10% due to Zn deficiency (Subedi *et al.* 2009) [20]. And it shows very much response on corn growth parameters and yield components when supplied as foliar (Tahir *et al*. 2016 and Wasaya *et al.* 2017) [21, 22].
Iron is also an essential component of certain enzymes and numerous proteins that present during respiration and photosynthesis. Iron is immobile in plants and mobility decreases in soil with increasing pH and excessive Phosphorus may induce deficiency. In crops, especially in those grown on calcareous soils, iron deficiency is a major nutritional disorder that causes decreases in vegetative growth and marked yield and quality losses (Abadia *et al*. 2011) [1]. However when iron applied as foliar method it shows very much response on corn growth parameters (Jamshidzadeh *et al.* 2014 and Daliwal *et al.* 2013) [12, 9].
Boron is also extremely immobile in plants and is not translocated to new growth, but moves easily in soil. Sinha *et al*. (2009) [18] boron reduced the economic yield and deteriorated the

quality of the product by lowering the concentration of starch, protein in seeds and storage organs, and ascorbic acid in tomato fruits and oil in oil seeds. However, deficiencies can be corrected by foliar application of boric acid which directly enhances several growth traits in plants (Zhang, 2009; Ahmad *et al*. 2012) [23, 2].

A pot culture experiment was carried out at the Agricultural Farm and Laboratory of the Dept. of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University during 2016-17 to investigate the effect of foliar applications of three nutrients zinc, iron and boron in maize crop. Keeping in view the above facts and the importance of micronutrients on the growth and development of maize was carried in pot culture with the following objectives to determine effect of foliar spray of zinc, iron and boron on morpho-physiological and certain biochemical parameters in maize.

Materials and Methods

The field experiment was conducted during the khiref season 2016-2017 to investigate the effect of Zn, Fe and B application as single nutrient in two different concentrations on morpho-physiological and biochemical parameters like Plant height, number of leaves, leaf area, total dry matter per plant, chlorophyll, sugar, starch and protein content at farm of institute of agricultural sciences, Banaras Hindu University. The experimental site is located at is situated 25^015' North Latitude and 60^003' East Longitude with an altitude of 128.93 m above sea level. Plastic pots of diameter 20 cm were filled with well pulverized 5 kg of field soil and Maize (*Zea mays* L.) variety K-65 was used for the experimental purpose. The experiment was laid out in completely randomized design (CRD) with three replications. The seeds were sterilized with 70% alcohol for 5 minutes and washed with distilled water twice before sowing.

The depth of sowing in the soil was 2-3 cm. 5 Seeds were sown in each plastic pot at equidistance positions. Two foliar applications of Zn, Fe and B each of two different concentrations were given at 30 DAS and 40 DAS as the following treatments. The time of foliar application was in the morning at 6.00 a.m. and observations pertaining to at 10 days after each foliar spray (treatment).

The observations on plant height and number of leaves plant^{-1} were recorded manually on randomly chosen three plants per treatment and then average value is calculated per treatment. Physiological parameters like leaf area and total dry weight per plant character were recorded as per the standard method. Leaf area per plant was determined using graphical and leaf area factor method. The dry weight of plant samples recorded after putting them into an electric oven, first at the temperature of 110 ^{0}C for an hour followed by the constant temperature of 70 ^{0}C for a period of 72 hours. For weighing digital electronic balance was used till a constant dry weight of the plant material was attained.

The different types of chlorophyll content and related compounds in leaves were estimated by the method of (Arnon, 1949) [7]. Anthrone method (Dubois *et al.*, 1956) [10] was employed for estimation of sugar content in leaf samples. Starch content was determined by anthrone method (Dubois *et al*., 1956) [10]. Starch content was estimated from the residue retained from the samples that was used for soluble sugar estimation by standard anthrone reagent method. Protein estimation was done according to the Coomassie Brilliant Blue G-250 dye binding method (Broadford, 1976) [8]. The enzyme nitrate reductase activity was assayed *in vivo* by the method of Srivastava (1975) [19] in the fully expanded leaf.

To test the significance of the treatment over control, completely randomized design was used and one way analysis of variance (ANOVA) was performed for statistical analysis of morphological and biochemical parameters (Panse and Sukhatme 1967) [15]. Critical difference (C.D.) values were calculated at 1 percent probability level.

Results and Discussions

The data can be recorded and analyzed for morpho-phyiological characters of maize crop in relation to different treatment application (Table 1). Data in Table 1 showed that plant height was significantly affected by foliar application of treatment T_2 at 1% concentration as compared to control values. The highest plant height 42.33 cm and 45.56 cm was obtained due to first (40 DAS) and second (50 DAS) foliar application respectively. The results indicated that the highest number of leave per plant was obtained when plants were treated with T_3 (Fe 0.5%). Number of leave per plants increased 26.60% after first spray (40 DAS) with T_3 treatment followed by 21.65% increment observed after second spray (50 DAS) with T_1 and T_3 as compared to control 6.67 and 8.22 respectively. Similar finding was reported Manasa (2015) [14]; Singh *et al*. (2013) [17] and Amanullah *et al*. (2016) [4].

The highest leaf area was achieved by foliar application of T4 treatment (Fe 1%) and showed increment 23.98% and 20.87% after first and second foliar application respectively. However the control treatment indicating the vales 122.91 cm^2 and 126.80 cm^2 at the two growth stages of the maize crop. With respect the control values of total dry matter per plant 4.40 g and 5.23 g at two growth stages (40, 50 DAS respectively), the highest increment in dry matter was obtained with T_2 treatment (Zn 1%) 91.67% and 87.38% after first and second treatment respectively. Similar results were found Kumar *et al*. (2016) [3] and Verma *et al*. (2015). Due to micronutrient foliar application (Zn) noticeable increase in synthesis of plant hormones like auxin which directly stimulating growth of plants.

Data in Table 2 show that treatment had significant effect on chlorophyll 'a' content was recorded for T_4 (Fe 1%) treatment and values obtained were shown 48.35% and 39.03% increment after first and second foliar sprays respectively with relative to the (T_0) control values (1.54, 1.71 mg g^{-1} FW). Moreover chlorophyll 'b' content over control value of 0.61 (mg g^{-1} FW) was recorded and observed 23.53% and 23.44% percent increased after first and second foliar application respectively in the T_4 treatment. Similar results were found Rengel *et al*. (2012) and Alijani *et al.* (2016) [3].

The data can be recorded and tabulated in TABLE 2 for total soluble sugar content with respective to spray order over control values of 18.34 (during first treatment) and 21.33 (during second treatment) (mg g^{-1} FW) was recorded and observed highest percent increment with the T_1 treatment and the values are 70.81% and 71.03% after first and second foliar application respectively. With respect to spray order starch content of untreated plants (control) 2.28 and 3.09 (mg g^{-1} FW) was recorded (TABLE 3) and observed highest percent increment with the T_5 treatment and the values are 73.73% and 76.38% after first and second foliar application respectively. Similar results were found Alijani *et al.* (2016) [3] and Amirinejad *et al.* (2015) [5].

With respect to spray order protein content of untreated plants (control) 66.09 and 70.55 (mg g^{-1} FW) was recorded (TABLE 3) and observed highest percent increment with the T_1 treatment and the values are 17.18% and 18.48% after first and second foliar application respectively. And lowest percent increment observed in T_4 treatment with respective to spray order 2.83% and 4.41% values over control. Similar results were found Esmaeili *et al.* (2016) [11] and Tahir *et al.* (2016) [21]. However all treatments recorded non-significant effect on carotenoid content and nitrate reductase activity.

Table 1: Morpho-Physiological parameters of maize crop in relation to different treatments application

Treatments	**plant height (cm)**		**number of leaves (plant⁻¹)**		**leaf area (cm² plant⁻¹)**		**total dry matter per plant (g Plant⁻¹)**	
	Days After Sowing#							
	40	**50**	**40**	**50**	**40**	**50**	**40**	**50**
T_0: control	28.78	31.11	6.67	8.22	122.91	126.80	4.40	5.23
T_1:0.5%Zn	40.67 (41.30)	43.89 (41.08)	8.11 (21.61)	10.00 (21.65)	143.57 (16.82)	152.26 (20.08)	7.63 (73.48)	8.90 (70.17)
T_2: 1% Zn	42.33 (43.62)	45.56 (46.43)	7.78 (16.61)	9.33 (13.54)	145.46 (18.35)	147.56 (15.46)	8.43 (91.67)	9.80 (87.38)
T_3:0.5%Fe	34.89 (21.23)	38.22 (22.86)	8.44 (26.60)	10.00 (21.65)	148.32 (20.67)	150.79 (17.99)	8.20 (86.36)	9.48 (81.33)
T_4:1% Fe	37.44 (30.11)	40.22 (29.29)	7.63 (14.44)	8.89 (11.75)	152.38 (23.98)	154.48 (20.87)	6.63 (50.76)	7.97 (52.33)
T_5:0.3% B	33.33 (15.82)	36.11 (16.08)	7.22 (8.28)	9.11 (10.84)	140.67 (14.45)	142.67 (11.64)	5.87 (33.33)	6.83 (30.66)
T_6:0.6% B	33.78 (17.37)	36.33 (16.79)	8.00 (19.38)	9.67 (17.60)	133.20 (8.38)	141.75 (10.92)	6.17 (40.15)	8.03 (53.60)
SEm±	1.00	1.12	0.31	0.27	5.39	5.20	0.26	0.29
CD at 1%	3.05	3.43	0.95	0.83	16.49	15.94	0.80	0.90

(%) Values in parentheses denote percent increment over control.
T_0: control, T_1: 0.5% Zn, T_2: 1% Zn, T_3: 0.5% Fe, T_4: 1% Fe, T_5: 0.3% B, T_6: 0.6% B.
#As per the treatments, the first and second foliar spray was given at 30 DAS and 40 DAS respectively and the observations were recorded after 10 days of each spray applications coinciding at 40 DAS and 50 DAS.

Table 2: Biochemical traits of maize crop in relation to different treatments application

Treatments	**chlorophyll a content (mg g⁻¹ FW)**		**chlorophyll b content (mg g⁻¹ FW)**		**Carotenoid content (mg g⁻¹ FW)**		**total soluble sugar content (mg g⁻¹ FW)**	
	Days After Sowing#							
	40	**50**	**40**	**50**	**40**	**50**	**40**	**50**
T_0: control	1.54	1.71	0.61	0.61	0.12	0.11	18.34	21.33
T_1:0.5%Zn	1.90 (23.42)	2.05 (20.10)	0.63 (3.97)	0.62 (1.94)	0.12 (4.29)	0.12 (5.11)	31.33 (70.81)	36.48 (71.03)
T_2: 1% Zn	1.96 (27.29)	2.12 (24.09)	0.64 (5.24)	0.64 (4.80)	0.12 (1.61)	0.11 (2.23)	28.98 (58.02)	32.51 (52.43)
T_3:0.5%Fe	2.20 (42.92)	2.29 (33.84)	0.71 (17.08)	0.70 (15.00)	0.12 (2.06)	0.12 (6.55)	23.31 (27.08)	29.05 (36.18)
T_4:1% Fe	2.29 (48.35)	2.38 (39.03)	0.75 23.53)	0.75 (23.44)	0.13 (4.42)	0.11 (4.23)	27.32 (48.96)	30.55 (43.24)
T_5:0.3% B	1.55 (0.48)	1.74 (1.44)	0.62 (2.04)	0.62 (1.04)	0.12 (1.81)	0.11 (4.45)	18.37 (0.15)	21.34 (0.05)
T_6:0.6% B	1.56 (1.23)	1.75 (2.58)	0.62 (1.93)	0.63 (3.16)	0.13 (3.69)	0.11 (3.55)	18.46 (0.68)	21.65 (1.48)
SEm±	0.19	0.13	0.02	0.01	0.01	0.02	1.33	1.14
CD at 1%	0.57	0.38	0.06	0.04	NS	NS	4.10	3.50

(%) Values in parentheses denote percent increment over control.
T_0: control, T_1: 0.5% Zn, T_2: 1% Zn, T_3: 0.5% Fe, T_4: 1% Fe, T_5: 0.3% B, T_6: 0.6% B.
#As per the treatments, the first and second foliar spray was given at 30 DAS and 40 DAS respectively and the observations were recorded after 10 days of each spray applications coinciding at 40 DAS and 50 DAS.

Table 3: Biochemical traits of maize crop in relation to different treatments application

Treatments	**starch content (mg g⁻¹ FW)**		**soluble protein content (mg g-1 FW)**		**nitrate reductase activity (µ moles NO₂⁻ g⁻¹ FW h⁻¹)**	
	Days After Sowing#					
	40	50	40	50	40	50
T_0:Control	2.28	3.09	66.09	70.55	10.69	11.05
T_1:0.5% Zn	3.87 (41.00)	3.95 (27.72)	77.45 (17.18)	83.59 (18.48)	11.24 (5.14)	11.67 (5.57)
T_2:1% Zn	3.13 (22.05)	4.20 (36.03)	73.79 (11.66)	80.31 (13.83)	10.98 (2.74)	11.42 (3.36)
T_3:0.5% Fe	5.87 (65.98)	6.10 (66.13)	76.86 (16.29)	82.15 (16.44)	11.43 (6.95)	11.71 (6.00)
T_4:1% Fe	3.69 (36.35)	3.71 (19.13)	67.96 (2.83)	73.66 (4.41)	11.48 (7.42)	12.19 (10.34)
T_5:0.3% B	5.93 (73.73)	5.52 (76.38)	70.00 (5.91)	77.53 (9.90)	10.91 (2.09)	11.26 (1.92)
T_6:0.6% B	3.23 (24.46)	3.26 (5.61)	72.22 (9.28)	76.95 (9.07)	11.17 (4.49)	11.41 (3.25)
SEm±	0.58	0.38	2.35	2.67	0.44	0.71
CD at 1%	1.78	1.16	7.20	8.19	NS	NS

(%) Values in parentheses denote percent increment over control.
T_0: control, T_1: 0.5% Zn, T_2: 1% Zn, T_3: 0.5% Fe, T_4: 1% Fe, T_5: 0.3% B, T_6: 0.6% B.
#As per the treatments, the first and second foliar spray was given at 30 DAS and 40 DAS respectively and the observations were recorded after 10 days of each spray applications coinciding at 40 DAS and 50 DAS

Conclusion

The explosions of Indian population enhance the demand of grains. The high human population needs higher grain production for satisfying the nutritive requirements. Through new innovative technology we will produce more amounts of grains in upcoming decades. Experiment results revealed that the micronutrients (Zn, Fe, B) that are used for the experiment have directly elaborate importance on the morpho-physiological and biochemical parameters and which directly improves the productivity of the crop. And this piece of work is important that opens gate in new research areas and further extensions of application in more crops.

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