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Coffee seedlings growth and nutrient accumulation affected by application of different rates of nitrogen, zinc and boron fertilizers at Dilla, Ethiopia

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Abstract

A proper coffee seedling management is required for successful field establishment. Pot experiment was conducted to evaluate different rates of nitrogen (N), zinc (Zn) and boron (B) on the seedling growth of coffee varieties. It was conducted under lath house condition in complete block design (CBD) with three replications. Two varieties with three levels of nitrogen (0g, 0.295g and 0.59g), zinc, (0mg, 1.62mg, 3.24mg) and boron (0 mg, 0.77mg 1.54mg) were considered. The results indicated that the application of nutrients significantly affected the agronomic performances of coffee seedlings. The average number of roots plant^{-1} was significantly affected by varieties and zinc rates. The highest result (42 roots plant^{-1}) was recorded by the application of 3.24mg Zn plant^{-1}.The highest seedling height (48.2cm) was observed by the application of 1.54mg B plant $^{-1}$ and 0.59 g N plant $^{-1}$ on coffee Fayate variety while 48.1cm was observed by the application of 0.295g N plant $^{-1}$ and 0.77mg B plant $^{-1}$ on variety Odicha. The highest main branch length, 6.9cm and, 6.8cm, were obtained by the application of 0.59g N plant $^{-1}$, 1.54mg B plant $^{-1}$ and 1.62mg Zn plant $^{-1}$ on variety Odicha and Fayate, respectively. Regardless of varietal differences, the highest above ground biomass yield was obtained by the application of 0.59g N plant^{-1} (21.2g plant^{-1}), 0.77mg B plant $^{-1}$ (19.3g plant^{1}) and 1.62mg Zn plant^{-1} (17.8gm plant $^{-1}$). The highest biomass yield obtained from variety Odicha (18.2g plant^{-1}) followed by variety Fayate (15.9g plant). Three way interaction of variety, nitrogen and boron resulted in significant ($p<0.05$) effects on coffee seedlings biomass. The application of 0.59g N plant^{-1} and 0.77mg B plant^{-1} gave the best results on both varieties despite their differences. In most of the cases, 0.59g N, 0.77mg B and 1.62mg Zn plant^{-1}showed significantly the highest results which is recommended for the best and wider coffee seedling multiplication.

Keywords: “Fayate”, “Odicha”, Critical level, Biomass

Introduction

Ethiopia is the first largest producer among the 25 coffee producing countries in Africa and fifth of the world after Brazil, Vietnam, Indonesia and Colombia (AFDB, 2010). In Ethiopia, coffee shared 3.91% of the area under all crops and produces 419,980.16 tons with productivity of 0.76 $tonha^{-1}$ (CSA, 2015). Coffee is still one of the major agricultural export commodities, generating wealth and income, apart from its major social functions (Mauricio**,** 2014).

Despite the existence of enormous genetic diversity and importance of the crop in the national economy of the country, its production potential hardly exceeds 0.62 ton ha^{-1} clean coffee (CSA, 2018), which is by far lower than world average (0.85t ha^{-1}) (FAOSTAT, 2017). Such a low productivity of the crop stems from the use of weak and weepy seedlings with undesirable shoot and root growth for field transplanting (Anteneh et al., 2015). Optimized maintenance of coffee seedlings in nurseries is also very important for successful field establishment (Rochmah et al., 2015) and maintaining fertility of growth media of coffee seedling enhances the production of higher dry matter, healthy and vigorous seedlings for transplanting (Ewnetu et al., 2019). Growth, development and performance of coffee seedlings are determined by both macro and micro nutrients available in the soil or supplied as organic or inorganic fertilizers. Among this nitrogen, phosphorus (P) and potassium (K) are the dominant nutrients required by plants in larger amounts. Nitrogen is reported to directly increase the rate of carbon dioxide assimilation which in turn increases coffee yields (Masaba, 1998).

According to soil fertility status and fertilizer recommendation atlas of the Southern Nations, Nationalities and Peoples' Regional State, Ethiopia, the area where the experiment was conducted is highly deficient in both micro and macro nutrients like N, P, S (Sulphur) and B (ATA, 2016). The bulk soil nutrient analysis indicated the deficiency of Zn at the specific location of the experiment which is lower than the optimum concentration (Edward et al., 2005).

The main purpose of applying fertilizer to young trees is to supply the nutrients necessary to support vigorous and continuous growth of roots and leaves (Young Coffee Orchids, 2015). Nitrogen is regarded as one of the key factors limiting productivity of coffee. However, optimum levels of available N are seldom achieved in practice and there is poor synchronization of N availability and crop demand (Berry et al., 2002.). It was seen that zinc and boron 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

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production (Lakshmipathi et al., 2018). The functional role of Zn includes auxins metabolism, nitrogen metabolism, influence enzymes activity (e.g. dehydrogenase and carbonic anhydrase, proteinases, and peptidases), and cytochrome c synthesis, stabilization of ribosomal fractions and protection of cells against oxidative stress (Obata et al., 1999; Tisdale et al., 1997). Boron plays a pivotal role in a number of vital processes such as root development, cell wall formation, cell division and also in enhancing the uptake of primary and secondary nutrients. Boron also plays an important role in disease and insect pest resistance due to its role in thickening of the cell wall making it difficult for the larvae of insects to penetrate the bark (Aveen and Yogita, 2016).

Farmers are facing shortage of farm yard manure and compost in coffee growing areas (Anteneh et al., 2015). Chemical fertilizers are higher in nutrient content than organic fertilizers and are a more effective method of applying nutrients (Edward et al., 2005). However, no research was conducted by using inorganic nutrient sourced fertilizers for coffee seedling development in Ethiopia. Thus, it was paramount important to look for easy available, efficient and low labour demanding options. Nitrogen, boron and zinc have a great benefit in fastening the growth, increase vigour and other agronomic performance of coffee seedlings. Thus, optimum growth help so as to coincide the planting date with the actual coffee cropping calendar without which coffee producers might be challenged. Therefore, the experiment was conducted to evaluate the effects of nitrogen, boron and zinc fertilizers on the early performance of coffee varieties under lath house condition.

Materials and methods

The experiment was conducted at Dilla substation of Hawassa Agricultural Research Center which is located 100kms away from Hawassa. It's located at a Longitude of 38°18′37″E, Latitude 6°24′38″N and an altitude of 1,570 masl (meters above sea level).

The experiment was executed for six months (until the seedlings were ready for transplanting) under lath house condition and arranged factorial in CRD with three replications. Two coffee varieties (Fayate and Odicha), three levels of Zn (0, 1.62, 3.24 mg plant^{-1}), three levels of B (0, 0.77, 1.54mg plant^{-1}) and three level of N (0, 0.29, 0.59g plant^{-1}) were considered. These rates correspond to 200kg ha^{-1}N, 0, 1, 2kg ha^{-1}Zn and B (0, 0.5 and 1kg ha^{-1}). The two varieties were obtained from Wendo Genet Agricultural Research Center Awada sub-center, Ethiopia. The seedlings were raised in 140mm diameter and 250mm length black polyethylene pot filled with

70% soil and 30% manure. Recommended rate of phosphorus (0.102g P_2O_5 plant^{-1}) was applied uniformly for all pots after the development of two pairs of true leaves. Nitrogen was applied after emergence in split form within 15 days interval three times during the experiment, whereas Boron was sprayed after the development of two pairs of leaves within an interval of 30 days but two times throughout the growth period. The solution was made by using 200L ha^{-1} (Vagner, 2007) which is equivalent to 80mlplant^{-1}. Fifteen seedlings were allocated for each treatment (five seedlings in each replication) and all possible agronomic seedling managements like shading, mulching, weeding, cultivation, watering and regular follow ups were made as the recommendation of Ashenafi et al. (2017). A total of 810 seedlings were examined.

Initial soil analysis for nitrogen was made by using Kjeldahl Method and micro nutrients were analysed by using Mehlich-3 (Table 1).

Data collection

Collection of data was started after six months of emergence when the seedlings were ready for permanent field planting except the data on percent emergence. Emergence percentage, number of roots, main branch length, leaf area, stems girth, dry biomass yield and disease infestation level are some of the agronomic parameters recorded. Distractive measures were implemented for recoding above ground biomass yield, dry matter yield and number of roots plant^{-1}.

The agronomic parameters are described as follows:

Percent of seed emergence: the number of seedlings survived till transplanting stage and computed as proportion of emerged seedlings to the number of seedlings at transplanting stage over 100.

Number of roots/seedling: the number of both tap and adventitious root counted from three randomly selected seedlings and average to one.

Stem/seedling height at transplanting stage (cm): the plant height of three plants, taken at random was measured from soil level to the tip of the longest stem. The mean plant height was recorded after final transplanting stage.

Specific Leaf Area (ELA): the product of the conversion of leaf length and leaf width and expressed in centimetre square (cm^2). The conversion was made by using the following model developed by Carlos et al. (2015).

$$ELA=0.99927\times (L\times (-0.14757+0.60986\times W))$$

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Where ELS=specific leaf area in cm^2, L=leaf length in cm and W=leaf width in cm

Total leaf area (LA): it is the sum total of the value of specific leaf area in the plot. It was estimated by using the following formula:

LA=ELA x number of primary branch x number of leaves per branch

Stem girth (cm): the measure of the diameter of a seedling by using measuring caliper and expressed in centimetre. Main branch length in centimeters: The length of a primary branch of coffee seedling from stem to the tip of the branch and expressed in centimetre.

Biomass yield (g): the total amount of above ground yield of a seedling taken from three randomly selected seedlings and expressed in g plant $^{-1}$. Dry matter content (g): Dry weight of the biomass yield obtained from randomly selected three plants and then oven dry at 72 ^{o}C for 48 hr.

The experimental soil samples were randomly collected based on treatment from experimental pots and then bulked from each replication and mixed thoroughly and send to Horticoop Laboratory for nutrient analysis. The same is true with plant tissue analysis.

Table1. The fertility status of the study soil

Parameters		Unit	Result	Applied Standard for Measurement
Soil pH	pH-H_2O	-	5.70	pH metre
Available Nitrogen	N	%	0.21	Kjeldahl Method
Boron	B	mg/kg	0.65	Mehlich-3
Calcium	Ca	mg/kg	2037.85	
Cupper	Cu	mg/kg	1.28	
Iron	Fe	mg/kg	103.46	
Potassium	K	mg/kg	879.27	
Magnesium	Mg	mg/kg	317.63	
Manganese	Mn	mg/kg	196.68	
Sodium	Na	mg/kg	16.29	
Phosphorus	P	mg/kg	2.22	
Sulfur	S	mg/kg	8.06	
Zinc	Zn	mg/kg	4.32	
Aluminum	Al	mg/kg	858.31	

The nutrient accumulation and concentration was calculated by using the following formula:

Nutrient Accumulation: nutrient concentration x Dry matter content

Nutrient recovery = (nutrient applied-nutrient concentration)/Nutrient applied

Statistical analysis

Agronomic data were subjected to analysis of variance using SAS version 9.2 (SAS, 2008) and mean separation was carried by using Tukey's Test (Tukey, 1953).

Results and Discussion

Analysis of variances result indicated that variety and nutrient main effects showed significant difference in Number of roots plant $^{-1}$ (NR), Seedling height (SH), main branch length (MBL), Leaf area (LA), Stem Girth (SG) and Biomass yield (BY). Two way interaction of variety by nitrogen, boron, variety by zinc, nitrogen by boron, nitrogen by zinc and boron by zinc significantly affected SCHM, LACM2, MBLCM, BYG and DM. Similarly the three way interactions of variety, nitrogen and zinc, variety, nitrogen and boron, variety, boron and zinc, and nitrogen, zinc and boron significantly affected SHCM, SGCM, MBLCM, BYG and DM. Four way interactions of variety, nitrogen, boron and zinc have shown significant difference on MBLCM. Thus, the detail description, mean separation, discussion and recommendation were made on those characters which were significantly affected by different rates of boron, zinc and nitrogen fertilizers, varieties and their interaction (Table 2).

Variety and nutrients main effect

Average number of roots plant^{-1} was significantly ($P<0.05$) influenced by zinc fertilizer application and variety (Table 2).The highest average number of roots (37 roots plant $^{-1}$) was recorded from variety Odicha. Similarly, the highest number of roots (42 roots plant^{-1}) was obtained by the application of 3.24mg plant^{-1} followed 1.62mg Zn plant^{-1} (34.9 roots plant $^{-1}$).This finding confirmed the result obtained by Pedrosa et al. (2013). They stated that, among micronutrients, Zn is important for coffee plant cultivation, especially in the clayey acid soils, where coffee production is an important activity. The report of Taye and Jurgen (2013) also supports the current finding that the number of roots of coffee varied with varieties. In contrast to the present findings, improvement of the root number was achieved by increasing the B concentration (Josten and Kutschera, 1999).

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Table 2. Analysis of variance for variety fertilizer interaction

Source of variation	df	Mean Squares							
		EP	NR	SHCM	LACM^2	SGCM	MBLCM	BYG	DM
Var	1	0^{ns}	499.1^{hs}	174.9^{hs}	0.71^{ns}	0.02^{hs}	6.1^{hs}	212.7^{hs}	33.9^{hs}
N	2	2.5^{ns}	53.1^{ns}	3868^{hs}	663.4^{hs}	0.00^{ns}	300.4^{hs}	815.5^{hs}	2406.2^{hs}
B	2	2.5^{ns}	44.7^{ns}	506.5^{hs}	692.4^{hs}	0.16^{hs}	28.2^{hs}	239.8^{hs}	346.4^{hs}
Z	2	17.3^{ns}	2365.0^{hs}	59.64^{s}	87.2^{s}	0.04^{hs}	3.3^{hs}	31.1^{hs}	56.9^{hs}
Rep	2	39.5^{s}	49.0^{ns}	6.576^{ns}	265.6^{s}	0.01^{hs}	0.3^{hs}	5.96^{hs}	6.0^{hs}
Var*N	2	7.4^{ns}	48.2^{ns}	123.6^{hs}	118.4^{hs}	0.00^{ns}	2.3^{hs}	15.73^{hs}	11.0^{hs}
Var*B	2	7.4^{ns}	0.4^{ns}	4.292^{ns}	18.5^{ns}	0.00^{ns}	1.8^{hs}	17.3^{hs}	0.56^{ns}
Var*Z	2	7.4^{ns}	1.7^{ns}	0.652^{ns}	15.3^{ns}	0.00^{ns}	0.01^{ns}	0.9^{ns}	1.5^{s}
N*B	4	9.9^{ns}	37.7^{ns}	31.2^{ns}	46.5^{ns}	0.00^{ns}	8.3^{hs}	29.1^{hs}	37.5^{hs}
N*Z	4	13.6^{ns}	1.7^{ns}	5.3^{ns}	6.7^{ns}	0.00^{ns}	1.1^{hs}	2.3^{hs}	10.2^{hs}
B*Z	4	2.5^{ns}	5.4^{ns}	1.3^{ns}	18.2^{ns}	0.01^{ns}	0.46^{hs}	0.51^{ns}	11.1^{hs}
Var*N*B	4	14.8^{ns}	10.0^{ns}	49.1^{s}	10.2^{ns}	0.00^{hs}	1.5^{hs}	24.3^{hs}	5.0^{hs}
Var*N*Z	4	3.7^{ns}	8.5^{ns}	3.5^{ns}	4.8^{ns}	0.00^{ns}	0.09^{s}	2.1^{hs}	0.54^{ns}
Var*B*Z	4	14.8^{ns}	5.3^{ns}	0.7^{ns}	11.2^{ns}	0.00^{ns}	0.06^{ns}	0.39^{ns}	0.9^{ns}
N*B*Z	8	9.88^{ns}	7.5^{ns}	12.1^{ns}	5.2^{ns}	0.00^{ns}	0.34^{hs}	2.2^{hs}	8.3^{hs}
Var*N*B*Z	8	10.5^{ns}	14.4^{ns}	10.9^{ns}	11.0^{ns}	0.00^{ns}	0.13^{hs}	0.41^{ns}	0.5^{ns}
Error	106	9.9	17.5	15.08	21.95	0.0004	0.032	0.6	0.4
Total	161								
CV		3.1	11.87	11.28	11.58	6.00	7.8	4.5	5.9

Note: ES=Emergency percent, NR=number of roots/seedling, SHCM=stem height at transplanting stage in centimeters, LAM2=leaf area in square centimeter, SGCM=Stem girth in centimeters, MBLCM =main branch length in centimeters, BYG=biomass yield in grams, DM=dry matter yield in grams: hs=highly significant at p≤0.01, s=significant at p≤0.05, ns=non-significant.

Seedling height during field transplanting stage was significantly different among different rates of N (P<0.01), B (P<0.01) and Zn (P<0.05) fertilizers and between coffee varieties (P<0.01). Among the varieties, variety Fayate recorded 35.4cm which was higher than the value obtained from variety Odicha (33.4cm). The application of the higher rates of N (0.59 g N plant $^{-1}$) and the higher rate of B (0.77mg B plant $^{-1}$) resulted in higher seedling height. This implies that the soil of the study area is deficient in these nutrients and they are important for coffee growth.

There was no significant difference between the two varieties while the application of different rate of nitrogen boron, and zinc showed significant differences (Table 3). The application of 0.59g N, 0.77mg B and 1.62mg Zn plant^{-1} gave the highest value 44.5, 44.4 and 41.9 m^{-2}. Significant increase in leaf area could be due to increased metabolic activity by increased supply of nutrients (Wahdan et al., 2011).

There was statistically significant difference between varieties and among different rates of N, B, and Zn nutrients on the main branch length. The average main branch length of variety Odicha (2.5cm) was slightly longer than Fayate were (2.1cm). Similarly, fertilizer rates N (0.59gm plant^{-1}), B (0.77mg plant^{-1}) and Z (1.62 mg plant^{-1}) application were resulted in the highest main branch length of 2.6cm, 2.5cm and 2.9cm, respectively, regardless of variety differences. This implies that the nutrients application is required to obtain vigorous seedling growth with longer main branches (Table 3).

Variety, boron and zinc showed significant differences but their interaction with the exception of boron by zinc were none significant. The stem girth observed on variety Odicha was slightly higher than variety Fayate (Table 3). The application of 0.77mg and 1.62mg plant^{-1} gave the highest result 0.40cm and 0.37cm respectively. Application of B showed a further increase in stem diameter, with the thickest stem caused by 50 μM B while increasing the amount of B to 75 tended to decrease the stem diameter of cut rose (Mahboobeh et al., 2012).

Coffee seedling biomass yield was significantly different among rates of fertilizers nitrogen, boron and zinc application, and between the two coffee varieties. The highest biomass yield (18.2gm plant^{-1}) was obtained from coffee variety Odicha (Table 3). Regardless of varietal differences, the highest biomass yield was obtained by the application of 0.59g plant^{-1} N, 0.77gm B and 1.62g Zn plant^{-1} gave the highest biomass yield 21.2g plant^{-1}, 19.3gplant^{-1} and 17.8g plant^{-1}, respectively. The report by Jacqueline et al. (2019) indicated that lower biomass accumulation was observed only for deficient seedlings at the end of the experiment. The nutritional deficiency of some essential nutrient prevents the maximum potential productivity of plants. According to Serra et al. (2011), the fresh and dry biomass production from medicinal plant *Pfaffiaglomerata pedersen* (Spreng.) was negatively influenced by nitrogen (N).

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Table 3. Coffee seedling growth agronomic parameters as affected by variety, N, B and Zn

Treatments	RNP	SH (cm)	MBL (cm)	LA (cm^2)	SG(cm)	BY (gm plant $^{-1}$)
Variety						
Fayate	33.5	35.4	2.1	40.4	0.32	15.9
Odicha	37	33.4	2.5	40.5	0.35	18.2
MSD	1.3**	1.2**	0.06**	1.5 ^{NS}	0.006**	0.2**
CVSR	2.8	2.8	2.8	2.8	2.80	2.8
Nitrogen rates (g plant^{-1})						
0	36.2	26.1	0.0	39.0	0.33	13.4
0.295	35.4	34.1	2.2	38.0	0.34	16.5
0.59	34.3	43	4.7	44.5	0.34	21.2
MSD	1.9^{NS}	1.8**	0.08**	2.1**	0.009^{NS}	0.4**
CVSR	3.36	3.36	3.4	3.4	3.36	3.4
Boron rates g plant^{-1}						
0	35.1	31.2	1.6	39.7	0.29	15.1
0.77	34.5	37.3	3.0	44.4	0.40	19.3
1.54	36.3	34.8	2.3	37.4	0.34	16.8
MSD	1.9 ^{NS}	1.8**	0.08**	2.1**	0.009**	0.4**
CVSR	3.36	3.36	3.4	3.4	3.36	3.4
Zinc rates g plant^{-1}						
0	28.85	33.4	2.1	39.5	0.31	16.3
1.62	34.94	35.5	2.6	41.9	0.37	17.8
3.24	42.07	34.3	2.3	40.0	0.34	17.0
MSD	1.9 **	1.8*	0.08**	2.1*	0.009**	0.4**
CVSR	3.36	3.36	3.4	3.4	3.36	3.4

Note: RNP=root number per plant, SH=seedling height in cm, MBL=main branch length in cm, LA=leaf area in cm^2, SG=stem girth in cm, BY=biomass yield gm per plant, *=significant at $p<0.05$, ** highly significant at $p<0.01$, NS = non-significant

Variety and nutrient interaction effect

Seedling height at planting stage

In the same way seedling height was significantly affected by the interaction of variety x nitrogen ($P \leq 0.01$), variety x nitrogen x boron ($P \leq 0.05$). In the two way interaction, both varieties, Fayate and Odicha, recorded the highest seedling height (43.2 and 42.9cm, respectively) at 0.59g N plant^{-1} (Table 4). Similarly both varieties produced the highest seedling height at the combined application of 0.59g N and 0.77mg B plant^{-1} (Table 5). The results obtained in this experiment is in agreement with the findings of Quddus et al. (2014), who had conducted an experiment on lentils by the application of different rate of zinc and boron fertilizers. They stated that the yield contributing characters like plant height showed significant variation due to different levels of boron fertilizer application. In the same way, Baktear et al. (2001) indicated that plant height of rice was statistically significantly affected by the application of different rate of Zn and boron. The highest plant height (120cm) was obtained by the application of 6kg Zn ha^{-1} and 3kg B ha^{-1} in combined form. The plant height of tomato also increased from 77.22 cm to 88.14 cm with increasing B from 0 to 0.25%, but adding more B up to 0.5% decreased the plant height (84.78 cm) of tomato (Haleema et al., 2018). In line with this, Chemura et al. (2013) illustrated the importance of the readily available N from the inorganic fertilizer was fundamental in producing taller coffee plants.

Table 4. Seedling heights of coffee varieties affected by different rate of nitrogen

	Nitrogen level (g plant^{-1})		
Variety	0	0.295	0.59
Fayate	26.61	36.86	43
Odicha	25.57	31.37	43.15
MSD		3.1	

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Table 5. Variety, nitrogen and boron interaction effect on coffee seedling height (cm)

	V1 (Fayate)			V2 (Odicha)		
Boron/Nitrogen*	N1	N2	N3	N1	N2	N3
B1	21.94	34.92	40.74	23.94	28.84	36.65
B2	30.33	37.93	46.28	26.95	33.43	48.67
B3	27.55	37.72	41.57	25.81	31.85	44.15
CVSR	5.1					
MSD	6.5					

* N1=0gmNplant $^{-1}$, N2=0.295gmNplant $^{-1}$, N3=0.59gmNplant $^{-1}$, B1=0mgBplant $^{-1}$, B2=0.77mgBplant $^{-1}$ and B3=1.54mgBplant $^{-1}$

Leaf area

Plant leaf area is an important determinant of light interception and consequently of transpiration, photosynthesis as well as plant productivity. In other words, leaf area is the most important parameter for the prediction of the performance of a crop at its earliest stage. The two ways, varieties by nitrogen interaction, showed highly significant effects. Accordingly, the highest value 44.9 and 44 cm^2 from Fayate and Odicha; respectively were obtained by the application of 0.59g N plant^{-1} which suggest that the contribution of nitrogen was higher than any other nutrients in increasing the leaf area of coffee seedlings (Table 6).

Table 6. Variety by nitrogen interaction affecting the leaf area performance of coffee seedlings

	Nitrogen rates		
Variety	N1	N2	N3
1 (Fayate)	40.1	36.2	44.9
2 (Odicha)	37.9	39.7	44.0
CVSR	4.1		
MSD	3.7		

Stem girth

The interaction of boron and zinc were significant as the combined application of 0.77mg and 1.62mg plant^{-1} scored the highest stem girth as shown in Fig.1. Application of B showed a further increase in stem diameter, with the thickest stem caused by 50 μM B while increasing the amount of B to 75 tended to decrease the stem diameter of cut rose (Mahboobeh et al., 2012).

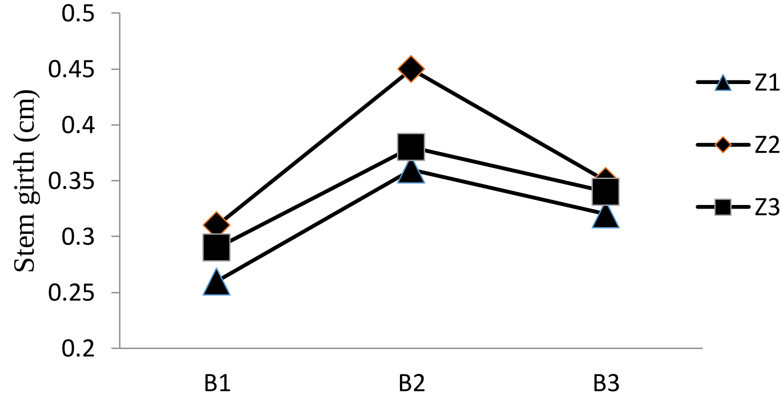

Fig. 1 The effect of Boron-Zinc interaction on the stem girth of coffee seedlings

Main branch length and biomass yield

Except for variety by nitrogen interaction, main branch length of coffee seedlings was significantly affected by both main and interaction effects of the treatments.

The highest main branch length of coffee seedlings were recorded when combined 0.59g N, 0.77mg B and 1.62mg Zn plant^{-1} applied to both Odicha and Fayate varieties (Figure 2). This indicated that main branch length of seedlings of coffee varieties could be governed by different rate of N and micro nutrients (B and Zn) (Figure 2)[1] as the application of N, B and Zn increases the internodes length of crops which is the component part of branches and stems (Barbara et al., 2018).

Biomass yield

The two way Nx V and VxB interactions indicated that the application of N3 and B2 gave the highest biomass yield. The application of N3 and B2 on variety Odicha recorded 21.7 and 19.8gm plant^{-1} (Table7). In the same way the highest biomass value 20.6gm plant $^{-1}$ and 18.8gm plant $^{-1}$ respectively were recorded on variety Fayate by the application N3 and B2. However, the difference between varieties was at par. The interaction of N and B to improve growth parameters of the crop suggesting better assimilation of enhanced nitrogen in the presence of sufficient boron contents, since boron was found to control most of elemental uptake and assimilation in the shoot tissues (Shaaban et al., 2004).

[1] V1=Fayate, V2=Odicha, N1=0g N plant^{-1}, N2=0.295g N plant^{-1}, N3=0.59g N plant^{-1}, B1=0mg B plant^{-1}, B2=0.77mg B plant^{-1}, B3=1.54mg B plant^{-1}, Z1=0mg Zn plant^{-1}, Z2=1.62mg Zn plant^{-1} and Z3=3.24mg Zn plant^{-1}

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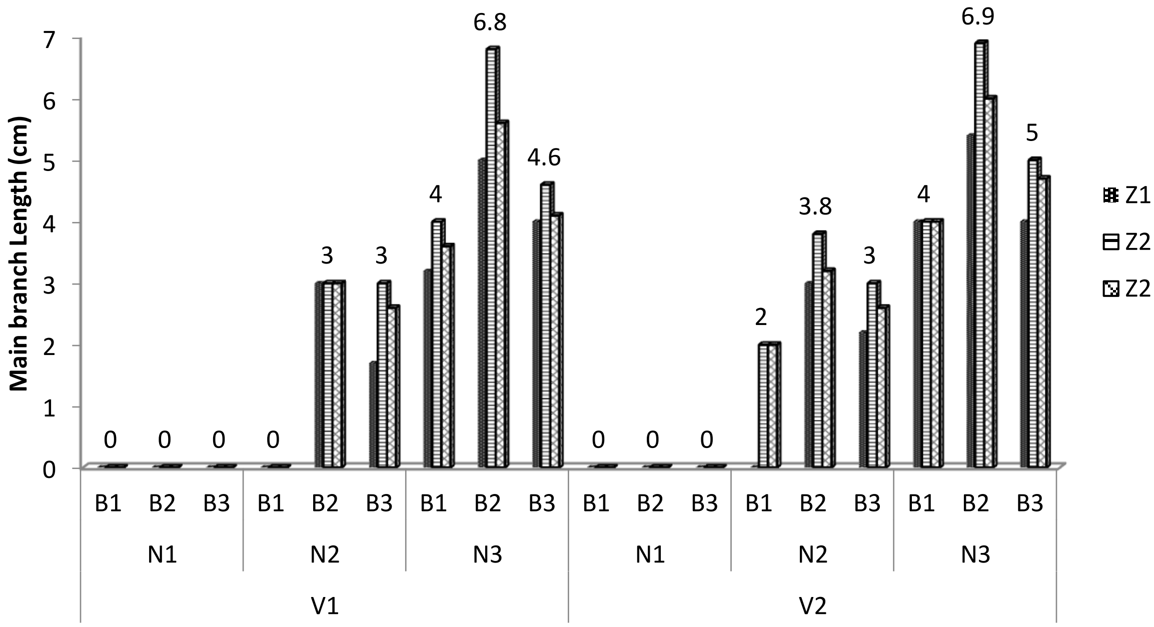

Figure 2. Variety, nitrogen boron and zinc interaction effect on main branch length of coffee seedlings

Table 7. Variety by Nitrogen and Variety by boron interaction affecting biomass yield of coffee seedlings

	Nitrogen			Boron		
Variety	N1	N2	N3	B1	B2	B3
V1	12.2	14.9	20.6	13.5	18.8	15.4
V2	14.6	18.2	21.7	16.7	19.8	18.1
CVSR		4.1			4.1	
MSD		0.6			0.6	

In the same way, the interaction of nitrogen with zinc also showed significant effects on biomass of the seedlings regardless of the difference of varieties. The highest value was recorded by the combined application of N3 and Z2 which yielded 22.4 gm plant $^{-1}$ (Table 8).

Table 8. Effect of different rate of N, B, and Z fertilizers on the biomass yield of coffee seedlings

	Boron			Zinc		
Nitrogen	B1	B2	B3	Z1	Z2	Z3
N1	11.5	15.1	13.7	12.6	14.2	13.5
N2	15.3	17.7	16.6	16	16.9	16.6
N3	18.4	25	20.0	20.3	22.4	20.9
CVSR		4.8			4.8	
MSD		0.8			0.8	

Even though the overall interaction of varieties by different fertilizers rate was statistically none significant, the three way interaction of variety, nitrogen and boron resulted in significant effects on coffee seedlings biomass. The application of 0.59gm N plant $^{-1}$ and 0.77mg B plant $^{-1}$ gave the best results on both varieties (Table 9). The result confirmed the report made by Lukas et al. (2015) that the application of micronutrients at the sites with sufficient content of those micronutrients in soil has almost no effect on achenes and biomass yield and the opposite is true with the sites devoid of micronutrients like Zn and B.

Table 9. Variety, nitrogen by boron and variety nitrogen by zinc three way interaction affecting biomass yield of coffee seedlings

	V1			V2		
N/B	N1	N2	N3	N1	N2	N3
B1	10.1	13.9	16.4	13	16.7	20.5
B2	14	15.7	26.6	16.2	19.6	23.5
B3	12.6	14.9	18.8	14.8	18.2	21.3
CVSR	4.95					
MSD	0.9					
N/Z	N1	N2	N3	N1	N2	N3
Z1	11.4	14.6	19.4	13.9	17.5	21.1
Z2	12.9	15.2	22.4	15.5	18.6	22.4
Z3	12.4	14.9	20	14.6	18.4	21.7
CVSR	4.95					
MSD	0.9					

Dry matter yield

Dry matter yield of coffee seedlings was significantly different among nitrogen, boron and zinc fertilizers application, and between the two coffee varieties (Table 10). Except for variety by boron interaction there were also all two way interaction (V x N, V x Zn, N x B, B x Zn), except V x B, were observed to significantly affect dry matter yield of coffee seedlings. Even though overall four way interaction was non-significant variety by nitrogen by boron and nitrogen by boron by zinc three way interactions showed statistically significant results. The combined application of N2, B2 and Z2 showed the highest dry matter (26.5g plant $^{-1}$). The difference in varieties by their dry mater yield was also reflected as the highest value was recorded by variety Fayate (22.1g plant $^{-1}$) compared with variety Odicha (21.6g plant $^{-1}$). The current study proved the study conducted by Lima and Malavolta. (1998). They reported positive interaction between B and Zn in affecting coffee seedling dry matter production, and when the nutrients were supplied together, the dry matter production was 21% higher than single application. Similarly, Lorenzo et al. (2019) indicated that the DW increase was 28%, 85%, and 20% in roots, stems, and leaves of coffee plants, respectively.

Table 10. Three way variety by nitrogen by boron and zinc by nitrogen by boron interaction effect on the dry matter yield of coffee seedlings

	N1			N2			N3		
Variety/zinc	B1	B2	B3	B1	B2	B3	B1	B2	B3
V1	3.3	5.2	4.4	14.1	22.1	16.5	5.9	10.4	7.4
V2	3.7	5.8	5.1	15.3	21.6	16.5	6.8	13.8	9.1
CVSR					5.1				
MSD					1				
Z1	2.9	5.4	4.4	14.2	18.4	15.8	5.9	10.8	7.4
Z2	4	5.7	5.1	15.2	26.5	17.3	6.9	13.6	9.1
Z3	3.6	5.5	4.7	14.6	20.5	16.4	6.3	12	8.2
CVSR					5.4				
MSD					1.4				

Residual soil B and Zn nutrients content

Compared with the initial soil content, the amount of boron and Zinc contents were increased with the increasing rate of B and Zn nutrients application to soils. The highest born in mgkg^{-1} of soil sample was obtained from the plot that received the highest born rate (1.54 mg plant $^{-1}$), which indicated that the entire amount of nutrients applied was not utilized by the crop (Table 11). Similarly, the amount of residual soil Zn content also varied with the amount applied and varieties. The soil content of Zn in the soil that received the highest amount was higher. The plot that received Z3, N2 and B3 has got 94.37mg kg^{-1} of soil sample followed by Z3, B3 and N1. The amount of Zn in this study might be a bit higher than the optimum amount (2-10mg/kg) recommended by Abayneh and Fiseha (2015) and 15 - 30 mg/kg, in the soil sample (Edward et al., 2005). The difference might be attributed to the extraction method employed by the two independent studies.

Table 11. Boron and zinc concentration, and recovery of nutrients in the experimental pot

Treatment	Concentration mg/kg	Recovery
Boron rate		
B1	1.2	1
B2	2.3	0.98
B3	2.5	1
CVSR	3.47	3.47
MSD	0.26	0.26
Zinc rate		
Z1	26.6	1
Z2	54.3	0.98
Z3	82.1	0.97
CVSR	3.47	3.47
MSD	17.1	0.26

Boron and zinc concentration, accumulation and recovery of coffee seedlings leaf

Boron content in leaves that received B fertilizer showed higher amount than the control treatment and the ability to uptake nutrient was different between varieties. On variety Fayate, B and Zn concentration was higher than Odicha (Table 12). The highest value of boron (72.19mg kg^{-1}) was recorded from the leaves of coffee variety Fayate that received 0.5kg ha^{-1}B, 1kg ha^{-1} Zn and zero nitrogen while the smallest value was observed from the treatment that received lower amount (zero B ha^{-1}). The same is true with coffee variety Odicha (Table 12). Application of B increased the total amount of B in the leaves that received higher amount but decreased with increasing rate of boron. Similar result was reported by Junia et al.(2018), who showed that index leaves of the coffee plants that received B as foliar spray or solid injections with B, B + Cu, or B + Zn presented higher contents of B than the control treatment without boron (WB). In the same way, Cong (2017) reported that supplying B to coffee on basaltic soil in Central Highlands of Vietnam remained appropriate B content in leaves. The amount Zn in leaves of coffee varieties also varied with the supplied amount and varieties. The highest value was observed in the coffee seedling leaves that received B2, N3 and Z3 which is higher than the optimum value of Zn for coffee as reported by Abayneh and Fiseha (2015). There was also the difference in the uptake of varieties. Variety Fayate has great ability to take up nutrients than Odicha as the average amount of Zn in the leaves of Fayate (66.04mg/kg) compared with Odicha (61.33). The result is an agreement with Hossain et al. (2011) who indicated that the absorption of nutrients is different between varieties. It is due to the differences of varieties ability to uptake nutrients based on the function of each plant part.

Table 12. Boron and Zinc concentration, accumulation and recovery of coffee seedlings leaf

Treatment	Concentration	Accumulation	Recovery
Boron rate			
B1	29.4	241.08	1
B2	44	580.8	0.99
B3	36.7	362.596	1
CVSR	3.41		
MSD	9.1		
Zinc rate			
Z1	32	236.8	1
Z2	66.5	605.15	0.89
Z3	92.5	758.5	0.94
CVSR	3.41		
MSD	19.05		

Conclusion

Coffee seedlings that received both macro nutrient (nitrogen) and micro nutrients (boron and zinc) have shown the highest performance in terms of number of roots plant $^{-1}$, seedling height at transplanting stage, leaf area, stem girth, main branch length, biomass yield in grams and dry matter yield. In most of the cases, the application of 0.59gm N plant $^{-1}$, 0.77mg plant $^{-1}$ and 1.62mg plant^{-1} resulted in the highest performance which can be applied by coffee farmers and large scale producers in the study area and areas with similar agro-climatic conditions.

Thus, it can be concluded that all nutrients contributed for the healthy growth and vigour of seedlings which intern have a great influence on the establishment and field performance of coffee crops. Chemical fertilizers are higher in nutrient content than organic fertilizers and are a more effective method of applying nutrients. To explore the benefit of both methods, it should not be denied the integrated nutrient application and wider environmental testing in the future.

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