Australian Journal of Crop Science AJCS

AJCS 5(12):1649-1654 (2011) ISSN:1835-2707

Application of boron and zinc in the tropical soils and its effect on maize (*Zea mays*) growth and soil microbial environment

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Abstract

An experiment was conducted at glasshouse of Universiti Putra Malaysia with boron (B) and zinc (Zn) deficient soil (Serdang series) to evaluate the effect of B and Zn on maize crop and the behavior of soil microbial communities with various levels of boron and zinc. Among the six tested soil series, Malawi soil was found the most deficient in boron (0.06 ppm). Four levels of B from Borax as 0, 0.5, 1.0, and 1.5 kg ha^{-1} and two levels of Zn from Zinc Sulphate, 0 and 5 kg ha^{-1} were applied. The highest plant height (109 cm), root length (30.67 cm), leaf area index, chlorophyll content, shoot (5.38 g) and root dry weight (0.23 g) were obtained at $B_{0.5}$ + $Zn_{5.0}$ kg ha^{-1} treatment. The interaction effect between boron × zinc in the soil was significant. Antagonistic effect occurred for B uptake at higher rates of B with 5 kg ha^{-1} of Zn application. The addition of B at lower level alone or in combination with Zn significantly increased soil bacterial population. The highest rhizosphere bacterial population was found at $B_{0.5}$ + Zn_5 kg ha^{-1} treatment. The addition of $B_{1.0}$ kg ha^{-1} with zinc Zn_5 kg ha^{-1} showed a toxic effect on soil bacterial population. Although B produced substantial biomass yield increment with extended rates, the excess amount caused toxicity in the soil bacterial environment. It can be concluded that 0.5 kg ha^{-1} of B in combination with 5 kg ha^{-1} Zn can exhibit higher yield of maize and is friendly to this particular soil environment.

Keywords: bacterial population, micronutrients, rhizosphere, toxic environment.

Abbreviations: ICP-Inductivity Coupled Plasma; LAI-Leaf area index; MOP-Murate of potash; SPAD-soil plant analysis division; TSP-Triple superphosphate; YEL-youngest expanded leaf.

Introduction

Maize (*Zea mays*) is widely cultivated grain crop throughout the world. In the tropics, more than 8 million hectares of acid soils are planted with maize. This crop is not acid soil-tolerant (Pandey and Gardner, 1992), but increases in the demand for cereals in developing countries have led to an increase in maize acreage on acid soils. Acidic soils, therefore, generally have a low pH, contain toxic levels of Al and Mn, and are deficient in Ca, Mg, P, K, and in micronutrients. The characteristics limit the fertility of acid soils and inhibit root development, leading to low water and nutrient uptake and low maize yields (Duque-Vargas et al., 1994). In addition severe boron and zinc deficiency is one of these incidences that also causes low yield of maize. The use of acid soil-tolerant maize cultivars and with appropriate fertilizer management practices can provide an environmental friendly, inexpensive, and permanent solution, of maize production on these soils. Micronutrients are those trace elements which are necessary for the normal healthy growth and reproduction of plants and animals. Among the micronutrients boron (B) deficiency is the second most widespread micronutrient problem (Alloway, 2008). Boron is important for crop production from the both point of view, its effects in deficiency and excess. When B is deficient plant root systems are often stunted and less effective. Deficiencies of B occur in a wider range of crops and climatic conditions than deficiencies of any other trace elements. Boron regulates metabolism of carbohydrates, activates certain dehydrogenase enzymes, aids in formation of pollen tube and feeder roots, involved in translocation of Ca, sugars, and plant hormones, it also facilitates the synthesis of nucleic acids which is essential for cell division and development (Reisenauer et al.,1973). At the opposite end of the biological spectrum, B essentially has been established for the growth of specific types of soil bacteria, such as heterocystous cyonobacteria (Bonilla et al., 1990). Microorganisms require B for the stability of the envelopes that prevent access of nitrogenase- poisoning oxygen (Bolanos et al., 2004). On the other hand a wide range of crops are affected by zinc deficiency, including cereals. When crops have a deficient supply of Zn, yield reduced and quality of the crop product may also suffer. Losses up to 30% in the yield of cereal grains in crops such as maize, wheat and rice have been observed (Alloway, 2001). Zinc is required in small but critical concentrations to allow several key plant physiological functions in plants. The soil fungi, bacteria and actinomycetes populations increased by the application of zinc (Reddy, 1968). However the toxic effects of zinc and boron in plants and soil are now well documented. In the recent years, several reports have been also documented for the harmful effects of zinc and boron toxicity on soil microorganisms and microbial activities (Takkar and Mann, 1978). In addition, application of additional quantities of N, P and micronutrients deficiencies can be maintained in maize, besides this can be increased the productivity, profitability and maintained sustainable soil resource base (Rajashekhara Rao, 2010).

However, there has been little research done on the effects of micronutrients such as B and Zn on the maize crop, especially under acidic conditions in tropical regions. On the other hand, the soil microbial community is a sensitive indicator of metal effects on bioavailability and biochemical process. Hence, present study was undertaken to evaluate the effect of different levels of boron and zinc on maize crop and the behavior of soil microbial communities at various rates of boron.

Results

Fertility status of soil series

Six soil series were selected for the fertility status before experiment and belong with different soil orders. The soils were low in fertility status and sandy to silty caly, and clay loam in texture with an electric conductivity that ranges from 0.5-2.81 dSm^{-1} (Table 1). Malawi and Saujana soil series were sandy in texture and contain very low amount of organic carbon. In general soils of the series were acidic to slightly acidic in nature and pH lies within 4.2- 5.1. The lowest soil pH (4.2) was recorded in Selangor soil series. Among the tested six series Bakau soil contains higher amount of phosphorus, zinc and boron compared to others (Table 2). For the present study Selangor soil series was selected which contain 0.51 ppm B and 1.54 ppm Zn which was considerably low in fertility status.

Effect of boron and zinc on the seed germination, leaf area index and leaf chlorophyll content of maize

The germination of maize seed was significantly influenced by the different rates of B and Zn fertilizers. The highest germination (80%) was observed at $B_{0.5} + Zn_0$ kg ha $^{-1}$ and $B_{1.0} + Zn_0$ kg ha $^{-1}$ followed by the $B_{0.5} + Zn_{5.0}$ kg ha^{-1} (76 %) respectively (Table 3). The leaf area index was affected by the rates of the fertilizers also. Significantly highest leaf area index (0.31 cm^2) was found at $B_{0.5} + Zn_{5.0}$ kg ha $^{-1}$, while leaf area index was decreasing with the increases rates of B. The interaction effect between B × Zn highly significant (P = 0.001). The leaf chlorophyll content (SPAD values) was taken at different times and it was varied with the time and treatments. Generally higher values were observed at 15 days and lower values were found after 45 days of sowing. The chlorophyll content was observed with wide range varied from 23.41 to 32.98 during the planting period. The significantly high value was recorded at $B_{0.5} + Zn_{5.0}$ kg ha^{-1} treatment.

Plant uptake of boron, zinc, plant height and biomass yield of maize

The concentration of B and Zn in plant tissues and its uptake were higher at the higher rates of applied fertilizers. An antagonistic effect found for B uptake with higher levels of B and 5 kg ha^{-1} of Zn. On the other hand a synergistic effect was found for Zn uptake at 0.5 kg ha^{-1} of boron. Significantly high uptakes values of B (1.09 mg plant^{-1}) and Zn (3.3**7** mg plant^{-1}) were obtained from the $B_{1.5} + Zn_0$ kg ha^{-1} and $B_{0.5}$ + Zn_5 kg ha^{-1} treatments (Table 4). While the lowest values obtained in control treatments. Plant tissue Zn uptake affected with different levels of boron application. The highest plant shoot, root length (Fig. 2) and total biomass obtained at $B_{0.5}$ +Zn_5 kg ha^{-1} treatment (Table 3). Plant dry biomass weights were decreased with the increased rate of B and Zn applications. The highest biomass yield increment (127.13%) was observed by $B_{0.5}$ + Zn_5 kg ha $^{-1}$ over control treatment.

Effect of boron and zinc on total bacterial population in maize

The total bacterial population was fluctuated with the B and Zn applications. The rhizosphere population was significantly higher than non-rhizosphere population. The highest rhizosphere and non-rhizosphere population found in $B_{0.5}$ + Zn_5 kg ha^{-1} treatment followed by control and $B_{1.5}$ kg ha^{-1} treatment, respectively (Fig. 3). The addition of B alone or in combination with boron and zinc significantly increased soil bacterial population at B 0.5 kg ha^{-1} and Zn 5 kg ha^{-1} treatments. The addition of $B_{1.5}$ kg ha^{-1} with zinc showed reverse or toxic effect on soil bacterial population.

Discussion

Results of this study proved a positive effect of B (0.5 kg ha^{-1}) and Zn (5 kg ha^{-1}) application along with other macro nutrients for maize crop growth in the acidic soil. The evidences of increased plant biomass, leaf chlorophyll content and plant height showed beneficial effect of addition of paramount dose of B and Zn in such soil. A synergism effect for Zn uptake observed between the two nutrients when boron was applied up to 0.5 kg ha^{-1}. However, the plant uptake of boron induced by 1.5 kg ha^{-1} B with decrease of plant biomass, showing a reverse effect. While the lowest total N % and plant biomass were found for the control plot while higher values were found in fertilized treatments. Application of B plays an important role to increase maize biomass as it has an important role for storage of assimilates. Kanwal et al. (2009) found a significant constant increase in shoot dry matter production of the four maize cultivars by adding Ca and B to the root medium. In the present study higher root and shoot biomass obtained at $B_{0.5\ kg\ ha^{-1}}$ +$Zn_{5.0\ kg\ ha^{-1}}$ treatment. The addition of B increased tissue B uptake up to 1.5 kg ha^{-1} applied boron while, Zn uptake affected. Similarly, the shoot dry matter yield of maize plant declined with higher rates of B application, where as increased with Zn application alone. Aref (2010) found a synergistic effect between B and Zn and increased in plant uptake with high soil concentration of these elements. This might be more B uptake of plants in B deficient soils, hence B toxicity is uncovered and the plant growth was affected negatively (Shaaban et al., 2004). On the other hand, application of 5 kg ha^{-1} Zn increased tissue Zn concentration as well as plant biomass in this deficient soil. Zn deficiency and B toxicity are elements concentration of maize plant and the interactions of nutrient are significant for plant nutrition (Alkan et al., 1998). Although there was an antagonistic effect for nutrient uptake found between B and Zn at higher levels of B with 5 kg ha^{-1} Zn. Zinc play important role in leaf chlorophyll synthesis (Cakmak, 2008). In the present study application of Zn increased leaf chlorophyll content while at the higher rates of B alone or with combination of Zn chlorophyll values decreased, this might due to toxicity effect. Similar findings were reported by Akta et al. (2004) who conducted wheat experiment in pots under greenhouse conditions and found that with the increase of B rates, the chlorophyll amount of leaf samples decreased. Soil microbes need micronutrient for their growth

Table 1. Chemical properties and texture of the soil series

S.No.	Soil series	EC (dS/m)	pH	Organic Carbon (%)	Texture
01	Munchong (Oxisols)	2.21a	4.7b	3.24b	Clay
02	Serdang (Ultisols)	1.12b	4.5b	1.37c	Sandy Clay Loam
03	Bakau (Entisols)	0.53c	5.1a	5.11 a	Silty Clay
04	Selangor (Inceptisols)	2.81a	4.2b	0.87d	Silty Clay Loam
05	Malawi (Entisols)	1.23b	4.9a	0.14e	Sandy
06	Saujana (Entisols)	1.37b	4.5b	0.16e	Sandy

Different letters in the columns are significantly different at $P \leq 0.05$

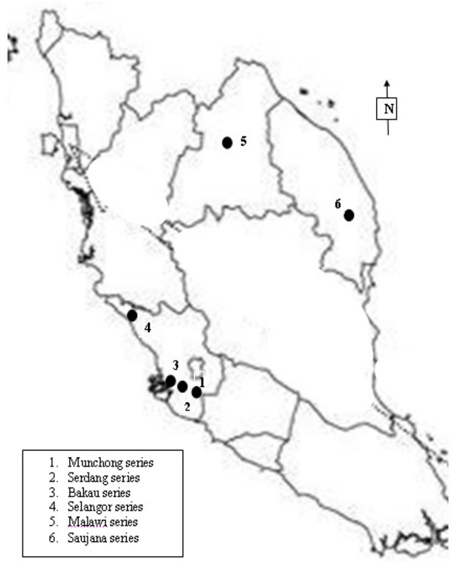

Fig 1. Map of west peninsular Malaysia is showing sample location of the different soil series

and activity but in trace amount. Excess amount causes toxicity. There were significant adverse ($P \leq 0.01$) impact of bacterial population found at higher rates of B and Zn fertilizer application. The study with micronutrients on rice rhizosphere bacterial population also showed similar effects (Reddy, 1968). In the present study it was found that highest rhizospheric bacterial population occurred only when 0.5 kg ha^{-1} B applied. A negative impact obtained at 1.0 kg $ha^{-1}$of B. Another evidence of an incubation study with increased zinc concentration from 50 to 150 mg kg^{-1} showed significantly decreases the total bacterial population (Cevik and Karaca, 2007). Higher rates of B alone and with Zn significantly reduced the soil and rhizosphere bacterial population.

Materials and methods

Sample collection from different soil series

Before experiment, a field survey was conducted to determine the status of boron (B) and Zinc (Zn) deficient soils. Soil samples were collected from six different soil series (Fig 1) as (1) Munchong series, (2) Serdang series from the UPM uncultivated field, (3) Bakau series from Tanjong Karang Selangor, (4) Selangor series from Banting Talok Datok Oil palm field, (5) Malawi series from Bachok, Kelantan and (6) Saujana series from Besut, Terengganа at the surface horizon (0-15 cm) of the soil. Samples were air dried and prepared for the laboratory analysis. After analysis Serdang series was selected for the experiment due to its low boron status and medium status of zinc and other nutrients with appropriate soil texture. Plants were grown for 45 days.

Soil chemical analysis

Soil pH was measured in soil; water (1:2.5) extract using PHM210 Standard pH meter at 300C (Benton, 2001). Total N in soil was determined by Kjeldahl digestion method (Bremner and Mulvaney, 1982). Phosphorus was extracted using Bray and Kurtz No. 2 extractants (Bray and Kurtz, 1945). Samples were analyzed for organic carbon using Carbon Analyzer (Benton, 2001). Soil texture was determined using the pipette method (Gee and Bauder, 1986).Available B was analyzed determined by hot water extraction method and was measured colorimetrically using azomethine-H (Bingham, 1982). Boron in plant tissue was determined by dry ashing followed by azomethine-H colorimetric (Benton, 2001). While, other micronutrients were determined by Double acid method on the Inductivity Coupled Plasma (ICP).

Seed surface sterilization

The seed surface sterilization method was modified from Amin et al. (2004). Maize seeds were soaked in 70 percent ethanol for 5 min. The ethanol was discarded and the seeds were agitated in hypochlorite solution comprising 3 percent of Chlorox ™ (2.6 % NaOCl) and washed with sterilized distilled water. Surface sterilized seeds were grown in Petri

Table 2. Nutrient analysis in the soils studied

S.No	Soil series	N (%)	Av P	Exch. K	B	Zn	Fe	Cu	Mn
			--------------------			mg kg^{-1}			-----------------------
01	Munchong	0.12a	26.49b	91.80c	0.83a	0.30e	138c	1.05c	4.42c
02	Sri Serdang	0.11b	24.58b	108.75a	0.50b	2.21b	199b	2.29a	5.96b
03	Bakau	0.13a	16.89c	101.25b	0.83a	6.56a	209a	1.55b	9.36a
04	Selangor	0.12a	32.23a	115.50a	0.51b	1.54c	188b	0.74d	7.09b
05	Malawi	0.07c	7.63d	20.70d	0.06d	0.90d	54c	0.26e	0.20d
06	Saujana	0.08c	2.51e	2.95e	0.27c	0.91d	25d	0.33e	0.05d

Different letters in the columns are significantly different at $P \leq 0.05$

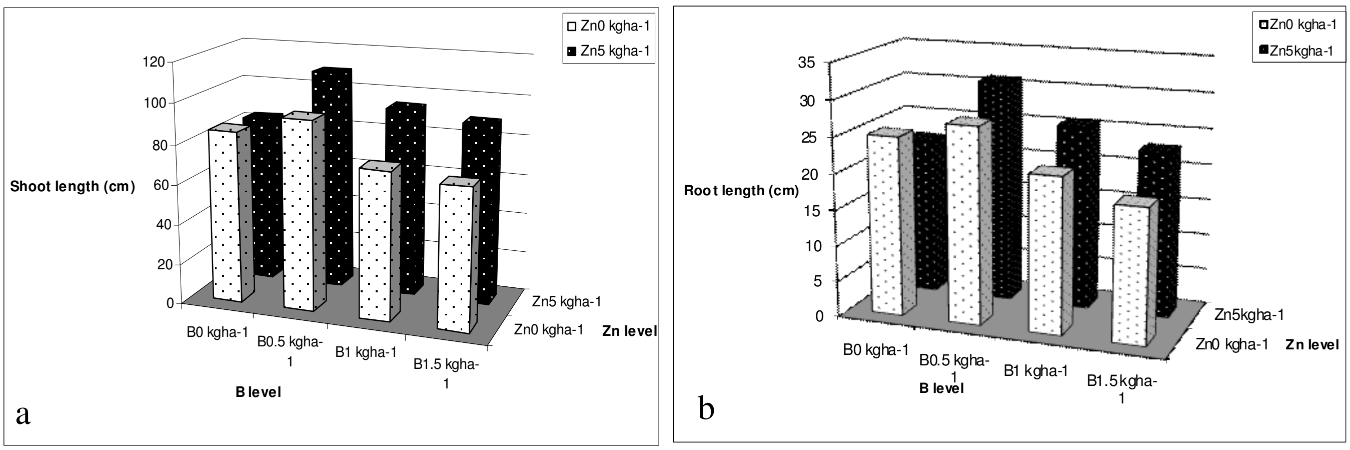

Fig 2. Effect of different levels of boron and zinc on plant height: (a) shoot length (b) root length of maize

dishes lined with filter paper. Sterile distilled water was added to wet the seed. The efficacy of sterilization was checked by germinating seeds on nutrient agar plates.

Soil preparation, planting and fertilizer application

The experiment was conducted in pots at glasshouse of Universiti Putra Malaysia. About 5 kg of soil (Serdang soil series) was used in each pot. Ten seeds of maize variety 'Sweet corn Mardi' were sowed in each pot for germination. Boron and zinc were applied at different levels, as 0, 0.5, 1.0, and 1.5 kg ha^{-1} B from Borax and 0, 5 kg Zn ha^{-1} from Zinc sulphate, respectively. A basal dose of nitrogen 120 kg ha^{-1} (Urea), phosphorus 80 kg ha^{-1} (TSP), and potassium 60 kg ha^{-1} (MOP) were applied before planting.

Estimation of population of bacteria from rhizosphere and non-rhizosphere soil

About 10 gm of soil was transferred to a conical flask containing 95 ml of sterilized distilled water. In case of rhizosphere 1 g of fresh root was transferred to the conical flask containing 99 ml of sterilized distilled water. The content was shacked on a shaker for 10 min. A series of 10 fold dilutions were prepared up to 10^{-10} of rhizosphere and non-rhizosphere soil and the bacterial populations were determined using total plate count method in nutrient agar (NA) medium.

Chlorophyll content and leaf area index

The leaf chlorophyll content was determined three times (15, 30 and 45 days after planting) using portable chlorophyll meter (MINOLTA^{TM} SPAD-502) (Peterson et al., 1993). The SPAD reading was taken from the youngest expanded leaf (YEL) of each plant. Each value was the mean of 4 replications. The leaf area index (LAI) of the plants was determined at 45 days when the plants were harvested. After harvest random leaf samples per treatment replicate were carried into the laboratory in plastic bags and leaf areas measured using leaf area meter model LI-3100 Area meter, LI-COR. Inc. Lincoln, Nebraska USA. Leaf area index were calculated using the following formulae:

$$LAI = \frac{Mean\ leaf\ area\ of\ whole\ plant}{Surface\ \ area\ of\ \ pot\ (cm^2)}$$

Plant tissue analysis and plant biomass

The plant tissue B was determined by dry ashing followed by azomethine-H colorimetric (Benton, 2001). The zinc amount was determined by wet ashing method (Ryan et al., 2001). After harvest the plant samples were carefully washed to remove all soil particles and dried in oven at 70 ^{0}C for 3 days until constant weight was achieved. The experiment was conducted in factorial using completely randomized design (CRD) with 4 replications. Data were analyzed using SAS statistical program version 9.1. Treatments means were compared using Tukey's test ($P \leq 0.05$).

Table 3. Effect of boron and zinc on plant germination and leaf area index

Treatments	Germination (%)		Leaf area index (cm^2)		Leaf chlorophyll content (SPAD value) 15 DAS		30 DAS		45 DAS	
	------ Zn kg ha^{-1} ------									
	0	5	0	5	0	5	0	5	0	5
B (0 kg ha^{-1})	71b	70b	0.15e	0.21d	27.77d	29.88c	26.80d	29.67c	23.41d	23.46d
B (0.5 kg ha^{-1})	80a	76a	0.24c	0.31a	29.72c	32.98a	31.13b	30.17b	27.40a	27.80a
B (1 kg ha^{-1})	80a	58c	0.27b	0.19d	31.05b	29.31c	32.07a	29.10c	25.30b	24.66c
B (1.5 kg ha^{-1})	63c	57c	0.20d	0.18d	31.33b	28.06d	32.23a	27.70d	23.53d	24.43c
B levels	***		***					***		
Zn levels	***		***					***		
B × Zn	***		***					***		

† DAS = Days after sowing

‡ Different letters in the columns are significantly different at $P \leq 0.05$ and *** denotes highly significant ($P \leq 0.01$)

Table 4. Effect of different levels of B and Zn on plant tissue uptake and biomass production in maize

Treatments	Plant tissue B (mg kg^{-1})		Plant tissue Zn (mg kg^{-1})		Plant B uptake (mg plant^{-1})		Plant Zn uptake (mg plant^{-1})		Total plant Biomass (g)		Biomass increment (%)	
	------ Zn kg ha^{-1} ------											
	0	5	0	5	0	5	0	5	0	5	0	5
B (0 kg ha^{-1})	3.62f	3.78f	24.67e	66.67b	0.09e	0.16e	0.61e	2.77b	2.47e	4.15b	-	-
B (0.5 kg ha^{-1})	11.54e	13.71d	32.33d	68.78a	0.65d	0.68d	1.81c	3.37a	4.96b	5.61a	100.81	127.12
B (1 kg ha^{-1})	24.82c	26.23b	36.43c	67.43b	0.91b	0.74c	1.32d	1.91c	3.67c	2.83d	48.58	14.57
B (1.5 kg ha^{-1})	27.76a	28.45a	32.12d	69.57a	1.09a	0.76c	1.25d	1.87c	3.92b	2.69d	58.70	8.91

† Different letters in the columns are significantly different at $P \leq 0.05$

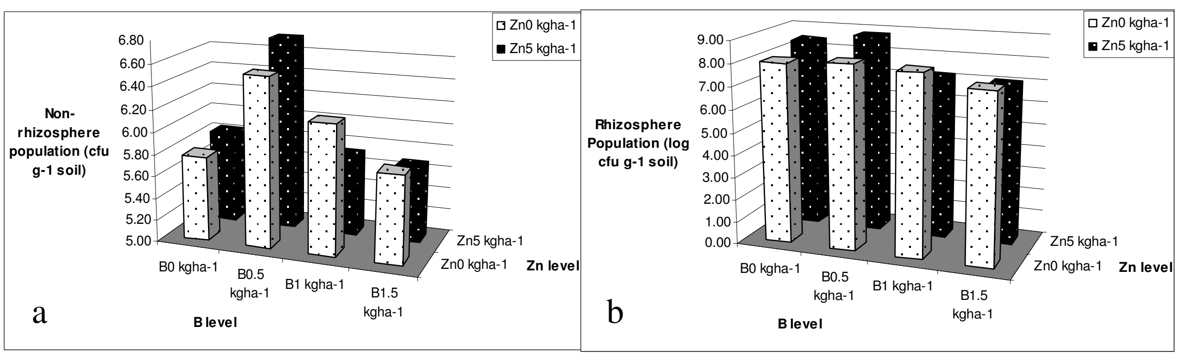

Fig 3. Effect of different level of boron and zinc on total bacterial population in (a) rhizosphere and (b) non-rhizosphere soil

Conclusion

The results of this study showed that the balanced mineral nutrition (through B and Zn fertilization) in the acidic soil for maize crop enhance growth of maize. Application of 0.5 kg ha^{-1} of B along with 5 kg ha^{-1} Zn, showed synergism effect and significantly increased maize plant height, leaf chlorophyll content, microbial population and plant biomass in Serdang soil series. The excess amount of the B and Zn application causes toxicity in the crop and soil environment.

Acknowledgements

The authors are grateful to Universiti Putra Malaysia, and the Ministry of Science, Technology & Innovation for providing the financial support for the project.

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