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Influence of Boron and Zinc nutrition on growth, yield and quality of turmeric (*Curcuma longa* L.) in Gangetic alluvial soil of West Bengal

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ABSTRACT

An experiment was carried out at Horticultural Research Station, Bidhan Chandra Krishi Viswavidyalaya during 2011-12 and 2012-13 to assess the interactive effect of two micronutrient-boron (B) and zinc (Zn) on growth, yield and quality of turmeric var. Suranjana. The micronutrients were sprayed once (90 DAS), twice (90 + 120 DAS) or thrice (90 + 120 + 150 DAS) to prepare an effective spray schedule for achieving higher production with better quality turmeric. Tallest plant (108.18 cm) and the highest number of tillers clump^{-1} (3.60) were observed in the treatment comprising combined application of B @ 0.2% and Zn @ 0.5% sprayed twice, while maximum number of leaves (17.88), highest girth of the plant (2.31 cm), leaf length (58.71 cm) and leaf width (16.02 cm) were recorded with combined application of B @ 0.2% and Zn @ 0.5% sprayed thrice. Combined application of B and Zn thrice, also proved to be most effective in achieving some important yield and quality attributing characters such as volume of rhizome per clump (448.42 ml), yield ha^{-1} (32.18 t), oleoresin content (12.94%), dry recovery (24.37%), curcumin content (5.21%) and enhancement in the antioxidant activity of phenolic extract (DPPH radical scavenging assay [IC_{50} value (µg ml^{-1})]).

Keywords: Boron, growth, quality, yield, zinc

Turmeric (*Curcuma longa* L.), also known as 'Golden Spice', is one of the most important and ancient spices of India, ranking first in area, production, consumption and export in the world. West Bengal is one of the major turmeric producing states of India. However, in spite of the ideal soil type and climate, productivity and quality is low compared to other major turmeric producing states. Nutrition plays a major role in the productivity of crops and the deficiency of zinc (Zn) and boron (B) and their inadequate use, may be one of the reasons for low productivity of rhizomatous spice crop like ginger and turmeric in West Bengal soils. Boron being important for stabilizing certain constituents of cell wall and plasma membrane, enhancement of cell division, and metabolism of nucleic acid, carbohydrate, protein, auxin and phenols (Marschner, 1997), its deficiency results in the reduction of yield of rhizome and curcumin (Dixit *et al*., 2002). Zinc plays a fundamental role in cellular functions such as protein metabolism, gene expression, photosysthetic C metabolism and indole-3-acetic acid (IAA) metabolism and its deficiency causes thickening of leaves, early loss of foliage and stunted growth (Tiwari *et al*., 1995).

Foliar application of micronutrients provides more rapid nutrient utilization and enables rapid correction of deficiency than soil application and results in yield increase, resistance to disease, insect and pest and enhances biological efficiency of crop plants and the quality of produce (Sebastian and Lourduraj, 2007). Despite importance of turmeric as commercial crops in the plains of West Bengal, information related to effect of boron and zinc on its sustained production to meet the market demand is limited. It was therefore felt necessary to undertake investigations to evaluate the effects of two important micronutrients (Zn and B) on yield and quality of turmeric including its antioxidant properties.

MATERIALS AND METHODS

A field experiment was conducted in a sandy loam soil having 6.9 pH, 0.74% soil organic carbon, 296 kg ha^{-1} available N, 28.5 kg ha^{-1} P_2O_5, 135 kg ha^{-1} K_2O, 0.4 mg kg^{-1} soil B and 0.57 mg kg^{-1} soil Zn, using turmeric (*Curcuma longa* L.) variety Suranjana as a test crop in the New Alluvial Zone of West Bengal. The field experiment was laid out in a Randomized Complete Block Design (RCBD) with 3 replications and 10 treatment combinations namely, 0.2% B Single spray (T_1), 0.2% B Double spray (T_2), 0.2% B Triple spray (T_3), 0.5% Zn Single spray (T_4), 0.5% Zn Double spray (T_5), 0.5% Zn Triple spray (T_6), 0.2% B + 0.5% Zn Single spray (T_7), 0.2% B + 0.5% Zn Double spray (T_8), 0.2% B + 0.5% Zn Triple spray (T_9) and Water spray-control (T_{10}). While Borax (8% Boron content) was used as the source of Boron, Zinc sulphate ($ZnSO_4$. $7H_2O$) [21% Zinc content] was used as the source of zinc. Well rotten FYM @ 30 t ha^{-1} and NPK @ 100:50:120 kg ha^{-1} (N, P_2O_5, K_2O) were applied in all the plots. The micronutrients were sprayed at 90, 120 and 150 days after sowing (DAS) to prepare effective spray schedule for achieving higher productivity with quality turmeric. The size of each plot was 1.5 x 1.5 m and the plots in each replication were surrounded by bunds of 0.3 and 0.5 m irrigation channels in between blocks with 30 numbers of plants per plot spaced at 25 x 30 cm. The

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crop was raised till maturity following recommended cultural practices. Growth and yield attributes were recorded following standard procedures. Oleoresin content (%) was estimated by extracting freshly ground dry turmeric with acetone by counter current extraction using soxhlet apparatus by ratio of the weight of oleoresin (g): Weight of the sample multiplied by 100 (ASTA, 1968). The phenol content was measured by the method established by Vinson *et al*.(1998). The total phenolic extracts, after suitable dilution, were subjected to measure antioxidant capacity of the phenolic extract using DPPH radical scavenging assay described by Dasgupta and De (2007) which was based on the decrease in absorbance of methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) at 517 nm in the presence and absence of the extract. The percent inhibition of DPPH radical was calculated as $[(A_o-A_e)/A_o] \times 100$ (A_o = Absorbance without extract, A_e = absorbance with extract). A graph of percent of inhibition vs. concentration was used to draw and calculate IC_{50} value (µg ml^{-1}), the concentration of sample required to decrease inhibition of DPPH radical by 50 per cent.

The curcumin content of turmeric were measured by the method established by Dixit *et al*.(2002) where 100 mg (dry) turmeric powder was extracted with 15 ml of 50 per cent aqueous methanol. The sample was centrifuge, heated at 90^0C with intermittent shaking for 2 hours in a water bath. The extracted materials, after cooling were centrifuged at 10,000 rpm for 30 minutes. The supernatant was decanted off in a graduated tube and the volume was made to 25 ml with respective solution. One way ANOVA for all studied parameters was carried out followed by Duncan's multiple range test (DMRT) to compare means using SPSS 16 software.

RESULTS AND DISCUSSION

Growth attributes

Perusal of the data (Table 1) revealed increases in all the growth attributes of turmeric due to application of micronutrients over control. Boron application @ 0.2% spray increased the plant growth which continued to increase with increasing number of sprays while there was no significant increase in growth due to sole application of Zinc @ 0.5%. A decreasing trend after double spray of Zn was observed. This indicated more effectiveness of B application in influencing growth of turmeric compared to Zn application.

Combined application of B @ 0.2% and Zn @ 0.5% was found to be most effective and among the treatment combinations, while double spray positively influenced plant height and number of leaves $plant^{-1}$, three sprayings proved effective in influencing the leaf parameters (Number of leaves $plant^{-1}$, leaf length and leaf width) of turmeric. Micronutrients are required in small amounts and they directly or indirectly influence photosynthesis, and the vital processes in plant such as respiration, protein synthesis, reproduction phase (Salem and Nasser, 2012) substantiating the effect of micronutrients (Zn and B) on growth of turmeric over control. Halder *et al*. (2007) also noticed progressive increase in the growth contributing parameters of turmeric with increased dosage of combined application of zinc and boron. Improvement in growth characters as a result of application of micronutrients might be due to enhanced photosynthetic and other metabolic activities leading to an increase in various plant metabolites responsible for cell division and elongation (Hatwar *et al*., 2003). The decrease in plant height and tiller from double to triple spray might possibly have been due to antagonistic affects, negative interactions and toxicity of some nutrients to plant as complex phenomena that occurred when nutrients were used in combination during growth (Malakouti, 2008).

Table 1: Influence of B and Zn on different growth parameters of turmeric (Pooled over two years)

Treatments	PH (cm)	NTC	NLP	LL (cm)	LW (cm)
One Spray of B (0.2%)	100.19	2.95	16.80	55.60	15.65
Two Sprays of B (0.2%)	102.24	3.10	17.07	56.63	15.84
Three Sprays of B (0.2%)	104.04	3.12	17.44	57.77	15.85
One Spray of Zn (0.5%)	98.91	2.78	17.18	55.35	15.53
Two Sprays of Zn (0.5%)	99.79	2.95	16.71	56.20	15.55
Three Sprays of Zn (0.5%)	99.35	2.78	16.40	56.35	15.52
One Spray of B (0.2%)+Zn (0.5%)	106.35	3.01	17.16	56.59	15.80
Two Sprays of B (0.2%) + Zn (0.5%)	108.18	3.60	17.35	57.64	15.90
Three Sprays of B (0.2%) + Zn (0.5%)	107.91	3.22	17.88	58.71	16.02
Control (Water spray)	97.86	2.59	15.43	54.68	15.31
SEm(±)	**1.92**	**0.11**	**0.26**	**0.67**	**0.10**
LSD (0.05)	**5.54**	**0.32**	**0.79**	**1.94**	**0.28**

PH: Plant height; NTC: Number of tillers per clump; NLP: Number of leaves per plant; LL: Leave length; LW: Leave width

Yield attributes

Perusal of the data (Table 2) revealed increase in all the yield attributes of turmeric in micronutrient applied

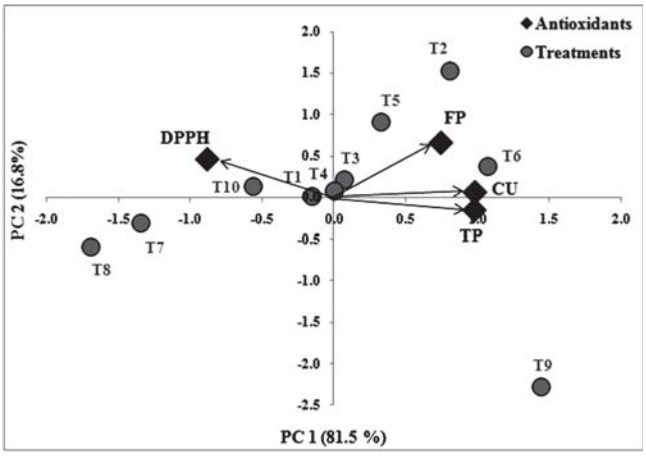

Fig. 1: Scattered diagram showing influence of B and Zn on the anti-oxidant properties of turmeric.

Notes: FP: Free phenol; TP: Total phenol; CU: Curcumin; T_1: One Spray of B (0.2%); T_2: Two Spray of B (0.2%); T_3: Three Spray of B (0.2%); T_4: One Spray of Zn (0.5%); T_5: Two Spray of Zn (0.5%); T_6: Three Spray of Zn (0.5%); T_7: One Spray of B (0.2%) + Zn (0.5%); T_8: Two Spray of B (0.2%) + Zn (0.5%); T_9: Three Spray of B (0.2%) + Zn (0.5%); T_{10}: Control water spray.

pots over control. Combined application of B @ 0.2% and Zn @ 0.5% proved effective in increasing the yield of turmeric over sole application of B @ 0.2% and Zn @ 0.5% which further increased with number of sprays. Three sprays of B and Zn recorded maximum values of certain yield attributing characters such as weight of mother rhizomes (112.94 g), weight of primary rhizomes (198.03 g), weight of secondary rhizomes (95.71 g), volume of rhizome clump^{-1} (448.42 ml), yield plot^{-1} (8.52 kg) and yield ha^{-1} (32.18 t). The increase in the rhizome yield might have been due to addition of micronutrients, which accelerated the cumulative effect of finger weight plant^{-1}, finger size and number of fingers plant^{-1}(Halder *et al*., 2007). As the experimental soil was deficient in boron and zinc, the turmeric responded highly to micronutrient application resulting in higher yield under combined application. Application of micronutrients brought about profound changes in various metabolic processes within the plant system and thereby influenced the yield potential (Tripathy *et al*., 1999). Increased rate of photosynthesis has been reported with micronutrients spray in turmeric (Jirali *et al*., 2007) directly influencing yield.

Comparatively B was found to be more effective in increasing the yield than Zn which may be because of the importance of B in cell wall structural integrity, cell expansion, pH regulation, transport of mineral *etc*. The B deficiency decreases translocation of the metabolites towards rhizome and roots and photo-assimilate portioning to essential oil in leaf and to curcumin in rhizome ultimately leading to decrease in overall rhizome yield and curcumin yield (Dixit *et al*., 2002). This indicated that spraying of micronutrients influenced the source-sink relation and improved crop yield. Significant and positive correlation (Table 3) between plant height with number of tillers, weight of secondary rhizome, yield ha^{-1} and dry recovery percentage suggesting that the growth parameters are influenced by the application of both the treatments over control and thereby increasing the yield and dry recovery, were also observed. Similarly number of tillers and plant height influenced the weight of secondary and mother rhizome and increased the yield of turmeric.

Qualitative attributes

The maximum oleoresin content was recorded from treatment comprising three sprays of combined application of B @ 0.2% and Zn @ 0.5%, followed by two sprays and single spray (Table 2). Sole application of B @ 0.2%, progressively increased the oleoresin content with increase in number of spray - once, twice and thrice, respectively. Similarly, Zn application @ 0.5% also progressively increased the oleoresin content with the number of sprays from single to double only but decreased on three sprays.

Combined application of B @ 0.2% and Zn @ 0.5% proved to be more effective in increasing dry recovery percentage (Table 2), than the application of single micronutrient and showed increasing trends with the increasing in the number of sprays. Sole application of B@ 0.2 % and Zn @ 0.5 % also recorded a gradual increasing trend. These findings suggest that B and Zn play an important role not only in increasing growth and yield but also in the qualitative parameters such as oleoresin and dry recovery. This has been substantiated from strong positive correlation between oleoresin with

Table 2: Influence of B and Zn on yield and quality attributing characters of turmeric (Pooled over two years)

Treatments	Wt. MR (g)	Wt. PR (g)	Wt. SR (g)	VRC (ml)	Yield (t ha^{-1})	Oleoresin (%)	DR (%)
One Spray of B (0.2%)	106.63	163.74	71.69	306.44	29.97	10.26	21.19
Two Sprays of B (0.2%)	109.64	175.06	77.80	340.03	30. 60	10.73	21.41
Three Sprays of B (0.2%)	109.92	179.25	86.88	363.97	31.10	10.91	21.67
One Spray of Zn (0.5%)	103.92	158.34	70.31	358.75	29.72	10.17	20.59
Two Sprays of Zn (0.5%)	104.55	160.78	72.65	357.50	29.84	10.86	21.00
Three Sprays of Zn (0.5%)	104.95	162.57	72.97	340.78	30.00	10.85	21.39
One Spray of B (0.2%) + Zn (0.5%)	110.19	176.89	89.61	381.83	31.48	11.15	22.19
Two Sprays of B (0.2%) + Zn (0.5%)	111.33	192.70	94.34	387.50	32.12	11.69	23.11
Three Sprays of B (0.2%) + Zn (0.5%)	112.94	198.03	95.71	448.42	32.18	12.94	24.37
Control (Water spray)	101.23	119.69	61.54	270.67	28.38	8.63	18.00
SEm(±)	**1.63**	**7.88**	**1.89**	**10.84**	**0.42**	**0.36**	**0.31**
LSD (0.05)	**4.69**	**23.65**	**5.66**	**32.53**	**1.21**	**1.09**	**0.93**

Note : Wt: Weight; MR: Mother rhizome; PR: Primary rhizome; SR: Secondary rhizome; VRC: Volume of rhizome per clump; DR: Dry recovery

Table 3: Correlation coefficients between growth, yield components and quality characters

	PH	NT	NL	Wt. MR	Wt. PR	Wt. SR	Yield	O
PH	1							
NT	0.361*	1						
NL	0.233	0.164	1					
Wt. MR	0.349	0.377*	0.444*	1				
Wt. PR	-0.015	0.150	0.106	0.117	1			
Wt. SR	0.630**	0.506**	0.309	0.751**	0.047	1		
Yield	0.530**	0.534**	0.203	0.651**	0.108	0.798**	1	
O	0.348	0.329	0.262	0.692**	0.117	0.749**	0.768**	1
DR	0.504**	0.555**	0.193	0.712**	0.134	0.779**	0.761**	0.842**

Note: PH: Plant height; NT: Number of tillers; NL: Number of leaves; Wt: Weight; MR: Mother Rhizome; PR: Primary Rhizome; SR: Secondary Rhizome; O: Oleoresin; DR: Dry recovery
*$p<0.05$; **$p<0.01$*

Table 4: Influence of B and Zn on phenolic content, curcumin and anti-oxidant capacity of phenolic extract of turmeric (Pooled over two years).

Treatments	Free phenol (mg CE g^{-1})	Total phenol (mg CE g^{-1})	Curcumin (%)	DPPH assay [IC_{50} value (μg ml^{-1})]
One Spray of B (0.2%)	34.04^{bcd}	66.24^{cd}	4.43^{ab}	7.31^{abc}
Two Sprays of B (0.2%)	43.98^{a}	70.60^{bc}	4.83^{ab}	6.95^{bc}
Three Sprays of B (0.2%)	36.23^{bc}	66.48^{cd}	4.49^{ab}	7.11^{abc}
One Spray of Zn (0.5%)	35.42^{bcd}	65.62^{cd}	4.49^{ab}	7.11^{abc}
Two Sprays of Zn (0.5%)	39.54^{ab}	66.40^{cd}	4.71^{ab}	7.10^{abc}
Three Sprays of Zn (0.5%)	40.31^{ab}	75.99^{ab}	5.10^{a}	6.68^{c}
One Spray of B (0.2%) + Zn (0.5%)	29.33^{cd}	54.49^{e}	3.59^{cd}	7.68^{ab}
Two Sprays of B (0.2%) + Zn (0.5%)	27.96^{d}	54.08^{e}	3.04^{d}	7.74^{a}
Three Sprays of B (0.2%) + Zn (0.5%)	33.66^{bcd}	80.26^{a}	5.21^{a}	5.61^{d}
Control (Water spray)	33.99^{bcd}	60.61^{de}	4.01^{bc}	7.35^{abc}

*Note : *Within the same column different letters indicate significant difference by DMRT ($p<0.05$)*

weight of mother rhizome, weight of secondary rhizome and yield ha^{-1} (Table 3).

While three sprays of Zn and combined application of B and Zn (three spray) were most effective in increasing the total phenol (75.9 and 80.26 mg CE g^{-1}) and curcumin (5.1 and 5.21 %) content of turmeric (Table 4), other treatments were statistically at par (two spray combine application of B and Zn; 54.08 CE g^{-1} to two spray of B; 70.6 CE g^{-1}) for total phenol and from two spray combine application of B and Zn (3.08 %) to two spray of B (4.83 %) for curcumin content respectively. Deficiency and sufficiency of each of the micronutrients, Zn than B, registered higher curcumin and total phenol content because of their similar chemical nature and their accumulation in rhizomes through common biosynthetic pathway.

Higher accumulation of curcumin in turmeric and phenol in potato (Dixit *et al.*, 2002) under B deficiency has been reported earlier. Dixit *et al.* (2002) also reported higher translocation and utilization of $^{14}CO_2$ assimilated metabolites into curcumin in rhizome in B applied plants compared to the plants without application on B. The interaction between B and Zn, each at a higher application rate, produced a synergistic effect leading to higher curcumin and total phenol content in rhizome of turmeric. Studies on precursor-product relationship or photosynthate partitioning to secondary metabolites in spice crops are scarce in the literature. Since photosynthates are incorporated into curcumin, the higher curcumin and total phenol content under deficiency or excess indicate that some steps of their biosynthetic pathway might have been selectively accelerated (Dixit *et al*., 2002). The development of rhizome and subsequent accumulation of phenol including curcumin depends on the translocation of photo-assimilate coupled with their biosynthesis. Deficiency of each of these micronutrients produced higher amount of free phenol. However, higher percent conjugated phenol was recorded with higher application rate of B and Zn. The higher percent conjugated phenol in rhizome with three sprays of B involves phenolic acids, such as caffeic or 5-hydroxy ferulic acid that possess structure with a cis-dihydroxyl configuration which favours the formation of cis-dihydroxyl-borate complexes (Lewis,1980). Thus, B deficiency may lead to increased availability of these molecules in free form as a result of decreased formation of cis-dihydroxyl-borate complexes (Cakmak and Romheld, 1997). However, in case of Zn application, composition and intrinsic property of complex mixtures of phenolic compounds to form conjugates with sugar, organic acids etc. probably determine the percent conjugated phenol.

The results further indicated that the deficiency and sufficiency of B and Zn that produced rhizomes with higher curcumin and total phenol content, exhibited highest antioxidant capacity of the phenolic extract as evidenced by their lower IC_{50} value (Fig. 1). The antioxidant capacity of the phenolic extract is expressed as IC_{50} value which is the concentration of sample (µg ml^{-1}) required to scavenge 50% of DPPH radical and is inversely related to activity. The effect was more pronounced with the three sprays of these micronutrients together (5.61 µg ml^{-1}) while other treatments were statistically at par (Three spray of Zn; 6.68 µg ml^{-1} to two spray of combined B and Zn; 7.74 µg ml^{-1}) respectively. The antioxidant capacity of the phenolic extract was directly related to total phenol and curcumin content which were in conformity with earlier reports (Ou *et al*., 2002; Imeh and Khokhat, 2002).

In order to identify the suitable treatment that can enhance the antioxidant property of rhizomes of turmeric, Principal Component Analysis (PCA) was performed to obtain a simplified view of the relationship. PC1, explains 81.5% of total variance where higher values of free, total phenol and curcumin reduces the IC_{50} value of total phenol. Based on scatter diagram (Fig.1), the treatment with higher application rate of both B and Zn appeared to be promising and can be used as a viable option to enhance the antioxidant property of rhizomes of turmeric with higher rhizome biomass yield.

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