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Foliar spray of Zn on tomato (*Solanum lycopersicum*) production at trans-Himalayan Ladakh region

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ABSTRACT

Physiological responses, root development, yield and growth of tomato (*Solanum lycopersicum* L.) (var. Tolstoy) to foliar applications of $ZnSO_4$ during 2016 and 2017 cropping season was evaluated at high altitude cold-arid agro climatic conditions of Ladakh region. Field experiments were conducted with foliar spraying of $ZnSO_4$ @0.5% (T_1); 1.0% (T_2); 1.5% (T_3) and 2.0% (T_4) during flowering and fruit formation stage on tomato plants over control treatment (T_0). Results indicated that projected root area was highest in T_3 followed by T_4 treatment. The total yield of tomato was significantly increased from 34.3 and 38.1 t/ha without foliar application of Zn to 88.8 (1.5% Zn) and 77.4 t/ha (2.0% Zn) in 2016 and 2017, respectively. Average marketable yield in T_3 treatment was recorded 153% and 116% higher in comparison to T_0 in 2016 and 2017, respectively. Increase in dry biomass (52.21%) was also observed in T_4 over control treatment (T_0). Foliar application of Zn application also improved physiological parameters of tomato. Significant enhancement in leaf chlorophyll content, relative water content (RWC), membrane stability index (MSI) and significant reduction in electrolyte leakage (EL) was observed in T_3 and T_4 treatment. The results showed that foliar application of $ZnSO_4$ @1.5% significantly improved growth, development and yield of tomato at cold desert Ladakh region.

Keywords: Cold arid, Foliar spray, High altitude, Root morphology, Zn deficiency

Among micronutrients, zinc plays an important role in pollination, fruit set and total yield (Motesharezade *et al.* 2001). Zinc has an important role either as a metal component of enzymes or as a functional, structural or regulatory cofactor of a large number of enzymes (Bowler *et al.* 1994). Zinc also plays an important role in chlorophyll formation and biomass production. Zn deficiency decreases the photosynthetic rate, chlorophyll content, activity of carbonic anhydrase, and protein biosynthesis (Fu *et al*. 2016). Thus, zinc deficiency has tremendously affected plant growth and prevents crops from attaining their potential yield. The problem of zinc deficiency is worldwide and mainly found in coarse textured sandy, calcarious soils of arid and semi-arid regions (Alloway 2008). Low solubility of Zn in soils rather than low total amount of Zn is the major reason for the widespread occurrence of Zn deficiency problem in crop plants (Cakmak 2008). Foliar spray is effective in regions where nutrient uptake through the root system is restricted due to stress conditions. Foliar application of micronutrients can improve the vegetative growth, fruit set and yield of tomato (Adams 2004). Singh (2008) reported that deficiency of Zn can be effectively controlled with 2-4 sprays of 0.5% $ZnSO_4$ salt solution on standing crops.

Trans-Himalayan region of India is popularly known as cold desert arid region with harsh climatic condition. In this type of climatic condition, main challenges in agriculture is very coarse textured sandy soil with little moisture, very poor water holding capacity with low fertility and drought stress. Little attention has been paid to study the response of zinc in improving vegetable production in trans-Himalayan high altitude Ladakh region. Tomato (*Solanum lycopersicum* L.) is one of the important vegetable which is regarded to be responsive to micronutrients application. In trans-Himalayan regions of India tomato is widely grown and is one of the important cash crops. Hence, the objective of the present study was to examine the effects of zinc (Zn) as foliar spray on plant physiological parameters, root morphology and growth and yield of tomato grown in cold arid trans-Himalayan Ladakh region.

MATERIALS AND METHODS

The experiments were conducted during 2016 and 2017 at experimental field of Defence Institute of High Altitude Research (DIHAR) in trans-Himalayan Ladakh (10526 ±32.30 to 13063 ±20.20ft. amsl). Longer photoperiod, about 325 sunny days and only one cropping season in a year (May to October) are typical characteristics of this region. Soils of the experimental fields were sandy, *p*H level

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7.92 and coarse textured. Soil phosphorus and zinc were estimated 18.8 kg/ha and 0.35 ppm, respectively, which are categorized as deficient level. Tomato hybrid variety Tolstoy was selected for the experiment. Zinc sulphate ($ZnSO_4$) was used as a fertilizer for foliar spraying with 21% Zn content. Following treatments were used for the present experiment: T_0: Control; T_1: Foliar spray of $ZnSO_4$ @0.5%; T_2: Foliar spray of $ZnSO_4$ @1.0%; T_3: Foliar spray of $ZnSO_4$ @1.5% and T_4: Foliar spray of $ZnSO_4$ @2.0%. Foliar spraying was done twice, first one at flowering and second one during fruit formation stage. The experiment was carried out based on completely randomized block design. All the agronomic practices were consistent with the local production practices. Plant growth parameters viz. plant height, number of leaves, number of branches were measured before first harvesting. After first flower formation, number of flowers per plant as influenced by various foliar treatments of zinc was also recorded at 60 days after transplanting (DAT). Tomato harvesting was done thrice during the experiment starting from August to first week of October. For the measurement of dry biomass of the plant, plants were weighed individually and kept in brown paper bags for 72 h at 70°C in oven for complete drying.

Leaf samples were collected after 50 DAT for determination of leaf chlorophyll content and physiological parameters as influenced by foliar application of zinc. Leaf chlorophyll content was determined by portable chlorophyll meter (CCM-200plus, ADC Bioscientific, UK). Leaf relative water content (RWC), membrane stability index (MSI) and electrolyte leakage (EL) was determined by methods described below. RWC was determined for tomato leaves using the method of Mata and Lamattina (2001). MSI was calculated according to the method described by Sairam *et al*. (1997). EL was measured as described by Lutts *et al.* (1996). Root morphology of tomato plant after harvesting was analyzed by image analysis software (Biovis PSM3000, Expert Vision Labs Pvt. Ltd., India).

All experimental data were expressed as mean ± standard deviation using statistical analysis with SPSS 16 (SPSS Corporation, Chicago, Illinois, USA) and MS excel 2007. Differences between mean values were evaluated using one way analysis of variance (ANOVA). The differences were compared using the Duncan's test with a significance level of 0.05.

RESULTS AND DISCUSSION

Tomato yield and yield components: Tomato is considered moderately sensitive in terms of relative sensitivity of crops to Zn deficiency. Results show that Zn has a profound impact on enhancing tomato yield (Table 1). Number of fruits per plant varied significantly among the treatments. Maximum number of fruits was found in T_3 (61.5) in 2016 and in T_4 treatment (55.1) in 2017, respectively. Flowering and fruiting process were greatly reduced under severe Zn deficiency as reported by Hafeez *et al.* (2013). Response of foliar application of Zn on fruit yield per plant is evident from the results (Table 1) which showed an increase from 1.8 kg/plant in control plots (T_0) to 2.9 kg/plant in T_1 in 2016 and from 2.0 kg/plant in T_0 to 3.3 kg/plant in T_1 in 2017, respectively. Total tomato yield in different treatments varied from 34.3 and 38.1 t/ha in T_0 to 88.8 (T_3) and 77.4 t/ha (T_4) in 2016 and 2017, respectively. Increase in marketable yield in T_3 treatment was 153% and 116% in comparison to T_0 in 2016 and 2017, respectively.

Zinc deficiency affects absorption of water and nutrients from soil thus resulting in growth and yield reduction in the plant. Cakmak (2008) also reported that low availability of zinc in the soil can lead to reduction of plant growth and its yield. Increased tomato yield as impacted by Zn foliar spraying may be attributed to enhanced photosynthetic activity and physiological responses, favorable effect on vegetative growth and retention of flowers and fruits which eventually increased number of fruits per plant and overall tomato yield.

Physiological parameters of tomato leaves: Plant physiological parameters of tomato were recorded as influenced by foliar application of zinc treatments (Fig 1). Significant variation was observed with respect to

Table 1 Response of yield attributing characters of tomato to foliar application of $ZnSO_4$

Treatment	No. of fruit/ plant		Fruit yield/plant (kg)		Total yield (t/ha)		Marketable yield (t/ha)		Non-marketable yield (t/ha)	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
T_0	34.7 ± 3.9[a]	32.5 ± 4.2[a]	1.8 ± 0·3a	2.0 ± 0.3[a]	34.3 ± 8.2[a]	38.1 ± 9.2[a]	31.5 ± 5.2[a]	33.4 ± 4.6[a]	2.8 ± 2.0[ab]	4.7 ± 2.3[b]
T_1	39.3 ± 4.5[a]	33.3 ± 4.7[a]	2.9 ± 0.3[b]	3.3 ± 0.4[b]	52.9 ± 9.5[b]	44.3 ± 4.3[ab]	47.5 ± 7.5[b]	41.5 ± 5.9[b]	8.6 ± 3.4[b]	2.8 ± 0.7[a]
T_2	53.2 ± 5.7[b]	47.3 ± 4.1[b]	3.5 ± 0.2[c]	3.5 ± 0.3[b]	68.2 ± 6.3[c]	59.2 ± 8.5[c]	63.9 ± 7.2[c]	52.7 ± 6.8[bc]	4.3 ± 1.5[a]	6.5 ± 1.4[b]
T_3	61.5 ± 7.5[b]	53.1 ± 5.8[b]	4.1 ± 0.4[d]	3.9 ± 0.3[b]	88.8 ± 9.2[d]	76.4 ± 6.4[d]	79.7 ± 6.3[d]	72.1 ± 6.1[d]	9.1 ± 2.6[b]	4.3 ± 1.5[ab]
T_4	59.2 ± 7.4[b]	55.1 ± 6.5[b]	3.7 ± 0.3[c]	3.8 ± 0.2[b]	82.9 ± 5.9[d]	77.4 ± 6.1[d]	75.3 ± 5.5[cd]	68.8 ± 5.1[d]	7.6 ± 3.6[b]	8.6 ± 2.2[bc]

The values with the different letter within same column are statistically significant by Duncan's test at $P \leq 0.05$

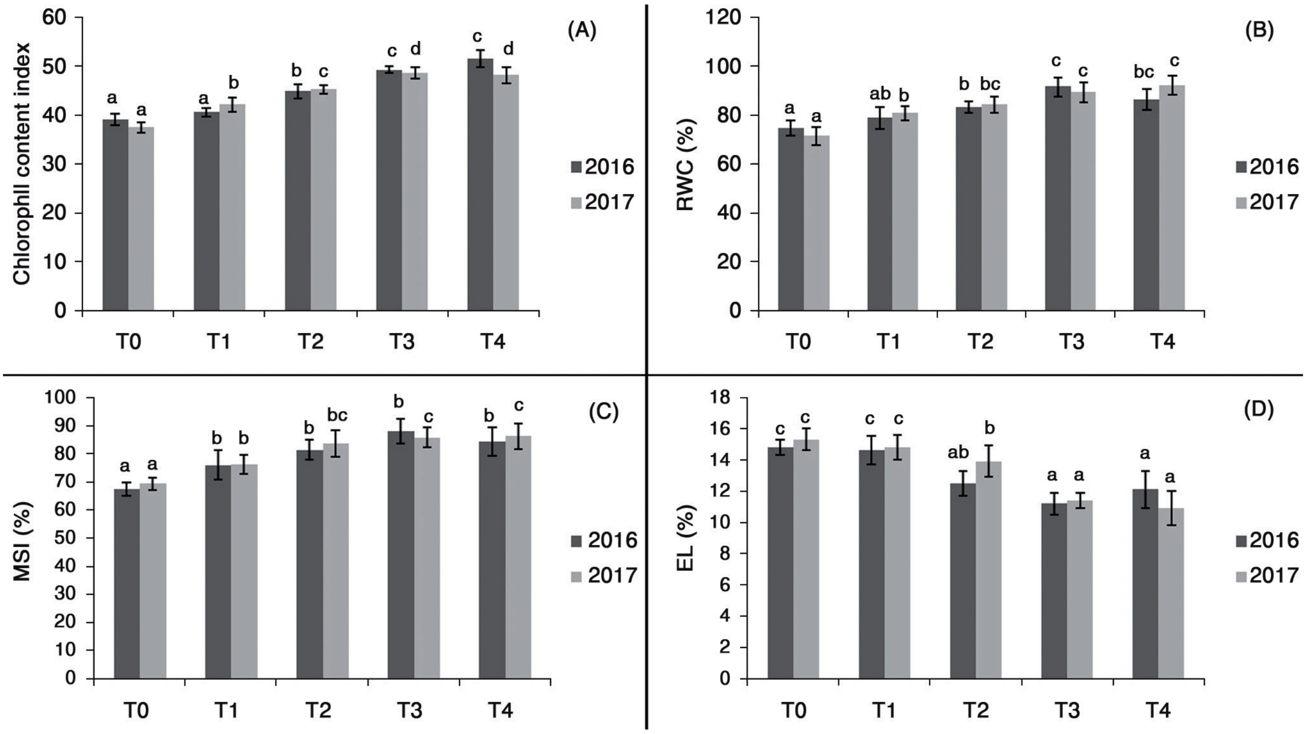

Fig 1 Chlorophyll content index (A), Relative water content (RWC) (B), Membrane stability index (MSI) (C), and Electrolyte leakage (EL) (D) of tomato leaves as affected by foliar application of $ZnSO_4$. Data were mean (n = 5) with standard deviation shown by vertical bars. Means with different letters are significantly different by Duncan's test at P≤0.05.

chlorophyll content of the leaves as to the main effect of foliar zinc application (Fig 1A). Except T_1, i.e. 0.5% $ZnSO_4$ spraying, all other higher doses of Zn significantly improved leaf chlorophyll content over control plots (T_0). Singh and Singh (2004) reported that zinc application increased chlorophyll content and raised the concentration of Zn, Ca, Mg, K, and P in plant tissues. Acharya *et al*. (2012) also reported increase in chlorophyll content of hydroponically grown *Jatropha curcas* seedlings when Zn was applied @0.1 and 0.5 mM, respectively.

The RWC indicates the status of water in the cell and is related to tolerance to any stress. Significant increase in (P<0.05) leaf RWC was observed T_2 onwards with highest RWC in T_3 treatment (91.5% in 2016) and T_4 treatment (92.1% in 2017) in respective seasons (Fig 1B). Similar to RWC, maximum leaf MSI was observed in T_3 treatment (87.9% in 2016) which was 30.4% higher than T_0 (Fig 1C). But among the Zn treatments no significant effect (P>0.05) of Zn on leaf MSI was observed. Application of Zn significantly increases plant water status as exemplified by improvement of RWC and MSI. Interaction of Zn with membrane proteins contributes to maintenance and stability of membranes and also helps to reduce adverse effects of short periods of environmental stress (Disante *et al*. 2011).

The data showed that maximum EL was observed in T_0, and the decreasing trend was observed in the order $T_0 > T_1 > T_2 > T_4 > T_3$ in 2016 and in the order $T_0 > T_1 > T_2 > T_3 > T_4$ in 2017, respectively. Zn helps in maintaining membrane permeability by lowering the EL of tomato leaves. From our results, it is clear that Zn plays an essential role in protecting cell membrane damage. Zn is an essential micronutrient involved in a wide variety of physiological processes (Stoyanova and Doncheva 2002). This effect of foliar spraying of Zn on improvement of physiological process of tomato in zinc deficient sandy soils of cold desert Ladakh region supports the findings of Singh *et al*. (2019) who also reported improvement of physiological characters of capsicum treated with Zn under water deficit condition of Ladakh. The improvement of plant water status could have contributed to the increased fruit yield in the Zn treated plants.

Plant growth parameters of tomato: Effects of foliar application of Zn on growth characteristics of tomato grown in cold desert of Leh-Ladakh region were also studied (Table 2). The results show that Zn foliar application has significant effect on tomato plant growth characters. Plants grown in the control plots (T_0) without Zn spray were stunted and had smaller leaves compared to plants that received Zn foliar spray. Several researchers also reported that Zn is an essential micronutrient for plant growth and its deficiency results in stunted growth (Hafeez *et al.* 2013). Foliar application of Zn at T_2 and higher doses corrected deficiency symptoms, and plants in this treatment showed healthy growth in both the seasons. In 2016, height of tomato plant was increased significantly (P<0.05) from 71.3 cm in T_0 to 109.7 and 126.1 cm in T_2 and T_3, respectively. In 2017 also, all treatments significantly enhanced plant height. No. of leaves also increased significantly in T_2 and other treatments in both the season. No. of branches per plant were also significantly enhanced in all treatments except

Table 2 Effect of foliar application of $ZnSO_4$ on growth response, flowering and plant biomass production of tomato

Foliar application of $ZnSO_4$	Plant height (cm)		No. of leaves/ plant		No. of branch/ plant		No. of flower/ plant		Dry plant biomass (g)		Projected root area (mm^2)	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
T_0	71.3± 12.5^{a}	65.9± 7.4^{a}	38.1± 4.8^{a}	40.3± 5.8^{a}	6.33± 0.5^{a}	5.33± 0.5^{a}	25.6± 5.2^{a}	23.1± 3.8^{a}	217.4± 33.2^{a}	231.9± 25.5^{a}	10417.3± 220.1^{a}	12510.5± 142.6^{a}
T_1	85.3± 10.6^{ab}	83.2± 11.2^{b}	45.3± 5.6^{ab}	46.8± 4.5^{ab}	6.67± 0.8^{a}	6.22± 0.7^{a}	34.3± 4.3^{ab}	25.6± 3.3^{a}	298.5± 26.6^{b}	282.8± 17.2^{b}	18689.1± 150.5^{b}	15935.9± 165.1^{b}
T_2	109.7± 15.9^{b}	95.6± 9.4^{bc}	52.5± 3.9^{b}	53.5± 3.5^{b}	8.67± 1.2^{b}	7.78± 0.7^{b}	43.6± 6.2b^{c}	47.9± 4.9^{b}	320.3± 25.3^{bc}	312.3± 12.5^{c}	20245.9± 184.6^{b}	20730.5± 205.2^{c}
T_3	126.1± 18.2^{bc}	106.2± 10.7^{c}	58.5± 6.8^{c}	55.6± 5.9^{bc}	10.3± 1.1^{b}	10.9± 0.8^{c}	55.6± 5.9^{c}	49.9± 5.7^{b}	344.1± 18.2^{c}	385.6± 21.4^{d}	22698.7± 123.4^{b}	20205.6± 138.5^{c}
T_4	120.3± 18.6^{bc}	110.2± 15.2^{c}	54.6± 6.1^{c}	60.8± 4.3^{c}	10.3± 1.4^{b}	9.7± 1.0^{c}	48.2± 7.6^{c}	52.9± 4.8^{b}	348.7± 22.6^{c}	335.2± 34.3^{cd}	20402.2± 212.7^{b}	17415.7± 142.6^{bc}

The values with the different letter within same column are statistically significant by Duncan's test at $P \leq 0.05$.

T_1. Maximum no. of branches was found in T_3 in both the seasons (10.3 and 10.9 for 2016 and 2017, respectively) followed by T_4 and T_2.

Flowering behaviour also showed significant increase in no. of flowers per plant due to increasing level of Zn foliar spraying. In T_3 it was 117.2% and 116% increase in number of flowers in 2016 and 2017, respectively. All the plants without foliar application of Zn produced significantly less dry plant biomass. Results (Table 2) clearly showed that plant dry biomass was significantly influenced by foliar spraying of Zn. Increased plant height, no. of leaves and branches, all contributed to higher plant biomass in Zn treated plants compared to control. Zn helps in the synthesis of tryptophan, the precursor of IAA and it is responsible for plant growth. Significant variations ($P<0.05$) have been noticed in projected root area of tomato in T_2, T_3 and T_4 over control (T_0) but among the treatments no significant differences were observed. It is clearly shown that zinc deficiency in soil in control plots had significantly reduced overall root development. In both seasons better root development was observed in the order of $T_3 > T_4 > T_2 > T_1 > T_0$ (Table 2). The results are consistent with the findings of Fageria (2004) who also observed that zinc deficiency significantly affects the root system, its distribution including root development. Soils of Ladakh have high *p*H, are sandy in nature with very low water holding capacity and rich in $CaCO_3$ content. In such soils, the supply of Zn to roots would be lower than the root capacity to take up Zn. Therefore, under such conditions, adding Zn to soil and/or foliar Zn application is important for better root development and crop yield.

In both the seasons, foliar spraying of Zn played vital role in significant enhancement of plant growth parameters. Reduction in plant growth in Zn deficient plots may be associated with inhibition of cell division and expansion as well as disruption of biochemical and physiological processes of plants. Singh and Tiwari (2013) also reported micronutrient spray including Zn significantly improved growth, yield and quality of tomato. Improvement in growth characters of tomato plant as a result of application of Zn might be due to enhanced photosynthetic and other metabolic activities which leads to an increase in various plant metabolites responsible for cell division and elongation (Hatwar *et al.* 2003). From the overall results, we conclude that application of Zn as foliar spray can correct the zinc deficiency of soils at cold desert Ladakh region and significantly increase plant growth and yield attributing characters of tomato when applied @1.5% twice during flowering and fruit formation stage. These results have important implications for guiding Zn fertilizer application for tomato production at different regions of high altitude trans-Himalayan Ladakh region.

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