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Original Article

# Zinc Influences Regeneration of *Talinum portulacifolium* Stem Cuttings in Nutrient Solution

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**Abstract**

Zinc (Zn) is an essential micronutrient for plant growth and development, but toxic at high concentrations. The present study focused to underline the direct effect of different concentrations (0-50 ppm) of Zn on the regeneration ability and morphological characteristics of *Talinum portulacifolium* stem cuttings in hydroponic solution over a period of 35 days without the interference of other soil factors. High concentrations of Zn (40-50 ppm) affected callusing, root initiation, root and shoot development to varying levels. At high concentrations, Zn caused stem decay, stunting and browning of roots, wilting, withering and necrotic spots on leaves. Increasing concentrations of Zn inversely affected the lateral shoot development, stem elongation, leaf, root numbers and total root length of the stem cuttings. Though Zn had no significant influence on fresh or dry weights of stem, low concentration (15 ppm) of Zn increased the fresh and dry weights of leaves by 11.17% and 26.79% respectively, compared to 0 ppm and with 77.06-243.80% and 47.92-255.00% compared with those raised in 20-50 ppm. Zn concentrations >10 ppm reduced the root fresh weight by 28.57-90.47% and dry weight by 27.27-90.91% than those at 0 ppm. The Zn content in leaves and stems increased linearly with increasing concentrations of Zn and ranged from 1.09-125.62 ppm and 1.00-110.26 in stems and leaves respectively. The tolerance index varied between 81 and 138 for different concentrations of Zn. The results of the study clearly indicate that high concentrations of Zn inhibit the regeneration of *T. portulacifolium* stem cuttings.

***Keywords:*** growth; heavy metals; hydroponics; rooting

## Introduction

Heavy metals (HMs) are exceptionally dangerous environmental pollutants which are toxic to plants and animals even at extremely low concentrations. Contamination by toxic HMs is of a great concern worldwide because of their long lasting toxic effect on the environment (Tchounwou *et al*., 2012). Consumption of crops and vegetables grown in HMs contaminated soils or drinking HMs contaminated water directly exposes humans to HMs (Nagajyoti *et al*., 2010; Balkhair and Ashraf, 2016). Being the member of Group-II of the periodic table, is an essential element for plant and animal metabolism and exists in the form of its oxides, sulphides and chlorides in the soil (Das and Green, 2013). On the other hand concentrations of Zn higher than 0.2 mg/g in plant dry matter can be toxic (Tsonev and Lidon, 2012). Excessive presence of Zn may result in leaf chlorosis (Cobbett and Goldsbrough, 2002) and also affect photosynthesis by interfering with the development of pigments, stomatal functioning, enzyme activities and electron transport through alterations in membrane architecture (Rout and Das, 2003). Zinc toxicity results in respiratory and gastrointestinal disorders in humans (Plum *et al*., 2010). Anthropogenic activities like mining, purification of Zn, excessive use of agrochemicals containing Zn and the use of untreated sewage water for irrigating agricultural crops are the major sources of Zn pollution (Lone *et al*., 2008; Tsonev and Lidon, 2012).

Remediation of Zn contaminated soils is receiving more attention recently both from the governmental bodies as well as the public, especially in developing countries (Yanez *et al*., 2002). Technologies involving plants that require long treatment period are used for removing HMs like Zn from areas where the concentrations of these metals are low, soils are shallow or when the medium is water (Li *et al*., 2014). However, when using a plant based system it is important to understand the toxic effects of Zn on the plants and also the ability of plants to grow or regenerate in such toxic environments.

Growing plants in nutrient solutions is a convenient method for studying plants under laboratory conditions and

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is termed as hydroponics (Conn *et al*., 2013). Manipulation of nutrient profile or incorporation of elements like HMs is easy in hydroponics than in the soil. Several factors like the physicochemical properties, total content of the HMs, microbial activities and plant species can affect the bioavailability of HMs in the soil (Jones, 1982). Therefore, HMs studies are often conducted in hydroponic system to better understand the effects of individual or combinations of HMs on plants as there is no interference from other soil factors (Shahid *et al*., 2014; Fasani *et al*., 2018). Moreover, the advantage of hydroponic culture includes reduction in the growth period, duration of treatments and also the space required to carry out the study (Niu *et al*., 2007). Hydroponics also allows us to understand the ability of plants to absorb, concentrate or precipitate toxic metals from contaminated growing medium (Dushenkov *et al*., 1995).

Many plants are propagated vegetatively due to their ease, quick regeneration and production of true-to-type plants. Several factors are shown to affect regeneration of stem cuttings (Hassanein, 2013). These include cutting material, environmental conditions and rooting media. The influence of HMs on the regeneration of stem cuttings have been examined in a wide range of plant species and different HMs (Rajkumar *et al*., 2009; Andrades-Moreno *et al*., 2013; Iori *et al*., 2015; Wang *et al*., 2016). However, information on the influence of Zn on the regeneration potential of stem cuttings is very limited compared to other HMs. A study on the interactive effect of Zn and Cu on the regeneration potential of *Portulaca oleracea* L., stem cuttings revealed that the toxicity of Zn was found to be higher with increasing concentrations of Cu (Jayanthi *et al*., 2015). A high concentration of Zn not only reduces the rooting capacity, but also alters metallothione gene expression in *Populus alba* L. cv. 'Villafranca' (Castiglione *et al*., 2007).

*Talinum portulacifolium* (Forssk.) Asch. *ex* Schweinf., of the plant family Portulacaceae is an erect under-shrub native to Africa (Lansdown, 2013). Though *T. portulacifolium* is categorized as a species of least concern with no major threats according to the IUCN red list of threatened species (Lansdown, 2013), the leaves and roots of this plant is used as food and an aphrodiasic (Miller and Morris, 2004). The extracts prepared from the leaves of *T. portulacifolium* possess antihyperglycemic and antioxidant activities (Babu *et al*., 2009). This plant can be propagated through stem cuttings (Kumar and Prasad, 2010).

The main objective of the present study was to investigate the influence of different concentrations of Zn on regeneration potential of *T. portulacifolium* stem cuttings and the ability of this plant species to accumulate Zn.

## Materials and Methods

*Experimental design and preparation of metal concentrations*

The influence of different concentrations of soluble form of Zn on the regeneration ability of *T. portulacifolium* stem cuttings was studied using nutrient solutions. Stem cuttings of *T. portulacifolium* of approximately equal length and thickness (12 cm long and 1 cm thick) were taken from healthy plants growing under natural conditions in uncontaminated soil at Bharathiar University Campus, Coimbatore, India using a sterile stainless steel sharp knife.

$ZnSO_47H_2O$ formed the source of Zn. Hogland nutrient solution ($KNO_3$, $Ca(NO_3)_24H_2O$, $MgSO_47H_2O$, $NH_4H_2PO_4$, $MnCl_24H_2O$, $H_3BO_3$, $MoO_3$, $ZnSO_47H_2O$, $CuSO_45H_20$, $FeSO_47H_2O$) without or with different concentrations of Zn was prepared by dissolving known quantities of $ZnSO_4$ in Hoagland nutrient solutions to yield the required concentrations of 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm, 30 ppm, 35 ppm, 40 ppm, 45 ppm and 50 ppm (Hoagland and Arnon, 1950). Hoagland nutrient solution without Zn (0 ppm) served as the medium for the growth of control plants.

The experiment was performed in 200 ml capacity glass containers each containing 100 ml of known concentration of Zn. Only the lower part of the twig (approximately one to two cm) was immersed in the Zn solution. The containers were covered with aluminium foil to prevent the growth of algae and development of chlorophyll in roots. There were five replicates for all concentration of Zn including control. Two cuttings were maintained per container. The solutions were replaced every $7^{th}$ day throughout the experimental period. The cuttings were maintained at room temperature (27 ± 4 °C) with 14 h day/10 h dark period at light intensity of 300 to 350 $\mu$mol $m^{-2}$ $s^{-1}$.

*Measurements of plant growth*

The treated cuttings were first observed 7 days after the initiation of the experiment and later regularly at weekly intervals for about 35 days. The regeneration parameters observed were sprouting, root initiation, number of shoots and roots, number of leaves developed, root length, callusing, and any stem decay.

*Harvest*

The plants were harvested after 35 days of the initiation of the experiment, separated into shoot and root parts, and fresh weight was measured. The shoots and roots were then dried at 78 °C for 48 hours in a hot air oven to obtain a stable dry weight.

*Plant elemental analysis*

Dried plant samples (leaves and stem) were ground into a fine powder and wet acid digested with $HNO_3$, $H_2SO_4$, and $HClO_4$ in the ratio of 9:2:1 (Antosiewicz, 1993; Piper, 1966) for quantifying total Zn concentration in plant tissues. The Zn content in the plant digestates were measured using an Atomic Absorption Spectrophotometer (Varian Techtran Spectr AA 10/20 BQ, Australia). Due care was taken to avoid metal contamination during the entire process of harvesting, washing, drying and grinding.

*Tolerance index (Ti)*

Tolerance index (Ti), the ability of a plant to grow in the presence of a given concentration of Zn was calculated according to Wilkins (1978) using the formula:

Ti = (Dry weight of plants raised in Zn solution/ Dry weight of plants raised in control solution)×100

*Statistical analysis*

Mean values of morphological growth parameters were calculated from five replicates in each concentration. The significant variation among means was assessed using Analysis of Variance (ANOVA) after testing for homogeneity (Levene's test). Duncan's Multiple Range Test (DMRT) was performed to separate means of variables where ANOVA was found to be significant. Pearson's correlation analysis and regression analysis was used to assess the relation of increasing Zn concentration on growth and Zn concentrations in stem cuttings.

## Results

*Effect of Zn on shoot and root regeneration and callusing*

Sprouts started to emerge on the stem cuttings on the third day, but root initiation was observed only on the sixth day in 0 and 5 ppm Zn solution (Table 1). Root initiation was further delayed with increasing concentrations (10-50 ppm) of Zn in the solution. The root initiation was delayed by 7 days in concentrations > 20 ppm and was further delayed by 13 days in higher concentrations of 40-50 ppm compared to control. The callus formation (Fig. 1) was maximum at 35 ppm, followed by control and callusing was minimum at 45 ppm. Though Zn concentrations significantly influenced root initiation and callus formation, it did not affect sprouting (Table 1). The delay in sprouting and root initiation was linearly related to increasing concentrations of Zn. However, variations in callus formation were not related to concentrations of Zn in the nutrient solution (Table 1).

*Morphological changes when exposed to various concentrations of Zn*

The stem cuttings showed various morphological changes in response to the exposure to different concentrations of Zn. Increasing concentrations of Zn caused wilting and withering of leaves (Fig. 2). At higher concentrations (40 ppm and above), Zn caused browning and decay of the stem cuttings.

Table 1. Days for initiation of shoots, roots and callus formation in the stem of *Talinum portulacifolium* exposed to various concentrations of Zinc

| Concentration (ppm) | Sprouting (days) | Root initiation (days) | Callus formation (cm) |
|---|---|---|---|
| 0 | 2.6 a | 6.0e | 2.06 a |
| 5 | 2.8 a | 6.0 e | 1.76 abc |
| 10 | 2.8 a | 6.2 e | 1.71 cd |
| 15 | 2.8 a | 6.4de | 1.86 ab |
| 20 | 2.8 a | 6.8 de | 1.80 bcd |
| 25 | 2.8 a | 8.4 cd | 1.64 de |
| 30 | 3.0 a | 9.2 bc | 1.86 ab |
| 35 | 3.0 a | 10.6 b | 2.09 a |
| 40 | 3.0 a | 13.2 a | 1.56 e |
| 45 | 3.0 a | 13.0 a | 1.40 f |
| 50 | 3.0 a | 13.0 a | 1.52 ef |
| $F_{10,44}$ | <1 ns | 14.17** | 2.69* |
| r† | 0.894*** | 0.953*** | -0.570 ns |

†Pearson's correlation coefficient.
*,**,*** Significant at P<0.05, P<0.01, P< 0.001 and ns, not significant.
Mean in a column followed by a same letter(s) are not significantly (P>0.05) different according to Duncan's Multiple Range Test.


Fig. 1. Callus formation of *Talinum portulacifolium* stem cutting exposed to (a) 10 ppm and (b) 40 ppm of Zinc. Note the browning and initiation of decay at the cut surface (white arrow heads) in image b. Scale bars =1 cm.

The decay started at the cut surface and spread upwards. At the maximum concentration (50 ppm) Zn caused withering off the leaves and the narrowing of leaves occurred at concentrations of 30-50 ppm.

*Influence of Zn on the morphological parameters*

*Number of lateral shoots*

Increasing concentrations of Zn significantly influenced the number of lateral shoots on *T. portulacifolium* stem cuttings. Nevertheless, neither growth period nor its interaction with different concentrations of Zn significantly influenced the number of lateral shoots developed on the stem cuttings (Table 2).

The highest number of lateral shoots was observed in 0, 10, 30, 35 and 45 ppm on the seventh day. However, the number of lateral shoots was 7.14-17.86% lower in other treatments. At 14$^{th}$ day, the number of lateral shoots in stem cuttings raised in different concentrations of Zn was 6.25-31.25% lower than the control stem cuttings. There was no lateral shoot formation after the 14$^{th}$ day of the initiation of the study except for 35$^{th}$ day where cuttings raised in 45 ppm of Zn had 7.14% more lateral shoots than at 28$^{th}$ day (Table 2).

*Elongation of stem cuttings*

The length of *T. portulacifolium* stem cuttings significantly varied with growth period and concentrations of Zn in the nutrient solution. But the interaction between these factors was not significant. Though the influence of increasing concentrations of Zn on the length of *T. portulacifolium* stem cuttings was not linear initially (seventh day), it was apparent during later stages of cutting development.

Different concentrations of Zn increased the length of the cuttings by 8.16-65.30% at the seventh day except for 25 ppm, 35 ppm and 50 ppm where the length of the cuttings was 4.05-13.95% lower than control. At 5 ppm concentration, Zn increased the length of the cutting by 8.27-15.85% between 15$^{th}$ and 35$^{th}$ day, whereas cuttings in other concentrations of Zn had reduced shoot length compared to control during the same period. Although cuttings in 15 ppm Zn had reduced shoot length compared to control during initial stages, a 1.75% and 2.69% increase in shoot length compared to control was evident during 28$^{th}$ and 35$^{th}$ day (Table 2).

*Leaf numbers*

Growth period, as well as concentrations of Zn significantly influenced the number of leaves on *T. portulacifolium* stem cuttings. However the interaction day × treatment for leaf number was not significant. The linear decline in the number of leaves with increasing concentrations of Zn was evident during different stages of cutting development except for the seventh and 28$^{th}$ day.

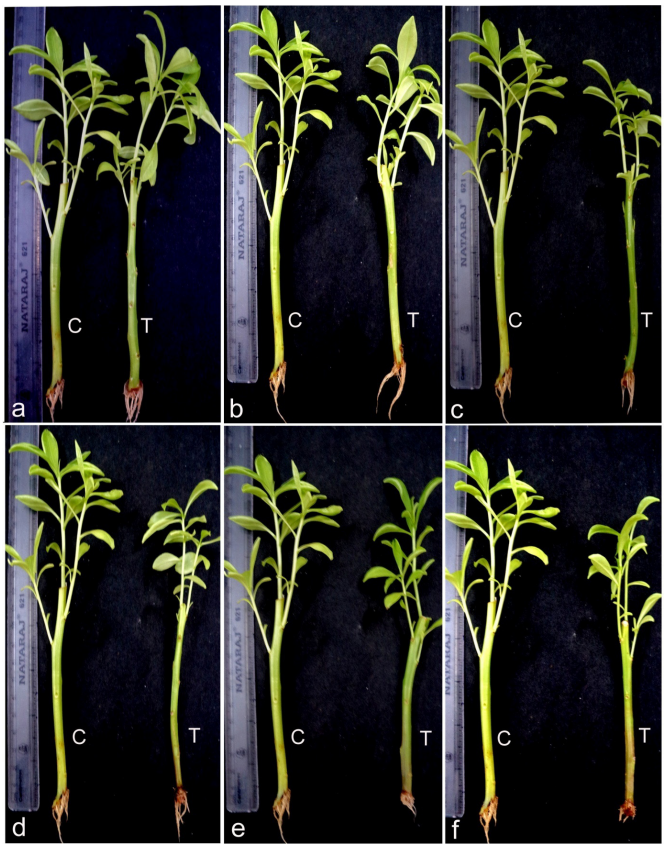


Fig. 2. Morphological aspects of *Talinum portulacifolium* exposed to 5 ppm (a), 15 ppm (b), 30 ppm (c), 40 ppm (d), 45 ppm (e) and 50 ppm (f) of Zinc. C, Control and T, Treatment

Table 2. Influence of different concentrations of Zn on the regeneration of *Talinum portulacifolium* stem cuttings over a period of 35 days

| Days | Concentration (ppm) | Lateral shoot number (per cutting) | Shoot length (cm) | Leaf number (per cutting) | Root number (per cutting) | Total root length (cm) |
|---|---|---|---|---|---|---|
| | 0 | 2.8 c | 0.98 c | 6.4 a | 34.8 e | 1.10 c |
| | 5 | 2.3 a | 1.14 fg | 8.2 bc | 24.2 d | 1.46 d |
| | 10 | 2.8 c | 1.16 gh | 8.4 c | 16.6 c | 1.32 d |
| | 15 | 2.4 a | 1.10 ef | 6.4 a | 17.0 c | 0.62 b |
| | 20 | 2.4 a | 1.06 de | 10.4 e | 1.0 b | 0.00 a |
| | 25 | 2.6 b | 0.94 bc | 10.2 e | 0.6 b | 0.00 a |
| | 30 | 2.8 c | 1.06 de | 9.2 d | 0.0 a | 0.00 a |
| Seventh | 35 | 2.8 c | 0.92 b | 7.6 b | 0.0 a | 0.00 a |
| | 40 | 2.6 b | 1.18 h | 11.8 f | 0.0 a | 0.00 a |
| | 45 | 2.8 c | 1.62 i | 9.4 d | 0.0 a | 0.00 a |
| | 50 | 2.4 a | 0.86 a | 8.2 bc | 0.0 a | 0.00 a |
| r† (n=11) | | 0.089 ns | 0.151 ns | 0.455 ns | -0.860** | -0.828** |
| | 0 | 3.2 f | 5.08 fg | 20.4 ef | 32.6 g | 4.90 a |
| | 5 | 3.0 e | 5.50 g | 21.4 f | 39.2 h | 2.98 g |
| | 10 | 2.8 d | 3.80 de | 17.2 cd | 26.4 f | 2.44 f |
| | 15 | 2.6 c | 4.40 e | 18.4 de | 22.8 e | 1.76 e |
| | 20 | 2.4 b | 3.68 cd | 15.8 bc | 5.4 a | 1. 76 e |
| | 25 | 2.8 d | 3.74 d | 17.0 cd | 13.4 c | 1.26 d |
| | 30 | 2.4 b | 3.28 b | 15.4 bc | 14.4 cd | 0.60 c |
| Fourteenth | 35 | 2.8 d | 3.16 b | 17.6 cde | 16.6 d | 0.44 b |
| | 40 | 2.6 c | 3.50 bc | 20.2 ef | 14.8 cd | 0.40 b |
| | 45 | 2.8 d | 2.08 a | 14.2 ab | 9.0 b | 0.28 a |
| | 50 | 2.2 a | 3.30 b | 13.8 a | 10.2 b | 0.40 b |
| r† (n=11) | | -0.628* | -0.844** | -0.654* | -0.779** | -0.907*** |
| | 0 | 3.2 e | 5.31e | 22.2 fg | 35.4 e | 5.20 g |
| | 5 | 3.0 de | 6.14 f | 23.4 g | 37.4 e | 3.42 f |
| | 10 | 2.8 cd | 4.42 d | 18.0 cd | 28.6 d | 2.86 ef |
| | 15 | 2.6 bc | 5.22 e | 19.2 e | 41.8 f | 2.40 e |
| | 20 | 2.4 ab | 4.30 cd | 17.0 b | 23.8 c | 4.46 g |
| | 25 | 2.8 cd | 4.31 cd | 17.4 bc | 24.8 c | 1.44 d |
| | 30 | 2.4 ab | 3.66 b | 17.4 bc | 25.6 cd | 0.82 cd |
| Twenty-one | 35 | 2.8 cd | 3.78 b | 19.0 de | 25.8 cd | 0.78 bc |
| | 40 | 2.6 bc | 4.50 d | 22.0 fg | 19.0 b | 0.42 a |
| | 45 | 2.8 cd | 3.06 a | 15.8 a | 18.6 b | 0.44 a |
| | 50 | 2.2 a | 4.18 bcd | 14.8 a | 13.4 a | 0.50 a |
| r† (n=11) | | -0.628* | -0.716** | -0.606* | -0.857*** | -0.871*** |
| | 0 | 3.2 e | 5.70 g | 23.6 gh | 41.4 h | 5.54 i |
| | 5 | 3.0 de | 6.40 i | 24.4 h | 39.6 g | 3.62 h |
| | 10 | 2.8 cd | 4.46 de | 18.6 cd | 29.0 f | 2.92 g |
| | 15 | 2.6 bc | 5.80 h | 19.6 de | 41.8 h | 2.58 f |
| | 20 | 2.4 b | 4.42 cd | 16.8 b | 23.8 de | 1.98 e |
| | 25 | 2.8 cd | 4.56 e | 19.0 de | 25.0 ef | 1.52 d |
| Twenty-eight | 30 | 2.6 bc | 4.34 c | 17.8 bc | 25.6 ef | 1.08 c |
| | 35 | 2.8 cd | 4.12 b | 20.4 e | 25.8 ef | 0.78 b |
| | 40 | 2.6 bc | 4.84 f | 22.6 fg | 20.2 c | 0.42 a |
| | 45 | 2.8 cd | 3.50 a | 17.6 bc | 18.6 b | 0.46 a |
| | 50 | 2.2 a | 4.40 cd | 14.8 a | 13.4 a | 0.54 a |
| r† (n=11) | | -0.628* | -0.725* | -0.575 | -0.890*** | -0.930*** |
| | 0 | 3.2 c | 5.94 f | 24.2 g | 41.4 g | 5.58 h |
| | 5 | 3.0 bc | 6.54 g | 24.6 g | 41.0 g | 3.92 g |
| | 10 | 2.8 abc | 4.70 cd | 19.0 cde | 29.0 f | 2.94 f |
| | 15 | 2.6 abc | 6.10 f | 19.6 de | 41.8 g | 2.80 f |
| | 20 | 2.4 ab | 4.58 c | 16.8 b | 23.8 bc | 2.12 e |
| | 25 | 2.8 abc | 4.92 de | 19.0 cde | 20.3 bc | 1.52 d |
| | 30 | 2.6 abc | 4.64 c | 18.0 bcd | 26.6 e | 1.42 d |
| Thirty-five | 35 | 2.8 abc | 4.36 b | 20.0 e | 25.8 de | 0.82 c |
| | 40 | 2.6 abc | 5.20 e | 22.8 f | 19.2 b | 0.58 b |
| | 45 | 3.0 bc | 3.74 a | 17.4 bc | 19.8 b | 0.46 a |
| | 50 | 2.2 a | 4.60 c | 15.0 a | 13.4 a | 0.54 b |
| r† (n=11) | | -0.526 ns | -0.644* | -0.605* | -0.864*** | -0.941*** |
| F statistics | *df* | | | | | |
| Days (D) | 4,220 | <1 ns | 193.51** | 24.94** | 58.47** | 9.41** |
| Concentration (C) | 10,220 | 4.80** | 10.35** | 1.66 ns | 11.29** | 2.30* |
| D x C | 40,220 | <1 ns | <1 ns | <1 ns | <1 ns | 1.61* |

†Pearson's correlation coefficient. *,**, *** Significant at P< 0.05, P<0.01, P< 0.001 and ns, not significant.
Mean in a column for a day followed by a same letter(s) are not significantly (P>0.05) different according to Duncan's Multiple Range Test.

The number of leaves on stem cuttings raised in 15 ppm of Zn was similar to control at the seventh day, while cuttings raised in other concentrations of Zn had 18.75-84.38% more leaves than control. Likewise, cuttings raised in 5 ppm of Zn had 1.65-5.41% more leaves than control during 14th to 35th day of growth. The number of leaves in other concentrations of Zn was 0.98-32.35%, 0.90-33.33%, 4.24-37.29%, and 5.79-38.02% lower than the respective controls respectively during 14th, 21st, 28th and 35th days of growth.

*Rooting*

Different concentrations of Zn and growth period significantly influenced the number of roots on *T. portulacifolium* stem cuttings. The decrease in root numbers was inversely related to increasing concentrations of Zn in the nutrient solution, but the interaction among these factors was not significant.

At the seventh day, different concentrations of Zn reduced root numbers by 30.46-100% when compared to control. Contrarily, 5 ppm of Zn increased the number of roots in *T. portulacifolium* stem cuttings by 20.25% and 5.65% at 14th and 21st days respectively. An 18.08%, 0.97% and 0.97% increase in root number than control was also evident in cuttings raised in the presence of 10 ppm of Zn during 21st, 28th and 35th days of growth. The number of roots in other concentrations of Zn was 19.02-83.44%, 19.21-62.15%, 4.35-67.63%, and 0.97-67.63% lower than the respective controls during 14th, 21st, 28th and 35th days of growth (Table 2).

Increasing concentrations of Zn inversely affected root length of *T. portulacifolium* stem cuttings. However, the root length of cuttings grown in the presence of 5 ppm and 10 ppm was 0.36% and 0.22% higher than the control at the seventh day. The roots of *T. portulacifolium* stem cuttings were 0.48% shorter at 15 ppm Zn. Different concentrations of Zn and growth period significantly affected root length of *T. portulacifolium* stem cuttings. The interaction day × treatment was also significant for root length. The roots of *T. portulacifolium* stem cuttings raised in different concentrations of Zn was 39.18-94.29%, 14.23-91.92%, 34.66-92.42%, and 29.75-91.76% shorter than the respective controls during 14th, 21st, 28th and 35th days of growth.

*Fresh and dry weights*

Significant differences existed for fresh weights of leaves, and roots of *T. portulacifolium* stem cuttings raised in different concentrations of Zn (Table 3). In contrast, the variation in fresh weight of stems was not significant. Leaf dry weight significantly varied among treatments for cuttings grown in different concentrations of Zn. Nevertheless, no such significant variation exited for stem dry weight. Different concentrations of Zn significantly influenced the root dry weights. Though different concentrations of Zn negatively influenced the leaf fresh and dry weights, such an influence was only evident for fresh weight of roots (Table 3).

Low concentration of Zn increased the fresh and dry weights of leaves which were respectively 11.17% and 26.79% higher than the control and 77.06-243.80% and 47.92-255.01% higher than those raised in other concentrations of Zn. Fresh and dry weights of roots of *T. portulacifolium* stem cuttings raised in different concentrations of Zn were 28.57-90.47% and 27.27-90.91% lower than the control root cuttings (Table 3).

*Tolerance index*

Maximum tolerance index (Ti) was observed for stem cutting raised in 40 ppm of Zn which was 9.88-69.07% higher than those raised in other concentrations of Zn. Similarly, cuttings raised in 45 ppm of Zn had the least Ti which was 4.28-41.25% lower than cuttings raised in other concentrations of Zn (Fig. 3).

*Accumulation of Zn in the stem and leaves*

Significant variation ($P<0.001$) existed between the accumulation of Zn in the stem ($F_{10,44} = 289.111$) and leaf ($F_{10,44} = 188.887$) of *T. portulacifolium* at various concentrations of Zn (Fig. 4). A linear increase in the accumulation of Zn was observed in the leaves and stems of *T. portulacifolium*, with the highest Zn accumulation occurring at 40 ppm in both the plant parts (Fig. 4).

Table 3. Fresh and dry weight of leaves, stem and roots of *Talinum portulacifolium* exposed to different concentrations of Zinc after 35 days of growth

| Concentration (ppm) | Fresh weight (g) | | | Dry weight (g) | | |
|---|---|---|---|---|---|---|
| | Leaf | Stem | Root | Leaf | Stem | Root |
| 0 | 1.271a | 4.13 ab | 0.042 a | 0.056 ab | 0.211 a | 0.011 a |
| 5 | 1.413 a | 4.87 ab | 0.030 b | 0.071 a | 0.264 a | 0.008 bc |
| 10 | 0.72b c | 3.48 ab | 0.016 c | 0.044 c | 0.208 a | 0.004 cde |
| 15 | 0.798 b | 3.93 ab | 0.018 c | 0.042 cd | 0.304 a | 0.005 cd |
| 20 | 0.552 bc | 3.68 ab | 0.010 c | 0.031 cd | 0.296 a | 0.003 de |
| 25 | 0.522 bc | 4.15 ab | 0.022 c | 0.022 cd | 0.202 a | 0.006 cd |
| 30 | 0.588 bc | 5.21 ab | 0.012 c | 0.028 cd | 0.252 a | 0.003 de |
| 35 | 0.508 bc | 4.24 ab | 0.004 c | 0.043 bc | 0.262 a | 0.001 e |
| 40 | 0.672 bc | 5.47 a | 0.010 c | 0.048 cd | 0.332 a | 0.003 de |
| 45 | 0.411c | 3.65 ab | 0.004 c | 0.034 d | 0.218 a | 0.001 e |
| 50 | 0.460 c | 3.94 b | 0.008 c | 0.020 cd | 0.218 a | 0.002 de |
| $F_{10,44}$ | 12.10** | 1.13 ns | 8.31** | 5.81** | <1 ns | 9.56** |
| r† (n=11) | -0.802** | 0.106 ns | -0.810** | -0.641* | 0.076 ns | 0.500 ns |

†Pearson's correlation coefficient.

*,** Significant at $P< 0.05$, $P< 0.01$ and ns, not significant.

Mean in a column for a zinc concentration followed by a same letter(s) are not significantly ($P>0.05$) different according to Duncan's Multiple Range Test.

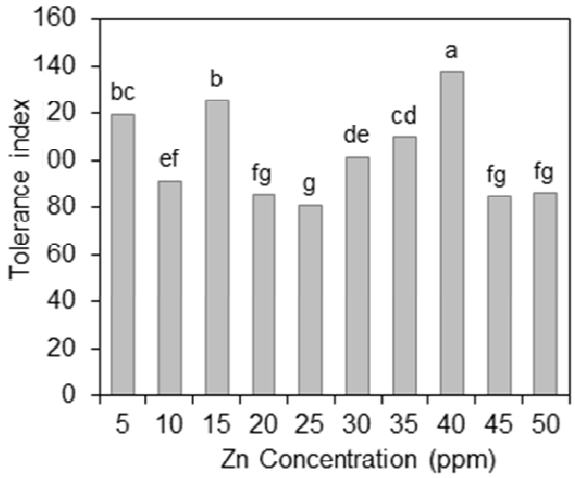


Fig. 3. Tolerance index of *Talinum portulacifolium* exposed to different concentrations of Zinc. Bars bearing same letter(s) are not significantly (P>0.05) different according to Duncan's Multiple Range Test

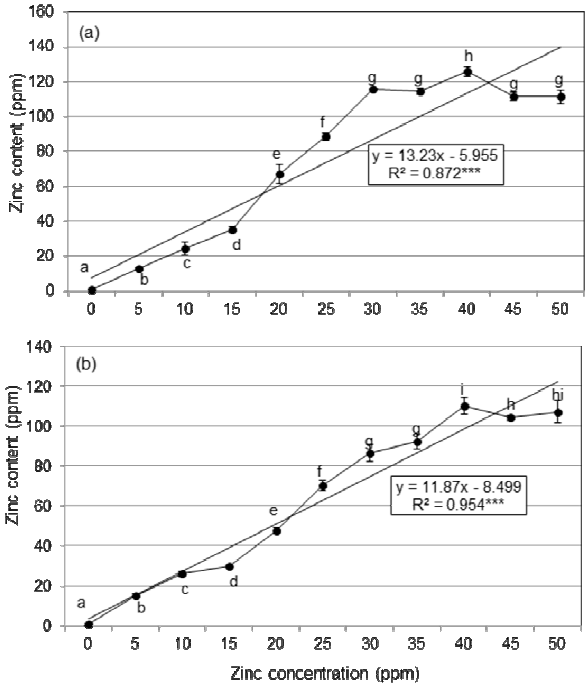


Fig. 4. Accumulation of Zinc in the stem (a) and leaf (b) of *Talinum portulacifolium* in nutrient solution. Error bar indicates ± standard error. Points followed by same letters are not significantly different according to DMRT (P>0.05). ***Significant at P < 0.001.

**Discussion**

The results of the present study clearly showed that increasing concentrations of Zn in the nutrient solution affected the regeneration of *T. portulacifolium* stem cuttings and the inhibition of development was at many instances proportional to the increase in concentrations of Zn in the nutrient solution. Root initiation on *T. portulacifolium* stem cuttings was delayed by almost seven to eight days in higher concentrations of Zn. This is similar to the results of a study where higher concentrations of Cu delayed root initiation in *P. oleracea* (Mohanapriya *et al*., 2006). The inhibitory effect of HMs on the plant hormone metabolic pathways can induce changes in the production of key plant growth hormones like auxins and cytokinins that are responsible for rooting (Vysotskaya *et al*., 2007). This could have delayed rooting as observed in *T. portulacifolium* stem cuttings. A moderate delay was observed in the development of leaves, as the leaves appeared on 2nd to 3rd day on the stem cuttings, which is in accordance with the results obtained by Mohanapriya *et al*. (2006) in *P. oleracea*. Sprouting of the stem cuttings even at the highest concentrations of Zn tested indicates the lack of critical concentration of Zn accumulation at the site of sprouting in the stem. Moreover, as roots are in direct contact with the metal solution, roots exhibit symptoms of metal interaction much earlier than shoots (Mohanapriya *et al*., 2006).

The decrease in the number of shoot buds and leaves with increasing concentrations of Zn may be attributed to the effect of Zn on the phytohormone cytokinin. Cytokinin is known to promote axillary and adventitious shoot formation due to its effect on cell division and shoot morphogenesis (Wang *et al*., 2017). Similar findings were also reported for *P. oleracea* stem cuttings when raised on different concentrations and sources of copper (Cu) (Mohanapriya *et al*., 2006) and for *Talinum triangulare* (Jacq.) Willd., stem cuttings exposed to various concentrations (Rajkumar *et al*., 2009).

At higher concentrations, Zn not only inhibited the development of roots on *T. portulacifolium* stem cuttings completely during the first week of exposure but also reduced the development of roots during later stages as well. This is similar to the observations made in *P. oleracea* and *T. triangulare* stem cuttings where exposure to HMs not only delayed root initiation but also reduced root growth (Mohanapriya *et al*., 2006; Rajkumar *et al*., 2009). Jewell (1994) also showed that Zn could reduce root growth in *Festula rubra* L. and *Calamagrostis epigejos* (L.) Roth. The negative influence of Zn on the development of roots can be due to its influence on the division and elongation of plant cells (Seregin *et al*., 2011). Although Zn is involved in the synthesis of tryptophan a precursor of auxin (Tsonev and Lidon, 2012), excess Zn in roots can influence auxin transport and its distribution in root tips thereby affecting root growth (Zhang *et al*., 2018). The linear reduction in root length with increasing concentrations of Zn in the nutrient solution is in line with the observations of Zhang *et al*. (2018) who showed that high concentrations of Zn in root tips of *Arabidopsis* reduced the abundance of PIN4 (peptidylprolyl cis/trans isomerase, NIMA-interacting 4) which is involved in the distribution of auxins thereby inhibiting root elongation. The lack of any significant effect of Zn at lower concentrations on rooting in *T. portulacifolium* stem cuttings is similar to the findings of Rajkumar *et al*. (2009) in *T. triagulare* where only higher concentrations of Cd was shown to inhibit root growth

while lower concentrations failed to show any marked effect. The toxic effect of Zn directly either on the membrane integrity or cell division might have contributed to the reduction in the development of roots on the stem cuttings. Subsequently, the effects of Zn on respiration, photosynthesis, and synthesis of proteins in roots may also contribute to the retardation of normal root development on the stem cuttings of *T. portulacifolium* (Agarwal *et al*., 1987). The observed inhibition of root growth indicates the metal penetration and accumulation in roots (Mohanapriya *et al*., 2006). Roots developed in the presence of Zn were much paler and finer than those developed in its absence.

Dry mass of the stem, leaves and roots reduced with increasing concentrations of Zn. The suppression of growth and decrease in dry weight of different plant parts is well supported by the results obtained by Kubota and Takenaka (2003). When toxic elements enter plants, the toxic effect of these elements on plant metabolism often results in reduced biomass production. The reduction in plant biomass may occur either at an organ level or on the whole plant (Hagemeyer, 1999; Sadeghzadeh, 2013). The proportion decline in plant biomass was correlated to an increase in the accumulation of Zn in plant parts. The negative influence of Zn on plant growth can result from the blocking of the metabolic pathways that are associated with the growth and development of plants due to the direct toxic effects of the HMs (Wierzbicka and Obidzinska, 1998). Although not examined in the present study, high concentrations of Zn in the plant growth medium are known to compete with plant's uptake of nutrients like phosphorus, Cu, iron, magnesium and manganese causing their deficiencies in plants (Broadley *et al*., 2007). This could have also contributed to the decline in growth of *T. portulacifolium* stem cuttings with increasing concentrations of Zn in this study. Tolerance index has been a useful indicator for characterizing plant tolerance to HMs. In Zn, the high Ti at 40 ppm (133.68%) indicates an increase in biomass suggesting that plants express a growth dilution effect due to its tolerance to high Zn concentrations (Audet and Charest, 2007).

The phytotoxic effect of Zn was well pronounced in *T. portulacifolium* at high concentrations of Zn in the nutrient solution. Generally, toxic symptoms become visible when the concentrations of Zn exceed 300 ppm in the leaves (Broadley *et al*., 2007). Nevertheless, certain plant species like *T. portulacifolium* as observed in the present study can express toxicity symptoms even at less than 100 ppm of leaf Zn concentration. The toxicity threshold for Zn can vary significantly even for the same plant species under different growing conditions and Zn toxicity at less than 100 ppm plant tissue concentration has been reported in certain crop species (Takkar and Mann, 1978). Studies have shown that leafy vegetables are more sensitive to Zn toxicity due to their inherent ability to uptake and accumulate high concentrations of Zn in their tissues (Broadley *et al*., 2007). Various morphological changes like wilting and withering in addition to the necrotic spots was evident on leaves at high concentrations of Zn. The accelerated senescence in leaves may be due to the increased membrane permeability induced by Zn (Langille and MacLean, 1976). Chlorosis of leaves was also seen at higher concentrations of Zn. This may be due to the reduction in the abundance of the chloroplast due to a decrease in their number per cell or a change in cell size. Baryla *et al.* (2001) showed that *Brassica napus* L., growing in HMs contaminated soils induced chlorosis due to the interference of the HM with chloroplast replication and cell division. The toxicity symptoms of Zn manifested by the appearance of necrotic spots and chlorosis of leaves, weak development of root branches and browning of roots similar to those induced by Cd toxicity (Das *et al*., 1997). Koleva *et al*. (2010) also reported necrotic spots in leaves of *Triticum durum* Desf., in response to Zn toxicity.

Higher concentrations of Zn caused the decay of the stem cutting which started from the base and progressed upwards. The initiation and progress of decay from the cut surface of the stem with increasing exposure time and concentrations of Zn is similar to the findings reported in *P. oleracea* by Makeshkumar *et al*. (1996). The initiation of decay in the stem cuttings in response to exposure to high concentrations of HMs has been ascribed to the failure in the functional integrity of cells resulting in microbial colonization and degradation (Rajput and Rao, 2006). Nevertheless, the decay process also depends on the concentrations of the metals and the gradient effect (Klapheck *et al*., 1994). Pink coloration and disappearance of colour in the metal solution deserve further investigation. However, the pink coloration may be due to the leaching out of plant pigments which were also noted by Rajkumar *et al*. (2009).

The accumulation of Zn in the leaves of *T. portulacifolium* stem cuttings was almost similar or slightly higher than those in the stems at lower concentrations of Zn exposure (up to 10 ppm). However, accumulation of Zn was always higher in the stems than in the leaves exposed to >10 ppm of Zn. The increased Zn accumulation as seen in stems and leaves of *T. portulacifolium* with increasing concentrations of Zn was also observed in *Brassica rapa* L., exposed to different concentrations of Zn in a hydroponic system (Coolong *et al*., 2004). The high Zn content in the leaves of *T. portulacifolium* was also observed in *Salix purpurea* L. (Dos Santos Utmazian *et al*., 2007) which could be due to the high mobility of Zn in plant parts (Clemens, 2017).

## Conclusions

It can be stated that Zn studied interfere with the regeneration potential of *T. portulacifolium* by affecting root development and/or their decay. Further studies on the significance of substrate in HMs accumulation would bring more evidence in the phytoremediation of HMs and their effects on the plants.

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