OPEN ACCESS Pakistan Journal of Agricultural Research

Research Article

Influence of Foliar Application of Zinc on Growth, Yield and Zinc Concentration in Strawberry

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Abstract | Deficiency of zinc (Zn) in the soils of Pakistan is now well recognized and hence its application in crop husbandry is essentially required. In a field experiment, the impact of foliar Zn fertilization was quantified on growth, yield, and Zn concentration in strawberry (*Fragaria x ananassa* cv. Silder) at Nasarpur, Sindh, Pakistan during 2015-16. The foliar rates comprised of 33, 66, and 99 mg Zn L^{-1} (equivalent to 100, 200 and 300 mg $ZnSO_4$ L^{-1}) and distilled water (control treatment) and sprayed to strawberry plants before flower initiation. A continual increase in number of leaves, number and weight of berries, fresh weight of strawberry plants, and Zn concentration in strawberry fruit was observed as a function of foliar application of zinc. Plants sprayed with 66 and 99 mg Zn L^{-1} had significantly more number of leaves (27.4 ± 1.14 and 29.9 ± 1.36) and berries plant^{-1} (12.0 ± 1.02 and 13.0 ± 0.61), heavier berries plant^{-1} (129.7 ± 8.15 g and 139.5 ± 2.85g), and higher plant fresh weight (33.8 ± 5.40 g and 40.2 ± 5.74 g) respectively, when compared with plants that were sprayed with distilled water. However, the zinc concentration in strawberries was significantly highest in plants that were treated with 99 mg Zn L^{-1} (37.2 ± 1.98 µg g^{-1}) than all other treatments. We suggest that the foliar application of Zn (99 mg Zn L^{-1}) may be included in strawberry farming for profitable yields and high Zn concentration in strawberry fruit.

Received | October 07, 2020; **Accepted** | March 18, 2021; **Published** | June 05, 2021
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Citation | Bhatti, S.M., M.A. Panhwar, Z.R. Bughio, M.S. Sarki, A.W. Gandahi, and N.A. Wahocho. 2021. Influence of foliar application of zinc on growth, yield and zinc concentration in strawberry. *Pakistan Journal of Agricultural Research,* 34(2): 486-493.
DOI | http://dx.doi.org/10.17582/journal.pjar/2021/34.2.486.493
Keywords | Strawberry, Zn deficiency, Foliar application of Zn, Growth and yield, Zn concentration

Introduction

Strawberry (*Fragaria x ananassa*) is a delicious and nutritious fruit of the world (Bibi *et al.*, 2016). It is widely consumed as fresh and/or used as flavor in food products i.e., ice-creams, jams, jellies, cakes, and milk shakes (Codrea *et al.*, 2019). Strawberries serve as an important source of vitamins (A, B1, B2 and C), fiber, calories and minerals (Kazemi, 2015; Singh *et al.*, 2015; Hossain *et al.*, 2016). Moreover, it also possesses some medicinal properties like anticarcinogenic, antidiabetic and antioxidant (Kumar *et al.*, 2017). This fruit is achieving popularity among the consumers of all age groups. The nutritional studies suggest that one hundred grams edible portion of strawberry may contain about 90 g water, 0.5 g fats, 59 g ascorbic acid, 8.4 g carbohydrates and 0.07 g proteins (Hossain *et al.*, 2016; Tariq *et al.*, 2018).

Strawberries are cultivated worldwide on an area of 255 thousand hectares with annual production of 7.7 MT (FAOSTAT, 2015). The major strawberry

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cultivating countries are China, USA, Mexico, Turkey, and Spain (Memon, 2014; FAOSTAT, 2015). In Pakistan, strawberry is an emerging fruit crop and is planted in temperate and subtropical areas of the country (Rajwana *et al*., 2017). It is planted on an area of 227 ha with the production of 96 tonnes (GOP, 2016). The major strawberry cultivation areas in the country include Swat, Mansehra, Abbottabad, Mardan, Haripur, Peshawar, Charsadda, Gujrat, Jhelum, Sialkot, Chakwal, Karachi, Dadu, Khairpur and Sukkur (Memon, 2014). The cultivated strawberry in Pakistan is sold either locally or exported to various countries, such as Afghanistan, Denmark, Norway, UAE and U.K. (Memon, 2014). The export potential of this fruit indicates the possibility of earning revenue for the country, provided the cultivated area and yield is increased.

Strawberry cultivation may offer numerous benefits including (i) the crop starts producing fruit within months of planting, (ii) many plots may be established because of having small sized plants, (iii) growth and yield components may easily be monitored because of crop morphology (May and Pritts, 1993). Afridi *et al*. (2009) suggested that strawberry production has relative advantage over wheat and sugarcane. These researchers estimated that the net income of strawberry was approximately nine times greater than wheat crop and approximately four times greater than sugarcane.

Micronutrients perform an essential role in the production of fruit crops, and their deficiencies largely affect the quality of fruits. Among micronutrients, horticultural crops suffer from Zn deficiency worldwide (Suman *et al*., 2017). The soils of Pakistan are deficient in many essential macro and micro nutrients. Among various essential micronutrients, Zinc (Zn) has been reported to exist in deficient levels in Pakistan soils. Zinc deficiency in Pakistan was the first micronutrient disorder recognized in 1967 as a cause of *hadda* disease in rice (Yoshida and Tanaka, 1969). Afterwards, extensive research has been carried out in Pakistan and it has been reported that 70% soils of Pakistan are Zn deficient (Imtiaz *et al*., 2010). The positive impacts of Zn fertilization on growth and yield have been reported in many crops including rice and wheat (Khan *et al*., 2007; Abbas *et al*., 2009; Ali *et al*., 2013).The positive effects may be attributed to role of Zn in plants physiological functions. Zn is a component of various enzymes, promotes growth hormones, starch formation, seed maturation and production (Brady and Weil, 2002; Ibrahim, 2013).

To our knowledge, no study has been found in Pakistan where the influence of Zn has been determined on the growth and yield of strawberry. Studies from overseas indicate that the application of Zn increases growth, yield, and quality of strawberry (Bakshi *et al*., 2013a; Kazemi, 2015; Rahman *et al*., 2016). We hypothesize that, the foliar Zn fertilization will enhance yield and Zn concentration in strawberry. Based on this hypothesis, the current study was designed to determine the effect of Zn on growth, yield, and Zn concentration in fruit of strawberry as a function of Zn foliar application.

Materials and Methods

Experimental details

The study was conducted at Syed Muhammad Alam Shah (SMAS) fruit farm KT near Nasarpur, Matiari during October 2015 to March 2016. The experiment was organized in Randomized complete block design (RCBD) for strawberry (*Fragaria x ananassa*, cv. Silder hybrid) plantation. Ridges of 2 meters length were prepared, and the runners of strawberry were planted on the top of ridges at 6 inches. This constituted 12-13 runners per ridge. The runners were transplanted during the 3rd week of October 2015. The N, P_2O_5, and K_2O fertilizers were applied after land preparation at recommended rates of 120, 135 and 50 kg ha^{-1} respectively, by Ahsan (2014). Full dose of P and K were applied as single super phosphate (SSP) and sulphate of potash (SOP) prior to strawberry planting. Nitrogen was applied in four equal splits as urea; 1st dose was applied prior to strawberry planting, while 2nd, 3rd and 4th splits were given with succeeding irrigations. The experiment involved four treatments, each having four replications. The treatments were: T1: Sprayed with distilled water, T2: Sprayed with 33 mg Zn L^{-1} (equivalent to 100 mg $ZnSO_4$ L^{-1}), T3: Sprayed with 66 mg Zn L^{-1} (equivalent to 200 mg $ZnSO_4$ L^{-1}) and T4: Sprayed with 99 mg L^{-1} (equivalent to 300 mg $ZnSO_4$ L^{-1}). Foliar application of Zn was applied once only i.e., before flower initiation. All the plants were sprayed until the runoff point (75 ml per plant) using a hand sprayer. Canal water was used for irrigation. First irrigation was given after the transplantation of runners. Succeeding irrigations were given at seven days interval.

Plant sampling

At harvest, two plants from each ridge (one ridge represents one replication) were selected for following observations; number of leaves and berries plant^{-1}, weight of berries plant^{-1} (g), and fresh biomass of strawberry plant (g). The leaves were counted after Zn application on fortnightly basis till harvesting. In this sequence, numbers of leaves were counted five times. These observations were summed up and reported in the result section as total number of leaves produced after spraying with Zn treatments. At maturity stage (when fruits turned red with waxy layer), the strawberries were picked one by one as they matured and recorded for number of berries and weight of berries. This process was initiated during the 1st week of March 2016 and remained continued for the entire month. The process of picking was performed on daily basis. The picked berries were counted and were placed on electrical balance for the fresh weight. After picking of mature berries, the aboveground plant part was removed by scissors at the soil surface level and weight of plant was determined by electrical balance.

Zinc concentration in berries

For Zn concentration, harvested berries were oven dried at 68°C for 48 hours. The oven dried strawberries were ground using Grinder Machine (ANEX, Germany). To digest berries samples, the method proposed by Estefan *et al.* (2013) was adopted. One gram of oven dried berries was digested with di-acids (HNO_3-$HClO_4$,2:1), by using hot plate. The samples were cooled and diluted with 25 mL distilled water. The prepared samples were run on Atomic Absorption Spectrometer (Model Novaa, 400, Analytik Jena, Germany) at Nuclear Institute of Agriculture (NIA), Tando Jam.

Soil analysis

The composite samples were taken only prior to land preparation at the depth of 0-15 cm and 15-30 cm. Soil samples were examined for different physico-chemical properties. Texture of soil samples was determined by Hydrometer method (Bouyoucos, 1962). For measurement of electrical conductivity and pH, soil water extracts of 1:2.5 were prepared. For EC measurement, EC meter (SensoDirect Con 100, Lovibond, Germany) and for pH measurement, pH meter (SensoDirect pH 110, Lovibond, Germany) with glass electrode was used. Soil organic matter content was determined as proposed by Walkley-Black (1934). In short, two grams of soil was added proposed amount of 1 N potassium dichromate ($K_2Cr_2O_7$) solution, concentrated sulphuric acid (H_2SO_4), distilled water, orthophosphoric acid (H_3PO_4) and few drops of diphenylamine indicator. This content was titrated against 0.5 N ferrous ammonium sulphate till green color appeared. For zinc analysis in soil, AB-DTPA procedure as outlined by Estefan *et al.* (2013) was adopted. In brief, forty grams of soil was added with 80 ml of AB-DTPA solution, followed by shaking and filtration. The filtrate was used for Zn analysis by Atomic Absorption Spectrophotometer (Novaa 400, Analytik Jena, Germany) at NIA Tando Jam.

Statistical analysis

The collected data was statistically analyzed to observe differences among the treatments. The analysis of variance (ANOVA) was performed using Minitab 17 (Minitab Ltd. USA) computer software. The variations among the treatments were assessed by Tukey's test at 0.05 *P* value.

Results and Discussion

The physico-chemical characteristics of the experimental site, prior to strawberry plantation indicate that the soil at both depths (0-15 and 15-30 cm) was alkaline in nature (pH 8.84 ± 0.08 and 8.64 ± 0.05), non-saline (EC 1.00 ± 0.003 dS m^{-1} and 0.58 ± 0.022 dS m^{-1}), moderate in organic matter content (1.20 ± 0.14% and 1.02 ± 0.26%) and sandy loam in texture. Extractable zinc content at the depth of 0-15 cm was marginal (1.2 µg g^{-1}) and low (0.9 µg g^{-1}) at the depth of 15-30 cm.

Total number of leaves plant^{-1}

The influence of foliar application of Zn remained highly significant for number of leaves plant^{-1} ($P < 0.05$; Figure 1). There was a constant increase in leaf number with the increase in Zn applications. However, the number of leaves were significantly higher in plants that were treated with 99 mg Zn L^{-1} (29.9 ± 1.36) than the plants which were treated with 33 mg Zn L^{-1} (24.0 ± 1.06) and control treatment (22.6 ± 1.11). There was no significant difference for number of leaves when the strawberry plants were sprayed with 66 mg Zn L^{-1} and 99 mg Zn L^{-1}. With respect to control treatment, the foliar application of Zn improved the number of leaves from 6 to 32% when sprayed with various levels of Zn (33 to 99 mg L^{-1}).

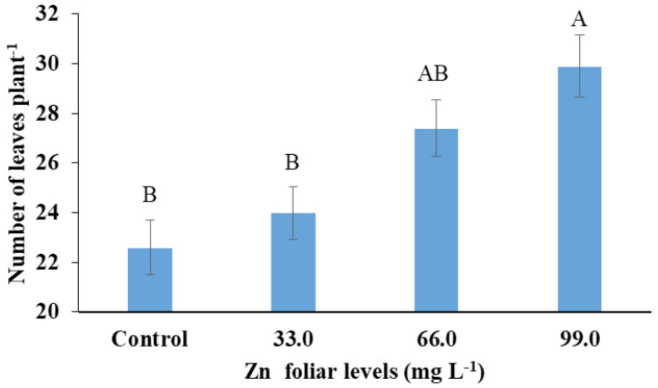

Figure 1: *Effect of varying levels of Zn on total number of leaves plant^{-1} in strawberry; each value is a mean ± SE (n = 4).*

Total number of berries plant^{-1}

The impact of Zn treatments remained significant for number of berries plant^{-1} ($P < 0.05$; Figure 2). The numbers of berries were constantly increased with the increase in Zn application levels. However, the number of berries were significantly higher in plants that were treated with 99 mg Zn L^{-1} (13.0 ± 0.61) than the plants which were treated with distilled water (10.0 ± 0.41) and 33 mg Zn L^{-1} (10.9 ± 0.42). Over control, the foliar application of zinc increased number of berries from 9 to 30% when sprayed with 33 mg L^{-1} to 99 mg L^{-1} Zn.

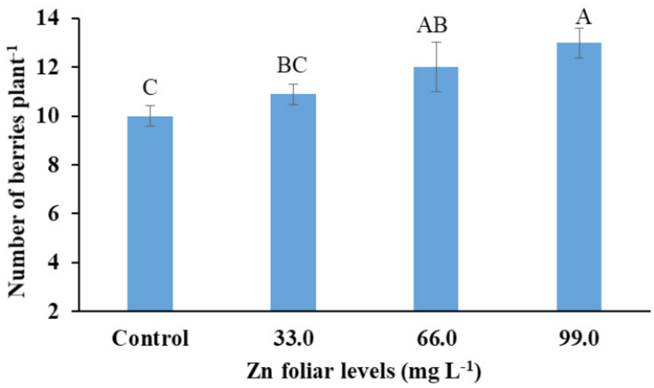

Figure 2: *Effect of varying levels of Zn on total number of berries plant^{-1} in strawberry; each value is a mean ± SE (n = 4)*

Weight of berries plant^{-1} (g)

The effect of treatments remained highly significant for weight of berries plant^{-1} ($P < 0.05$; Figure 3). The weight of berries plant^{-1} was increased as a function of zinc application whereby berries weight was reached to 37% over control plants. The weight of berries was significantly higher in plants that were treated with 99 mg L^{-1} Zn (139.5 ± 2.85 g) than the plants which were treated with 33 mg L^{-1} Zn (113.0 ± 4.05 g) and distilled water (101.9 ± 3.35 g).

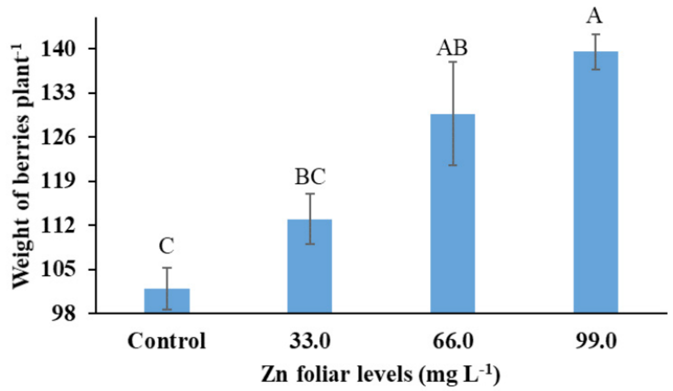

Figure 3: *Effect of varying levels of Zn on weight of berries plant^{-1} (g) of strawberry; each value is a mean ± SE (n = 4).*

Fresh weight of plants (g)

The influence of zinc treatments remained highly significant for fresh weight of plants ($P < 0.05$; Figure 4). The fresh weight of plants was constantly increased with the increase in Zn application. The fresh weight of plant was significantly higher in plants that were treated with 99 mg Zn L^{-1} (40.2 ± 5.74 g) and 66 mg Zn L^{-1} (33.8 ± 5.40 g) than the plants which were treated with distilled water (28.3 ± 1.26 g). With respect to control treatment, the foliar application of Zn increased fresh weight of plants by 13, 19, and 42% when sprayed with Zn applications of 33, 66 and 99 mg Zn L^{-1}.

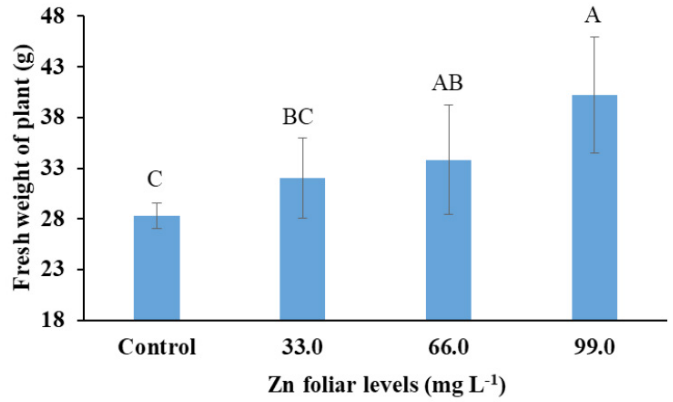

Figure 4: *Effect of varying levels of Zn on fresh weight of strawberry plants (g); each value is a mean ± SE (n = 4).*

Zn concentration in strawberry fruits (μg g^{-1})

The impact of foliar application of zinc remained highly significant for zinc concentration in strawberry fruits ($P < 0.05$; Figure 5). There was a constant increase in Zn concentration in strawberry fruits with the increase in Zn application. However, the zinc concentration in strawberries was significantly highest in plants that were treated with 99 mg Zn L^{-1} (37.2 ± 1.98 μg g^{-1}) than all other treatments. Furthermore, the Zn concentration in applications of

66 mg Zn L^{-1} (24.9 ± 0.20 μg g^{-1}) and 33 mg Zn L^{-1} (23.4 ± 0.44 μg g^{-1}) was significantly higher than the plants which were treated with distilled water (19.5 ± 0.48 μg g^{-1}). With respect to control treatment, the foliar application of Zn increased Zn concentration in strawberry fruits from 20 to 91% when sprayed with Zn applications (33 mg L^{-1} to 99 mg L^{-1}).

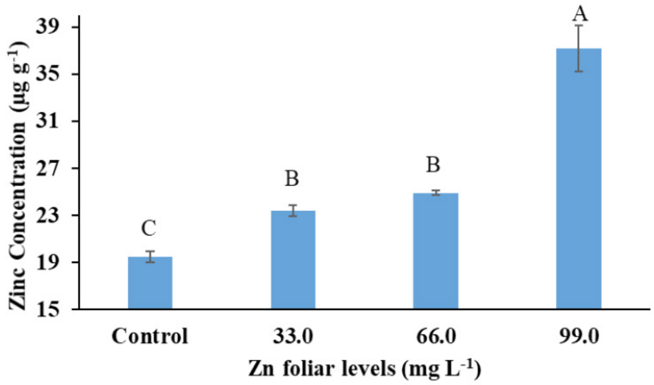

Figure 5: *Effect of varying levels of Zn on Zn concentration (μg g^{-1}) in strawberry fruits; each value is a mean ± SE (n = 4).*

The influence of foliar Zn fertilization on growth and yield parameters of strawberry plants was found significant with increasing the rates of Zn. The possible reason for increment in growth and yield parameters might be associated to the involvement of Zn in many physiological and metabolic activities in plants, e.g., enzymatic activities, photosynthates production, fruit production and maturation, plant hormones stimulation, and starch formation (Brady and Weil, 2002; Tabasum *et al*., 2013; Shivay *et al*., 2007). Positive effects of Zn fertilization on strawberry production have been reported by several scientists. For example, Mohamed *et al.* (2011) reported that the growth and yield of the strawberry plants were significantly increased with increasing Zn rates. Kazemi (2014) observed that the zinc sulphate treatments increased growth and yield parameters as compared to control treatment. Mishra *et al.* (2016) stated that the zinc fertilization improved growth and yield parameters (plant height, number of leaves $plant^{-1}$, number of flowers $plant^{-1}$, number of fruits $plant^{-1}$ and cumulative yield) of strawberry over control treatment. Rahman *et al.* (2016) reported that the zinc application produced more number of runners, leaves, flowers and fruits $plant^{-1}$, fruit length, fruit diameters, individual fruit weight, total fruit weight $plant^{-1}$, and yield (t ha^{-1}) over control. Similarly, Rafeii and Pakkish (2014) noted that the $ZnSO_4$ application improved vegetative and reproductive characteristics of strawberry with respect to control treatment.

In our study, among three levels of Zn, 99 mg Zn L^{-1}, produced more leaves and berries $plant^{-1}$, weight of berries $plant^{-1}$, and fresh weight of an individual plant. This indicates that foliar application of Zn with 99 mg L^{-1} is effective for enhancing growth and yield of strawberry (cv. Silder) in our experimental conditions. Variable rates of Zn or $ZnSO_4$ has been proposed by other researchers which may be associated to many factors including different environmental conditions, crop cultivars, soil types, zinc rates, time, and methods of applications. Bakshi *et al.* (2013b) observed the influence of different iron (0, 0.2% and 0.4%) and $ZnSO_4$ levels (0, 0.2% and 0.4%) on strawberry (cv. Chandler). These researchers found that zinc sulphate at the level of 0.4% increased growth, yield and runners production. Kazemi (2015) noted that application of 150 mg $ZnSO_4$ L^{-1} had more profound impacts on the growth and yield of strawberry (cv. Pajaro) than other $ZnSO_4$ levels (50, 100 mg L^{-1}). Mishra *et al.* (2016) concluded that among various levels of foliar Zn fertilization (Zn 0.2%, 0.4%, 0.6% and 0.8%), strawberry plants sprayed with 0.4% Zn performed better in terms of growth and yield as compared to other treatments. In another study, the effect of humic acid (0, 20 and 40 mg L^{-1}), $ZnSO_4$ (0, 50 and 100 mg L^{-1}) and boric acid (0, 50 and 100 mg L^{-1}) on growth and yield parameters of strawberry was observed (Rafeii and Pakkish, 2014). These researchers reported that growth and yield attributes were high when the $ZnSO_4$ was applied at the rate of 100 mg L^{-1}. Rahman *et al.* (2016) stated that zinc applied at the rate of 225 mg L^{-1} performed better for growth and yield parameters as compared to boron and control treatment.

There was a constant increase in zinc concentration in strawberry fruits with the increase in $ZnSO_4$ application. However, the zinc concentration in strawberries was significantly highest in plants that were treated with 99 mg Zn L^{-1} Zn (37.2 ± 1.98 μg g^{-1}) than all other treatments. The ingestion of strawberries produced in current study by the foliar application of 99 mg Zn L^{-1} will satisfy significant amount of daily requirement of Zn in humans. To our knowledge, no study was found where Zn concentration in strawberry fruit was reported. However, one study was found where zinc concentration in strawberry leaves was determined. Mohamed *et al.* (2011) investigated the phosphorus and zinc interaction effect on production of strawberry (cv. Sweet Charlie). The fertilizers application included phosphorus (0, 60, 80, and 100 kg P_2O_5/fed) and Zn (0, 5, 15, and 25 kg Zn/fed) through irrigation (fertigation). The zinc concentration in leaves significantly increased with increasing the rates of zinc where no phosphorus was applied. The maximum concentration of Zn (33.4 ppm) was observed at 25 kg Zn/fed and minimum concentration (20.4 ppm) was noted at control.

Conclusions and Recommendations

It is suggested that the application of Zn at the rate of 99 mg L^{-1} should be included in strawberry cultivation for enhanced growth, yield, and Zn concentration in fruits. Since there was a constant increase in growth, yield, and zinc concentration in strawberry fruits with increasing Zn applications, further studies may be designed with higher rates of Zn (foliar as well as soil applied) to investigate the response of strawberry.

Acknowledgements

The data presented in this manuscript is a component of MSc Thesis, submitted to Sindh Agriculture University Tandojam by second author (M. A. Panhwar).

Novelty Statement

Strawberry yield and quality can be improved by foliar application of zinc.

Author's Contribution

Saleem Maseeh Bhatti: Conceived the idea, Overall management of the work (Main Supervisor of the student, 2nd Author).
Muhammad Aslam Panhwar: Executed the trial and write up of Thesis (as MSc student).
Zohaib ur Rehman Bughio: Helped in manuscript management (formatting, latest references).
Muhammad Saleem Sarki: Technical input at every step of the work and manuscript management.
Allah Wadhayo Gandahi: Technical input at every step of the work (as Co-Supervisor I of the student).
Niaz Ahmed Wahocho: Technical input at every step of the work (as Co-Supervisor II of the student).

Conflict of interest

The authors have declared no conflict of interest.

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