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Article *in* Pakistan Journal of Botany · December 2024

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COMPARATIVE INFLUENCE OF TWO IRON SOURCES VIZ $FeSO_4$ AND IRON NANOPARTICLES ON QUALITY PRODUCTION OF STRAWBERRY

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Abstract

Nanotechnology has revolutionized the agriculture, food, and medicine sectors by offering altered and profitable products. Strawberry fruit, owing to its attractiveness, well-defined, and highly nutritious, is appreciated worldwide. The nutritional and quality characteristics are the focus of the production system and breeding programs. Strawberry Plants cv. Chandler was subjected to the application of nanoparticles under greenhouse conditions. Four doses of Nanoparticles viz: 15 mg L^{-1}, 30 mg L^{-1}, 45 mg $L^{-1,}$ and 60 mg L^{-1} were applied. $FeSO_4$ soil and foliar were kept as control treatments. The experiment was based on CRD design. Iron nanoparticles, a readily available alternative to iron sulfate, have also positively impacted strawberry plants. Iron Nanoparticles at the rate of 45 mg l^{-1} per plant were found to be best regarding the vegetative parameters like plant height (, number of leaves, and leaf area, and reproductive growth parameters like fruit yield and number. Statistical analysis regarding vegetative growth parameters viz., stem length (14.70 cm), the number of leaves (5.27), leaf area (14.1 cm^2), and the fruit weight (13.73 g) revealed the statistically significant improvement to Iron Nanoparticles application as compared to $FeSO_4$. In the case of stem length, leaf area, and fruit weight, there is an increase of 5.27 %, 6.77 %, and 14.87 respectively. The study concluded that iron nanoparticles are better for coping with iron deficiency than traditional sources such as $FeSO_4$ application. The study will facilitate future research regarding the development of iron management strategies for crop production in the alkaline soils of Pakistan.

Key words: Iron nanoparticles, Strawberry, Plant nutrition.

Introduction

Strawberries (*Fragaria x ananassa* Duch.) belong to the family Rosaceae. China is the leading strawberry producer, with a total production of 3801865 metric tonnes, grabbing 41 percent of total world production (Anon., 2019). The total area under strawberry production in Pakistan is 387 hectares, totaling 795 tonnes (Anon., 2018-19).

The major global problem is increasing food production with limited resources and the minimal and efficient use of fertilizer and pesticides without polluting the environment. Strawberry (*Fragaria ananassa*) is one of the most popular fruits. Strawberries give promising returns in a limited time compared to other berries (Boriss *et al*., 2006). Strawberry has experienced one of the highest consumption rates in the past few years owing to its rich protein, minerals, calcium, phosphorus, and potassium and rich sources of Vitamins such as Vit A, B_1, B_2, Niacin, and Vitamin C. It is regarded as one of the best sources of antioxidants (Kumar *et al*., 2017). Proper plant nutrition is one of the prerequisites for quality crop production in open or protected conditions. Quality plays a vital role in meeting the requirements of competitive export and domestic markets. Micronutrients are as important as macronutrients in plant growth and development. The significance of micronutrients has been realized recently due to their unavailability from the soil due to intensive cultivation; salinity and soil pH make them unavailable to plants despite being present in the soil (Ahmad *et al*., 2010).

Quality food production requires the application of modern technologies to meet ever-increasing food requirements in an environment-friendly manner (Wheeler, 2005). Nanotechnology can potentially address the food security problem (Anon., 2009). Moreover, they can reform agriculture and food systems through disease detection, disease resistance, targeted delivery, promoting nutrient uptake efficiency, enduring environmental pressure, and efficient processing and storage systems (Mousavi & Rezaei, 2011). In addition, it provides a new dimension for selection and privilege to improve

Agricultural production has gone through incredible changes owing to the application of modern labor-saving techniques. Modern technology has facilitated intensive on-farm mechanization, improved varieties of crop irrigation, and post-harvest handling. Despite the marvelous progress in the agricultural production sector, many developing countries still face poverty and food insecurity issues. The research on nanoparticles has wide application in the science and technology sector for manufacturing new items at the nanoscale level (Albrecht *et al*., 2006). Nanotechnology deals with maneuvering material at the nanoscale, i.e., 1-100 nm (Margabandhu *et al*., 2015). Moreover, due to their distinct properties, research on the impact of iron micronutrients and the application of nanoscale particles has recently increased in agriculture (Sabaghnia & Janmohammadi, 2015). They can be rapidly absorbed and readily available for plants (Askary *et al*., 2016).

Iron plays a vital role in biological function for many cellular enzymes involved in plant photosynthesis, respiration, and product quality. Most agricultural lands contain sufficient iron; however, about 30% of world soils pose iron-limiting conditions for plant growth. Iron deficiency negatively affects plant growth and development and may cause anemia in animals and humans. Therefore, it is indispensable that an eco-friendly

and efficient fertilizer may be developed to enhance the efficiency of iron application to agricultural lands. Iron-based nanoparticles showed great prospects as antibacterial agents and highly reactive sites (Velmurugan *et al*., 2010). The positive charge on iron nanoparticle's surface helps them to bind with negatively charged bacteria, resulting in improved bactericidal properties (Stoimenov *et al*., 2002; Seil, and Webster, 2012).

Nanotechnology has opened new avenues for enhanced nutrient use efficiency and minimizing the expenditure on environmental protection (Naderi & Shahkari, 2011). In addition, it has enabled the manufacture of higher value-added products and removing environmental toxicity (Garda-Torredey *et al*., 2002). Nano-fertilizers showed superiority over conventional fertilizers for the following reasons: 1. Increase uptake of nutrients with properties of slow element release, 2—reduction of cost, 3. Prevent the deposition of soluble salts in higher concentrations in the root environment, saving plants from damage; 4. To reduce the conversion of available form to unavailable form in response to soil reactions, 5. Reduce the loss leaching effect of nutrients in roots and ultimately cause a reduction in environmental pollution. Iron has been found to play an important role in Catalase, peroxidase, and cytochrome oxidase activity (Blakrishman, 2000). Iron is a co-factor of many antioxidant enzymes' structures, and previous studies showed that a lack of micronutrients increased plant sensitivity to environmental stresses. Mineral fertilizers play a vital role in the survival of humanity in terms of improved yield (Smil, 2001; Stewart *et al*., 2005), improving soil productivity and maintaining fertility (Balmford *et al*., 2005). The developing world faces the problem of lower yield owing to nutrient depletion. According to an estimate, about 50 % of losses in Pakistan occur due to poor nutrient use efficiency.

Moreover, it is crucial to increase nutrient use efficiency due to socio-economic constraints. Hence, a significant challenge for world agriculture is improving nutrient use efficiency, and nanotechnology could address this issue. Owing to the role of nanoparticles toward quality, the present trial was planned to find the appropriate dose of Iron nanoparticles for the growth and yield of strawberries. The hypothesis is that it will improve the iron supply to the plant. The study will facilitate future research on the efficient use of nanoparticles for crops, especially in Pakistani Soils, which are alkaline and prone to iron deficiency. It will be especially helpful for improving the nutritional value of short-duration crops.

Material and Method

The studies were conducted at the greenhouse located in PMAS-Arid Agriculture University Rawalpindi Pakistan during Fall 2017-18 & 2018-19 to examine the effect of Salicylic acid on the morphological and physiological characteristics of strawberries. The GPS location of the site is 33.6492° N, 73.0815° E. Strawberry plant runners were obtained from SWAT. They were grown in medium-sized pots containing a mixture of sand and farmyard manure. The experiment was designed in a completely randomized design with three replications and five plants per replication, and the results were subjected to one-way ANOVA at a 5 % probability level. The basic soil analysis at the start of the experiment is given in (Table 1).

Table 1. Chemical, Physical, and nutritional status of soil used for experiments.

Parameters analysed	Results
Soil texture	Loam
Saturation	45%
pH	7.5
EC	0.80dS m^{-1}
Organic matter	0.76 %
Available P	3.2 mg kg^{-1}
Available K	100 mg kg^{-1}

Preparation of nanoparticles: Nanoparticles were prepared using the sol-gel method with few modifications and used in solution form for foliar spray. Then, XRD was done to examine the crystallite properties of nanoparticles (Srivastava *et al*., 2013). To prepare Fe nanoparticles, 40 gm of $FeSO_4$ was weighed and dissolved in 1 liter of distilled water using a magnetic stirrer. After that, 0.1 molar NaOH solutions were poured dropwise into the $FeSO_4$ solution and titrated until pH was 8. The solution was stirred with constant stirring, and blue precipitates were formed, settled down, and washed with distilled water 4 to 5 times. These precipitates were transferred to crucibles and calcined in the furnace at 550°C for 3 hours. Black ash was obtained, and that ash was well grinded, and nanoparticles were prepared. The absolute intensity is shown in (Fig. 1). Then 15, 30, 45, and 60 mg l^{-1} solutions were prepared by dissolving nanoparticles in distilled water. They are compared with $FeSO_4$ Foliar and Soil application.

Growth and yield parameters

Plant height (cm): Plant height was noted from the ground to the highest level in its natural position with the help of scale. It is expressed in cm.

Number of leaves per plant: The number of leaves per plant was counted from five random plants per treatment, including visible and unopened leaves, and the average is calculated for examination.

Leaf area (cm^2): Leaf area measurements were done with ADC Area Meter (AM 100). Five leaves per treatment per replication were gathered arbitrarily to make the estimations.

Fruit weight (g): Average fruit Weight was determined for this research experiment carefully and noted down.

Quality characteristics

Total soluble solids (%): A hand refractometer was used to measure the total soluble solids (TSS) of harvested strawberry fruits according to the method illustrated by Anon., (1990). Briefly, on a dry and clean refractometer prism, a drop of juice was placed, and the reading was taken in % unit.

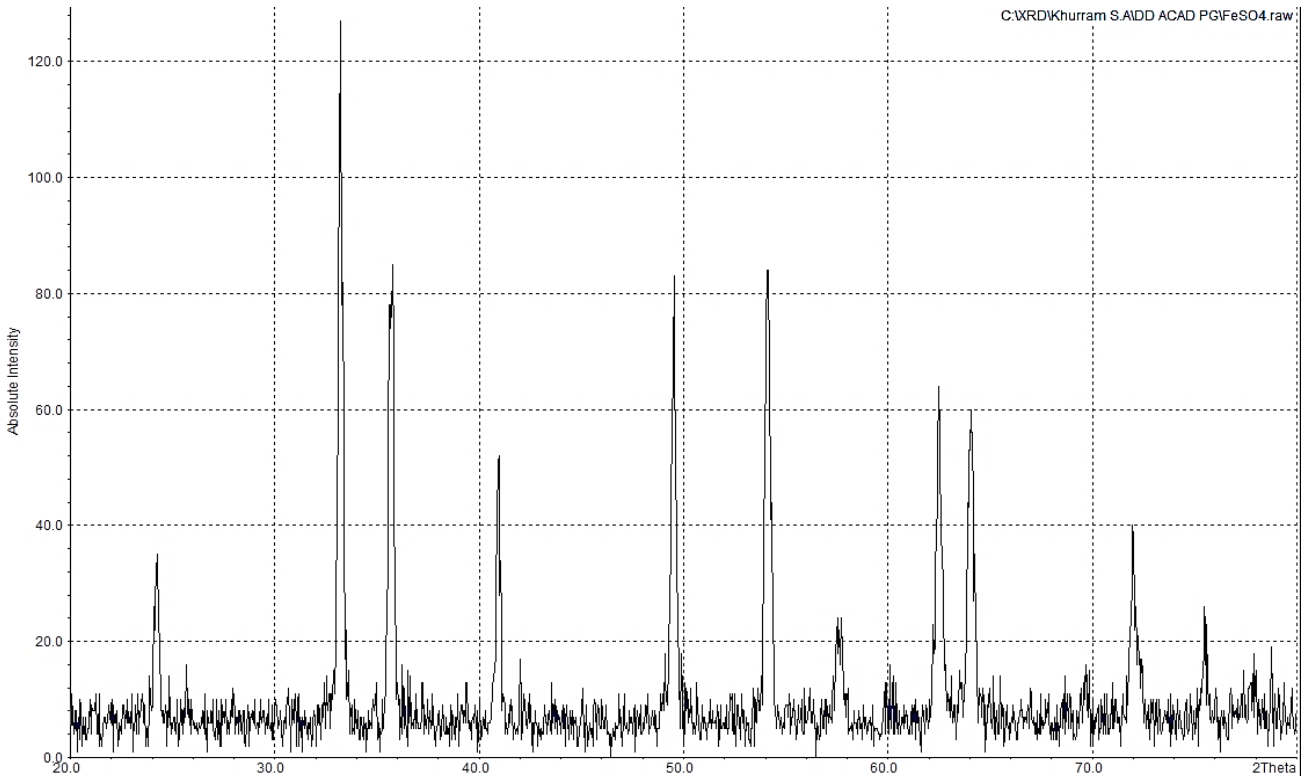

Fig. 1. Absolute intensity of Nanoparticles through XRD analysis.

Titratable acidity (TA): Fabro et al. (2006) described the titration method to estimate Titrable acidity. 50 ml volume was made by mixing 10 ml juice with 40 ml distilled water and adding 2-3 drops of phenolphthalein indicator. The solution was titrated against 0.1 N NaOH till the appearance of a light pink color, and the TA was estimated according to the following formula:

TA% = Vol of NaOH used x Normality of NaOH x 0.064 x 100/ml of juice used

Reducing sugars (%): Reducing sugars were estimated using the following method: 10 ml of juice, 10 ml of potassium oxalate (7 %), and 25 ml of lead acetate (2 %) were mixed in a 250 ml beaker. The final volume was made equal to 250 ml with distilled water. 10 ml Fehling Solution (% ml each of A & B) was used to titrate the above-prepared solution with slight warming until red color precipitates appeared. Fehling solution A composition includes 69.3 g copper sulfate. 5 H_2O dissolved in 1000 ml of distilled water, while Fehling B was Composed of 100 g NaOH anhydrous mixed with potassium sodium tartrate in 1000 ml of distilled water. The calculation of reducing sugar was done based on the below-mentioned formula.

Reducing sugar (g/100 ml) = 6.25 (X)/Y
X= Sample Volume used
Y= standard sugar

Total sugars (%): Total sugars were estimated according to the method described by Hortwitz (1960). According to this method, 25 ml of aliquot was taken in a volumetric flask (100 ml capacity), adding 20 ml of distilled water and 5 ml of pure HCL. The solution was kept overnight to convert non-reducing sugars to reducing sugars. The solution for neutralization was using 1 N NaOH (50 % concentrated), two drops of phenolphthalein indicator were added, and the total volume was equal to 100 ml using distilled water. The solution was then shifted to a burette and titrated against 10 ml Fehling solution by slight boiling. Again, 2 to 3 drops of Methyl blue were added, and the titration was done until a brick-red color appeared. The calculation of total sugar was done according to the formula:

Total sugars (%) = 25 × (X/Z)
Where X= ml of standard sugar solution used against 10 ml of Fehling Solution
Z= ml of sample aliquot used against 10 ml Fehling's Solution

Non-reducing sugars (%): Non-reducing sugars are calculated by the following formula:

Non-reducing sugars (g/100 ml) = Total sugars – Reducing sugars x (0.95)

Ascorbic acid (mg 100-1 ml juice): A method described by (Hans, 1992) was used to calculate ascorbic acid. Strawberry pulp (5 g) was mixed with 5 ml, 1% HCl (w/v), and homogenate was centrifuged at 10000 rpm for 10 minutes. The supernatant fluid was collected as vitamin C extract. The absorbance of the extract was estimated at 243 nm using a spectrophotometer (Model OPTIMA, SP-3000-plus).

Antioxidant enzymes analysis

Superoxide dismutase (SOD): SOD enzyme activity was assayed by measuring the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) using the method of (Abbasi *et al*., 1998).

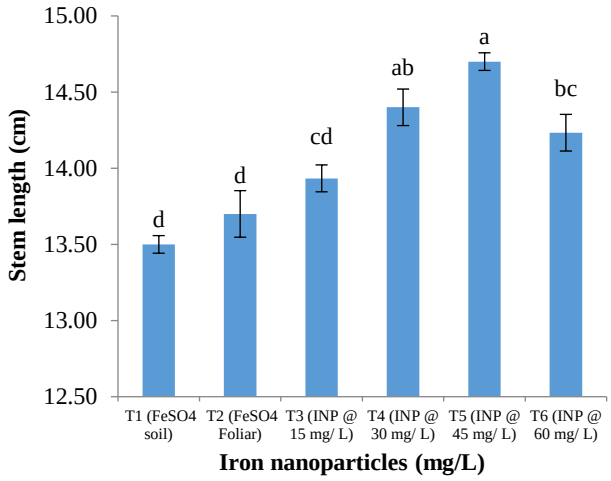

Fig. 2. Effect of $FeSO_4$ and FeNPs on stem length of strawberry.

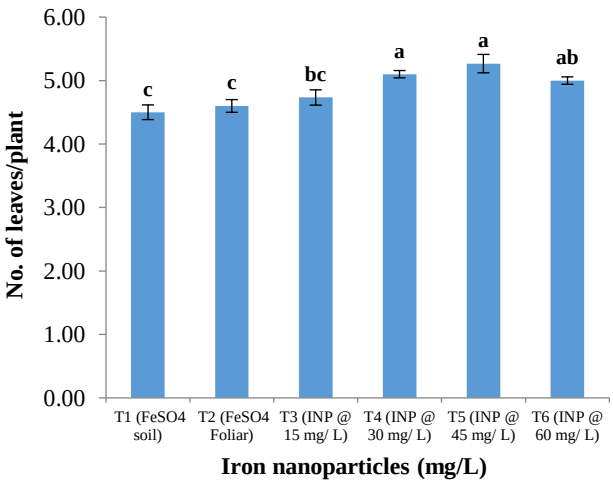

Fig. 3. Effect of $FeSO_4$ and FeNPs on Average Number of leaves of strawberry.

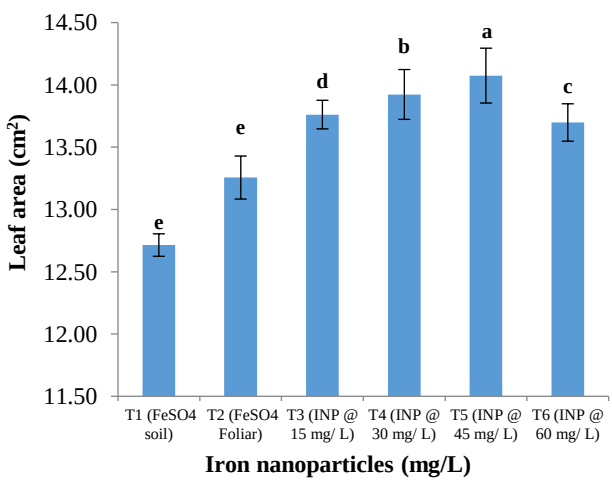

Fig. 4. Influence of $FeSO_4$ and FeNPs on strawberry leaf area (cm^2).

Peroxidase (POD): Peroxidase (POD) activity was determined according to the method prescribed by (Hassan *et al*., 2007) with few modifications.

Catalase (CAT): The CAT enzyme activity was determined via a method prescribed by Abbasi *et al*., (1998).

Post-harvest analysis: Fungal diseases, especially grey mold, have been a matter of concern regarding post-harvest storage of strawberries. The grey mold level observed on strawberries treated with iron nanoparticles vs traditional sources. The fruit was kept refrigerated, i.e., 4±1°C, followed by a shelf life evaluation of 25°C for 5 days at 90-95% Relative Humidity.

Statistical analysis

The experiment was completely randomized, with six treatments, three replications, and 15 plants per treatment. The observations were taken, and the results were compiled for comparison with the treatment. Uniform cultural practices were ensured for better growth and development of runners. The data was analyzed using the Statistic Software, and means were compared using an ANOVA LSD (Least Square Difference) test at a probability level of 5% (Steel *et al*., 1997).

Results and Discussion

Growth and yield parameters: Statistical analysis regarding vegetative growth parameters viz., stem length (14.70 cm), the number of leaves (5.27), leaf area (14.1 cm^2), and the fruit weight (13.73 g) revealed a statistically significant improvement to Iron Nanoparticles application as compared to $FeSO_4$. The application of 45 mg l-1 Fe Nanoparticles showed better results (Figs. 2, 3, 4). The size of nanoparticles was within the range of 1-100 nm (Fig. 5). Minimum values were recorded in $FeSO_4$.

Plant height was improved maybe due to proper nutrient availability, which resulted in strong vegetative growth, thereby improving the height of plants due to cell elongation, photosynthesis, cell division, and turgidity of plant cells. Fe plays an important role in the transfer of energy in plants. In addition to that, Fe is an enzyme and a protein component. Owing to these factors, it may be involved in modulating the height of plants (Abbas *et al*., 2009; Ali, 2012; Bameri *et al*., 2012).

Iron is an important element for chlorophyll synthesis and also serves as a structural component of chloroplast, resulting in increased growth and suggesting an increased number of leaves. Iron plays an important role in vegetative growth promotion, improvement in flowering, enhancement of yield, and better quality of strawberry fruits (Chaturvedi *et al*., 2005; Colombo *et al*., 2013).

Iron has a role in chlorophyll synthesis, catalyzing enzymatic reactions resulting in the biosynthesis of photoassimilates, thereby improving growth parameters (Kumar & Arora, 2000). Kumar & Haripriya (2010) attributed improved vegetative growth to better nutrient absorption and assimilation of water and nutrients. The vital role of iron in chlorophyll synthesis leads to the production of more photoassimilates, which leads to improvement in leaf area (Kumar & Arora, 2000). A similar pattern was observed by Kumar & Haripaya (2010), as in the case of *Nerium oleander* L.

Due to its miniature dimension, nanotechnology has a wide range of applications in the agricultural sector that control agricultural processes. The noteworthy interest in utilizing nanoparticles in agriculture is in using

nanopesticides and nanofertilization to track nutrients and product levels for increased productivity without compromising soil and water health (Prasad *et al*., 2017).

Moghadam *et al*., (2012) found similarity in the shape and size of epidermal cells of control plants, while Nano particles treated leaves showed an increase in the size of epidermal cells. This may be related to better nutrient availability, as iron is more stable. Fe plays an important role in nitrogen fixation, increasing leaf area and photosynthetic activity, leading to increased crop production. Mesophyll tissue thickness is also affected by iron treatment vs. control leaves.

Moreover, foliar treatment is much more effective than control treatment as it is readily absorbed and reaches the target area more efficiently and on time than soil application. Iron is a co-factor of around 140 enzymes facilitating exclusive biochemical reactions; hence, it plays an important role in the development of plants, viz. chlorophyll and thylakoid synthesis and chloroplast development. The better photosynthetic activity leads to better fruit size and yield. The increased fruit production may be attributed to better plant iron, which helps in auxins breakdown, protein synthesis, and photosynthesis.

Postharvest quality parameters: Data regarding postharvest parameters showed remarkable differences between Iron nanoparticle sprayed plants vs $FeSO_4$ plants. The concentration of 45 mg l^{-1} Iron Nanoparticles showed better results regarding Total Soluble Solids (6.57 ^{o}Brix), Ascorbic acid (12.99 mg/100 mL of juice) (Figs. 6, 7), Total Sugars (6.54 %) (Fig. 8). Minimum values were recorded in $FeSO_4$.

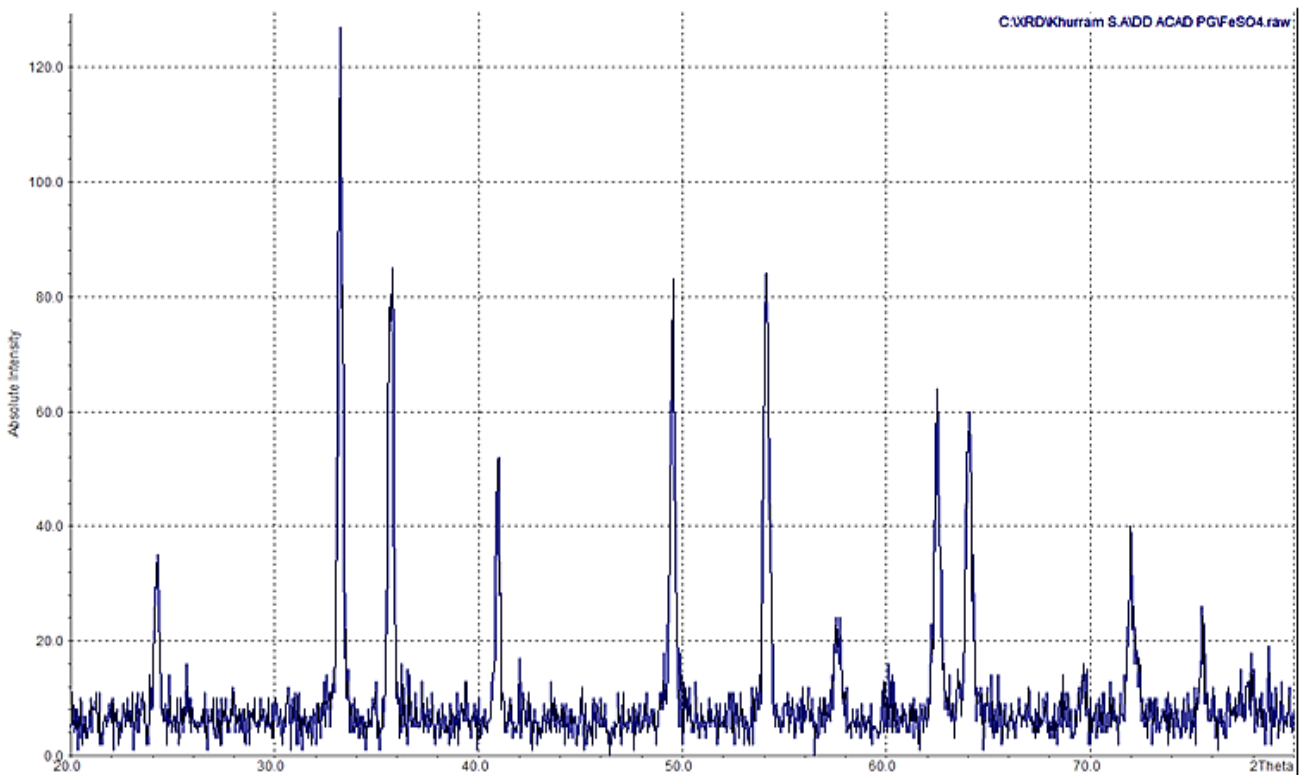

Fig. 5. Absolute intensity of nanoparticles through XRD.

In the current study, the TSS and AA contents (mg/100 ml of juice) were maximum with T_5, followed by T_4. Other nanoparticle treatments, such as T_3 and T_6, also showed better results than Fe SO_4 treatments. The influence of iron treatments on the amount of TSS and ascorbic acid contents may be due to its availability in plants' foliar feeding and its role in photosynthesis, leading to a higher photosynthetic rate. The acids and sugar contents are taste attributes that fascinate strawberry consumers *(*Wozniak *et al*., 1996).

Fresh strawberries comprise sucrose, fructose, and glucose, accounting for almost 99 % of total sugar contents (Maniken & Soderling, 1980). Current studies showed improved sugar contents with nanoparticle application compared to the control treatment. The positive influence of nanoparticles on strawberries may be attributed to improved soil fertility due to better availability of nutrients.

Davarpanah *et al*., (2020) suggested that proper iron nutrition accelerates the photosynthetic rate. Since sugar is a primary photosynthetic product, accelerating the photosynthetic rate leads to enhanced sugar compounds and increased soluble solids in fruit juice. The increase in total sugar may be attributed to increased reducing and non-reducing sugars.

Strawberry fruit is a rich ascorbic acid source containing more vitamin C than oranges. In the current study, the ascorbic acid content was maximum at T_5, followed by T_6. Other nanoparticle treatments, such as T_2 and T_4, also showed improved results compared to control treatments. Researchers found out that the ascorbic acid content increases with increasing iron concentration. The foliar iron application increases the flower bud formation, improving fruit size, sugar, and ascorbic acid contents of fruits (Ayub *et al*., 2010).

Antioxidant enzymes: The iron nanoparticle-treated plants showed better antioxidant activity than the FeSO4 plants. It may be attributed to improved plant growth because of their ability to scavenge free radicals. The results showed that activity was optimum at 45 mg $l^{-1,}$ though all other nanoparticle-treated plants also showed better activity than the $FeSO_4$ treatments (Fig. 9).

According to the statistical point of view, FeNPs concentration improved the noteworthy improvement in antioxidant enzymes activity viz., POD, SOD, and CAT of strawberries compared to control. The treated plants showed better antioxidant activity vs. control. It may be accredited to improved plant growth because of their ability to scavenge free radicals.

Antioxidant enzymes, viz., SOD, POD, and CAT, are vital for protecting against early senescence caused by ROS production. SOD plays a role in O_2 conversion to H_2O_2 molecules while the other two enzymes viz" CAT and POD, further accelerate the process of converting hydrogen peroxide (H_2O_2) molecules providing a protective mechanism against lipid peroxidation (Sharifzadeh *et al*., 2014).

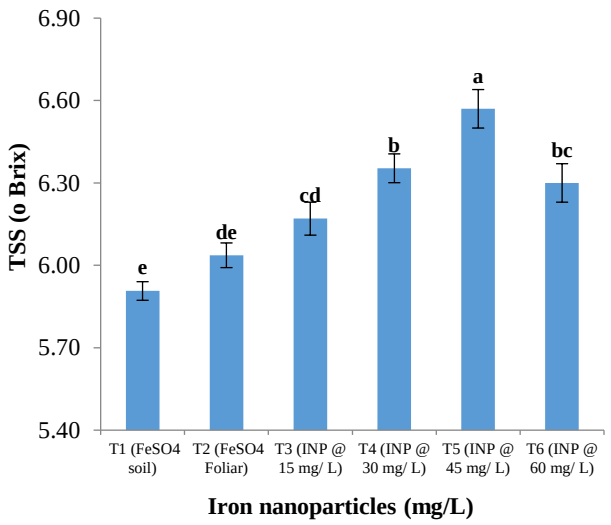

Fig. 6. Effect of $FeSO_4$ and FeNPs on TSS of strawberry.

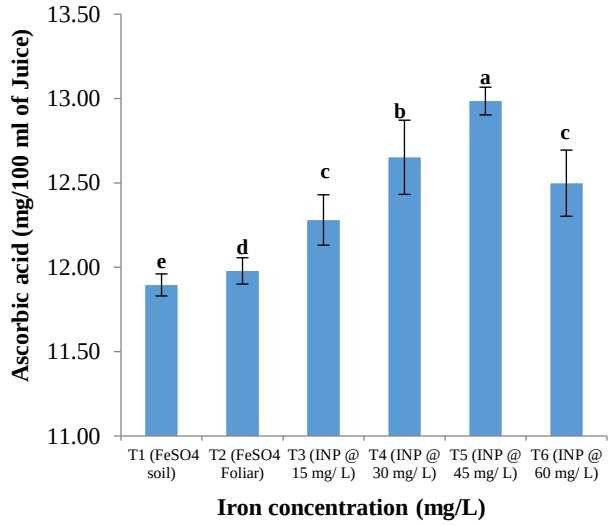

Fig. 7. Effect of $FeSO_4$ and FeNPs on ascorbic acid contents of strawberry.

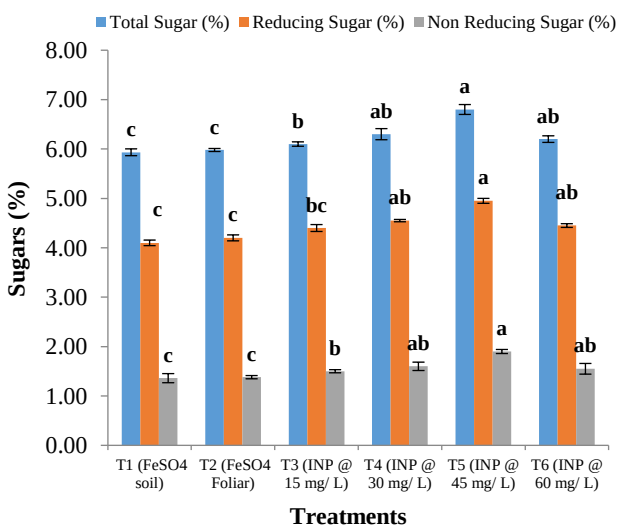

Fig. 8. Effect of $FeSO_4$ and FeNPs on strawberry sugar contents (%).

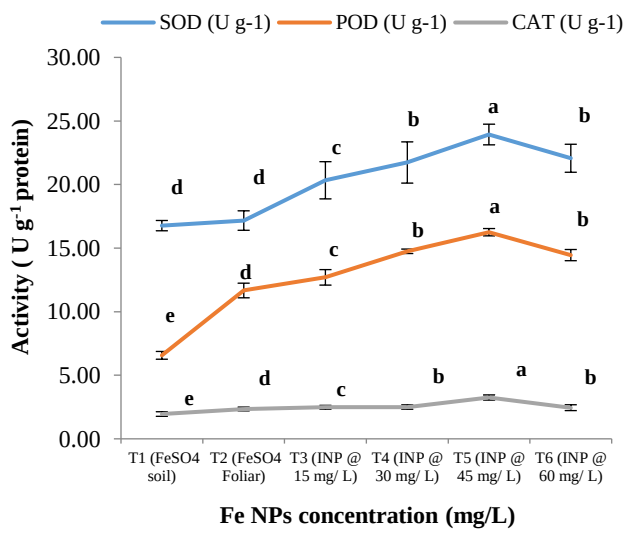

Fig. 9. Influence of $FeSO_4$ and Iron nanoparticles on antioxidants (SOD, POD and CAT) enzymes.

Acknowledgment

Higher Education Commission Pakistan for funding this research.

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(Received for publication 26 January 2024)