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Effect of Nano-Zinc Oxide on the Leaf Physical and Nutritional Quality of Spinach

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Abstract

Spinach (*Spinacia oleracea*) belongs to family *Amaranthaceae* and is one of the important and nutritious leafy vegetable consumed in India. The pot culture experiment is carried out during 2014-15 to study the effect of nano-zinc oxide particles on the leaf physical and nutritional traits of spinach. The spinach plants were sprayed with graded concentration of zinc oxide nanoparticles (ZnO NPs) after 14 days of sowing. The leaf physical parameters like leaf length, leaf width and leaf surface area are recorded at the time of maturity (45-50 days). The protein, carbohydrate, fat and dietary fiber content in leaf samples are determined. The plants sprayed with ZnO NPs at the concentration of 500 and 1000 ppm showed the increased leaf length, width, surface area and colour of leaf samples when compared to control leaf samples. Similarly treated plants with ZnO NPs at the concentration of 500 and 1000 ppm showed higher values of protein and dietary fibre content in comparison to control leaf samples of spinach. Hence our study suggests that the nano-zinc oxide sprayed spinach is more nutritious to vegetarian diet by providing, protein, fiber and required amount of vegetarian fat to diet.

Keywords: Nano-zinc oxide; Spinach; Leaf physical properties; Protein; Fibre

Introduction

Spinach (*Spinacia oleracea*) is a green-leafy vegetable belongs to family *Amaranthaceae*. It is often recognized as one of the *functional foods* for its wholesome nutritional, antioxidants and anti-cancer composition. The major micronutrients in spinach are vitamins A (from β-carotene), C, K and folate, and the minerals, calcium, iron and potassium. Spinach also provides fibre and is low in calories. Its tender, crispy, dark-green leaves are one of the favorite ingredients of chefs all around the world. Vegetables are also valuable in maintaining alkaline reserve of the body. They are valued mainly for high carbohydrate, vitamin and mineral contents [1].

Micronutrient fertilizers can increase the tolerance of plants to environmental stresses like drought and salinity [2]. Zinc has been considered as an essential micronutrient for metabolic activities in plants. It regulates the various enzyme activities and required in biochemical reactions leading to formations of chlorophyll and carbohydrates [2,3]. The crop yield and quality of produce can be affected by the deficiency of Zn [4]. Zinc nano-particle is used in various agricultural experiments to understand its effect on growth, germination, and various other properties. Most of the farmers are using either zinc sulfate or EDTA-Zn chelate for soil and foliar applications, however the efficacy is low [5] have demonstrated essentiality and role of zinc in plant growth, reproduction and yield. It has been indicated that the retention time of Zn in the plant system is low and hence, the bioavailability of Zn for long period is not sure with the use of $ZnSO_4$ fertilizer. Under high temperatures conditions $ZnSO_4$ has a large salt index and it may show burning injury if the plants are soft or sensitive [6].

Nano-particles with smaller particle size and large surface area are expected to be the ideal material for use as Zn fertilizer in plants. Application of micronutrient in the form of nano-particles (NPs) is an important route to release required nutrients gradually and in a controlled way, which is essential to mitigate the problems of soil pollution caused by the excess use of chemical fertilizers. A number of researchers have reported the essentiality and role of zinc for plant growth and yield [5,6]. Reynolds [7] demonstrated the use of micronutrients in the form of nano-particles can be used in crop production to increase yield.

It has been postulated that nano-particles are more effective, can be utilized in agriculture for the precision farming and enhance productivity crop yields [7,8]. Several studies are concerned with the synthesis of nanomaterials using biological routes. Only limited studies have been reported on the promotory effects of nanoparticles on plants in low concentrations. Nanoscale titanium dioxide (TiO_2) was reported to promote photosynthesis, and growth of spinach. The present study deals with the effect of nano-ZnO, particle suspension as micro-nutrient on the physical and nutritional quality of spinach leaves by foliar spray method. Foliar spray method is more practical from an agronomic standpoint as plant can absorb essential elements through their leaves more efficiently compared to root feeding.

Materials and Methods

Zinc oxide nanoparticles of mean size of 50 nm diameter (Sigma Aldrich) were characterized with zeta potential was used in the study. The stock solution of 10,000 ppm solution prepared and dilutions of 0, 100, 500, 1000 ppm were used for the study. The stock solutions were prepared by directly suspending the nano-particles in deionized water and dispersed by ultrasonic vibration (100 W, 40 KHz) for 1 hour. Magnetic bars were placed in the suspensions for stirring before use to avoid aggregation of the particles. The nano-particle solutions used for the experiment purpose were prepared from the stock solutions.

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Spinach seeds of variety 'All green variety' were used by sowing in pots (20 cm × 40 cm) filled with equal quantity of soil and watered to field capacity. Proper care was taken to use similar soil in all the pots to minimize soil heterogeneity effects. At 14 days after sowing plants were sprayed with the different concentration of ZnO nano-particles. The leaf samples were harvested at 45th day of sowing and were further processed for proximate analysis.

The physical properties of fresh spinach leafs *viz*., Length and width were measured using venire callipers and surface area of fresh spinach leafs was determined by using digital Planimeter (Make: Placom; model: KP90N roller-type digital Planimeter). The leaf parameters of fresh spinach leafs i.e., color and water activity were determined using Hunter lab colorimeter (Colour Flex EZ, Hunter Associates LAB INC., C04-1005-631, Taiwan) and Rotronic Hygrolab water activity analyzer (Model: a_w-HP23).

The proximate composition of fresh spinach leafs *viz*. crude protein, crude fat, total ash, crude fiber and carbohydrates were estimated by the recommended methods of the Association of Official Analytical chemists, (2005) in triplicate. Experiments were carried out in triplicate. Data recorded from three replications were subjected to single way analysis of variance (ANOVA) and critical differences were calculated at p=0.05 level.

Results and Discussion

Effect of different concentration of ZnO nanoparticles on leaf physical properties such as leaf length, leaf width and leaf surface area etc., were given in Figure 1. The leaf length was maximum in ZnO-NPs treated leaf samples at the concentration of 500 ppm and minimum in control leaf samples. With respect to leaf width, there is no significant variations were observed between treated and control leaf samples of spinach. However, there is slight improvement in leaf width was observed in ZnO-NPs treated leaf samples. The maximum leaf surface area was observed in ZnO-NPs treated leaf samples at concentration of 1000 ppm followed by at 500 ppm and lowest value observed in control leaf samples. Similarly, Prasad [9] reported that, groundnut seeds treated with nanoscale zinc oxide particles with a concentration of 1000 ppm have shown significant increment in germination, shoot length, root length and vigor index over the control samples. Raskar and Laware [8] studied effect of ZnO NPs on seed germination and seedling growth in onion and observed that seed germination increased in lower concentrations of ZnO NPs but showed decrease in values at higher concentrations.

The color values *viz*., L^*, a^* and b^* of the fresh spinach leaf was found to be is in the range of 37.01-47.11, from -9.79 to -8.97 and 19.68-24.92, respectively and concluded that green in color and water activity of fresh spinach leafs were found to be is in the range of 0.928-0.959. Since the initial moisture content of fresh spinach leaf was more, the presence of available moisture is sufficient to grow micro-organisms also. These values were in confirmation with those reported by Nangula [10] who reported the moisture content of fresh *Spinaceaoleracea*is found to be 92 g/100 g. Laware and Raskar [6] reported that ZnO-NPs can reduce flowering period by 12-14 days and produce healthy seeds of onion vegetable.

The results of effect of different concentration of ZnO nanoparticles on leaf nutritional traits were given in Table 1.

The Protein content of the raw spinach leaves ranged between 1.54%and 3.99% (Table 1). The plants treated with ZnO-NPs-1000 ppm was found to have the highest protein content (3.99%) and control (1.54%) had the lowest protein content. The protein content to be obtained in the spinach leaf was close to the values previously reported [1] was 2.10 ± 0.15 g. The fat, fiber and carbohydrates content of fresh spinach leaf were found to be is in the range of 0.10-1.57%, 2.01-6.97% and 0.20-0.58%, respectively. Similar results were also concluded by Nangula et al. [10] who reported the fat, fiber and carbohydrates of fresh *Spinaceaoleracea* was 0.4 g, 3 g and 1 g per 100 g, respectively.

Zinc is an essential micronutrient for normal growth, development, and health of plants and human beings. Zinc enhances cation-exchange capacity of the roots, which in turn enhances absorption of essential nutrients, especially nitrogen which is responsible for higher protein content. Zinc plays vital role in carbohydrate and proteins metabolism as well as it controls plant growth hormone i.e. IAA. Zn is also an essential component of dehydrogenase, proteinase, and peptides enzymes as well as promotes starch formation, seed maturation and production [5]. The application of slow/controlled release fertilizer coated and felted by nano-materials were reported to improve grain

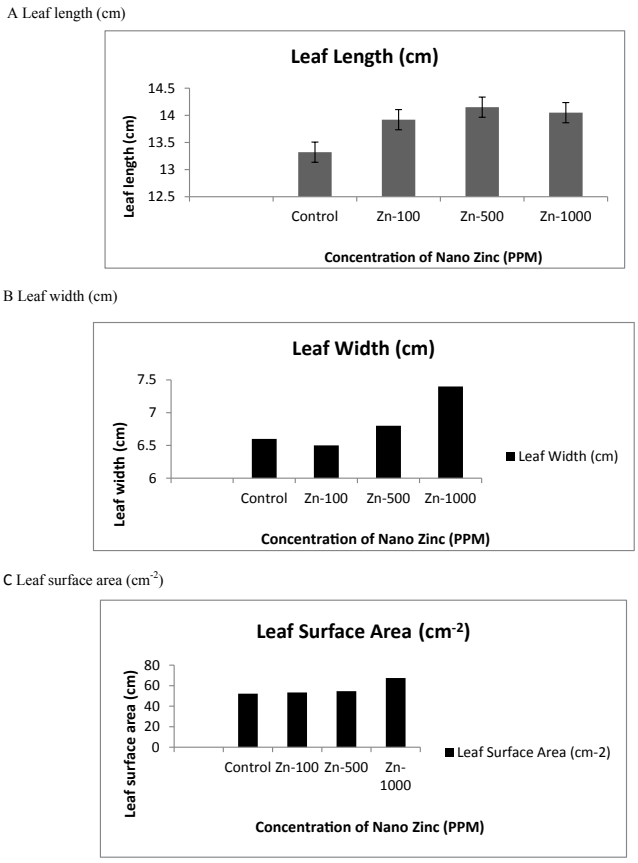

Figure 1: Effect of nano-zinc oxide spray on leaf physical properties of spinach.

S. No.	Traits	Control	Zn-100	Zn-500	Zn-1000
1	Protein (%)	1.54	2.69	2.95	3.87
2	Fat (%)	0.1	0.6	1.06	1.07
3	Fiber (%)	2.01	2.23	3.4	6.97
4	Ash (%)	0.18	2.16	1.90	2.06
5	Carbohydrate (%)	0.20	0.23	0.58	0.52

Table 1: Effect of nano-zinc oxide spray on leaf nutritional quality of spinach.

yield along with an increase in protein content and a decrease in soluble sugar content in wheat [9].

These results indicated that the nano-zinc oxide enhanced the leaf physical and nutritional properties of spinach leaves. Nano-zinc oxide (1000 ppm) has a potential to be used as a biofortification agent to improve protein and dietary fibre contents of spinach leaves and their by reduce the malnutrition.

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