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Effect of Nano-Zinc Foliar Spraying on Anatomical Characteristics of Soybean (*Glycine max*) Plants

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Abstract

In trendy farming, the use of Nano fertilizers is raising the productivity and strength of different crops by reducing the destructive effects of environmental changes. The current study is the first in Iraq to address anatomical characteristics and the Nanoscale effect on them. In the current study, the effect of Nano zinc oxide on the anatomy features of soybeans was tested. Concentrations of Nano zinc oxide were 0.75 and compared with control plants without spray. after doing the sections and examination, the results showed that the effect of Nano zinc oxide on the vascular bundle in the stem and midrib of leaf, also the length and width of stomata in leaf epidermis compared with control, as the stems of plants sprayed with Nano zinc were in an primary growth stage compared to the control treatment that were in secondary growth. Also, the diameter of bundles in control reached 297.3µ compared with the diameter of bundles treated with Nano zinc, which reached 116.2µ. On the other hand, the diameter of bundles in the midrib reached 135.7µ in the control and 148.1µ in the Nano zinc treatment, so the length and width of the stomata increased in the Nano zinc treatment.

Keywords: *Glycine max*, Cross-section, Stem, Leaf, Epidermis.

1.Introduction

Glycine max (L.) Merr or Soybean is an important food and industrial crop worldwide, from legume family, Fabaceae, native to Southeast Asia, including China and Japan (1, 2). The name of Glycine has had a convoluted taxonomic history, Carl Linnaeus, in 1753, was the first to introduce it (3, 4).

It is distinguished from other species of legumes by containing all eight essential amino acids necessary for the human body to make protein, which makes it an excellent source of complete protein, especially for vegetarians (5-7).

Soybeans are grown for their oil, which constitutes 14-24%, and also for their protein, which constitutes 30-50%. The United States of America ranks first in global soybean production, accounting for 66% of global production (8, 9).

Nanotechnology is one of the modern discoveries of the twenty-first century, as it is used in all areas of life. This technology is a distinctive scientific method that includes modifying the physical and chemical properties of matter at the molecular level. Nanomaterials have been used in the field of agriculture, as Nano fertilizers have been used instead of traditional fertilizers to meet the plant's need for the necessary nutrients to increase production and reduce the problem of harmful effects on the soil and the environment. The advantages available in Nano fertilizers are the high surface area of their particles, the increased density, and the increased interactions on the exchange surfaces of organic and mineral colloids (10, 11).

Foliar feeding is one of the most important methods of supplying plants with their nutritional needs. Foliar feeding is done by spraying the green parts of plants with diluted solutions of these nutrients several times. It is considered one of the important and successful methods for treating nutrient deficiencies, especially minor ones (12, 13).

Despite the known benefits of zinc, the precise effects of nano-zinc oxide on the anatomical development of soybean are not fully understood. Hypothesize that treatment with nano-zinc oxide will lead to an increase in the diameter of the vascular bundle and alter the properties of the stomata in soybean leaves. So the aim of the study is to demonstrate the effect of foliar spraying with Nano-zinc on some anatomical traits of soybean plants and this study considers the first and new study in Iraq.

2.Materials and Methods

2.1. Soil preparation and experiment implementation

field experiment was carried out in the fields of a farmer in the Baghdad Governorate, Abu Ghraib reign, located below longitude 44º21 east and latitude 33º31 north during the summer season of 2024, using Al-Shimaa soybeans produced by the Vietnamese project and registered with the Iraqi Ministry of Agriculture. The field soil was prepared by ploughing and smoothing it to obtain well-loosened soil and fertilized with triple superphosphate 48%. The experimental land was divided into experimental units. The experimental unit included two lines with dimensions of 2.5 * 2.25 and a distance of 75 cm between each hole and 25 cm between holes. 2-3 seeds were planted in each hole follows (14). Planting took place on 22.5.2024, and Nano zinc was sprayed at a concentration of 0.75 $gm.L^{-1}$ in the morning until completely wet, 40 days after planting. The form of zinc oxide (Zn_2O_3) 80% Zn and with a purity of 90% was purchased from Khazra Nano Chelated Fertilizer Company in Iran (it is a completely water-soluble powder consisting of 12% Nano zinc).

2.2. Anatomical study

The cross-sections of the plant parts including stems, leaf epidermis, and leaves, were prepared by hand sectioning (15) with some modifications according to (16), At the beginning, the stems and leaves specimens were carried out into small pieces, with a length ranging between 5 to 7 cm, then these pieces were placed in sodium hypochlorite (3%) for 10 minutes to get rid of chlorophyll pigment. Later the sections were transferred to a glass slide with a cover slide.

For Epidermis preparation, the epidermis of the plant leaf was peeled in the middle between the apex and the base by sharp scalpel. The sample was washed with distilled water and then placed in a Petri dish in a solution of sodium hypochlorite (industrial bleach) at a concentration of 5% for 5 minutes. After that, it was washed with distilled water and transferred to KOH 10% for 5-10 minutes. Then, the solution was replaced with ethyl alcohol 70% for 10-15 minutes. After that, it was placed on a slide and covered with a slide cover and became ready for Microscopic examination. The method was according to (17) with some modifications according to (18).

All the examination was done with a KRÜSS compound microscope and the imaging was done with an AmScope (Model MU 1000) camera mounted on the microscope.

3.Results

The results of **Figure (1A)** showed the stem of the soybean plant is quadrilateral in shape with angles in cross-section. It is composed of a thin layer of cuticle, followed by the epidermis which is composed of a single layer of oval-shaped cells with glandular and eglandular hairs (**Figure 1C**), followed by the cortex, and then the vascular bundles. The vascular bundles were composed of the secondary phloem to the outside and the secondary xylem to the inside. The vascular bundle is continuous because it is in the stage of secondary growth between early and complete. The midrib of leaf is crescent in shape; it is composed of a thin layer of cuticle, followed by the epidermis which is composed of a single layer of oval-shaped cells covered by glandular and eglandular hairs, followed by the cortex consist from collenchyma tissue under the epidermis followed by chlorenchyma tissue, and then the vascular bundles. The vascular bundles are composed of the phloem and xylem, the xylem consist from 14-17 rows of vessels and each rows consist from 8-10 vessel units, **Figure (1B)** The results of epidermis also showed the anticlinal walls in the surface view of the epidermal cells in the adixial and abxial epidermis were characterized by being wavy. The stomata were distributed on the adixial and abxial surface of the epidermis and the type of it paracytic type consisting of 2 subsidiary cells around it **Figure (1D and F)**.

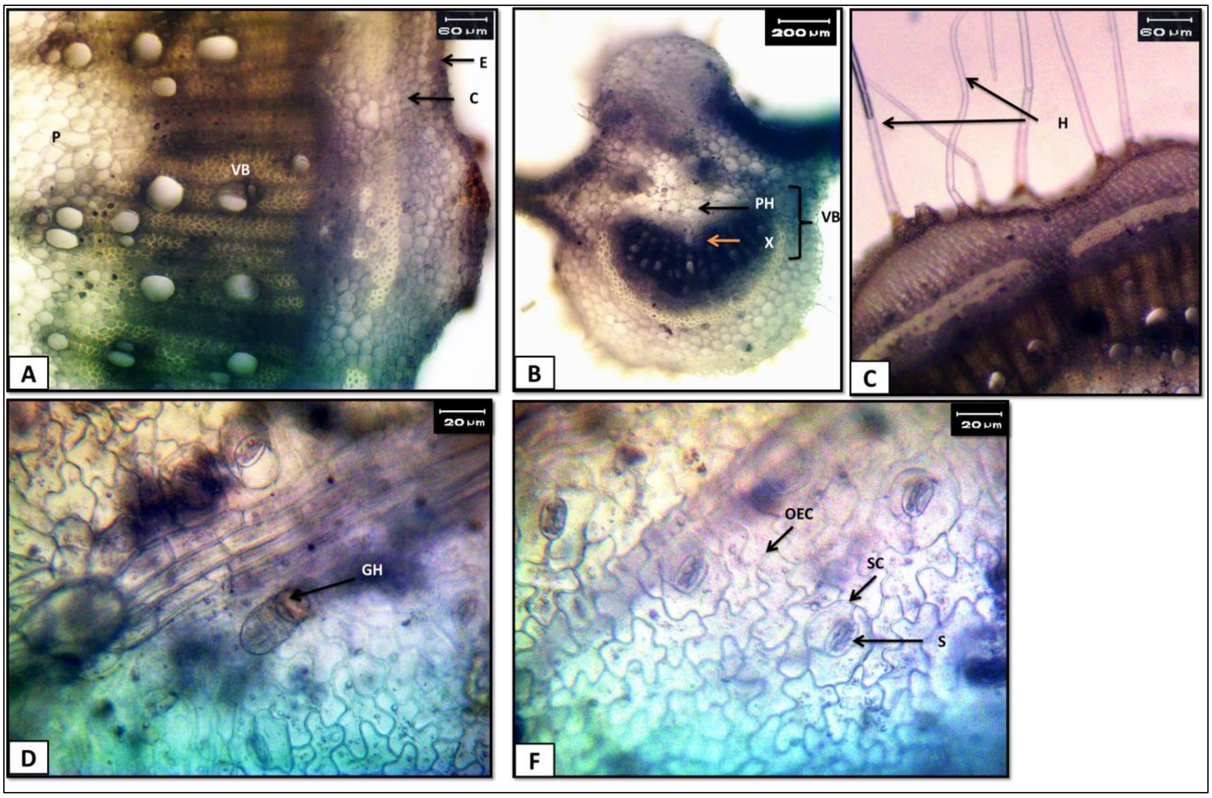

Figure 1. Control treatment shows: **A-**Stem, **B-**Midrib of leaf, **C-**Hairs in the epidermis of stem, **D-** Adixial epidermis, **F-** Abxial epidermis, E: pidermis, C: cortex, VB: vascular bundle, P: pith, PH: phloem, X: xylem, H: unglandular hairs, GH: glandular hairs, OEC: ordinary epidermal cells, SC: subsidiary cells, S: stomata.

The results of the study showed the presence of variations in the growth of the vascular bundle in the stem and midrib of leaf, also the length and width of stomata in leaf epidermis, by the treatments of Nano zinc compared with control, as the stems of plants sprayed with Nano zinc were in an primary growth stage compared to the control treatment that were in secondary growth. Also, the diameter of bundles in control reached 297.3µ compared with the diameter of bundles treated with Nano zinc reached 116.2µ. (**Table 1** and **Figures 2, 1A and 3A**) The diameter of bundles in the midrib reached 135.7µ in the control and 148.1µ in the Nano zinc treatment (**Table 1 and Figures 4, 1B and 3B**). On the other hand, the length of stomata increased in Nano zinc treatment reached 44.6µ and in control reached 34.1µ also, the width increased in Nano zinc treatment reached 30.8µ and in the control reached 21.1µ (**Table 1 and Figure 1D, F and 3C, D**).

Table 1: Effect of Nanozinc on anatomical characters of Soybean (the mean take it to 10 readings per sample) (Measurements in µm).

Treatments	Vascular bundle diameter in stem mean	Length of stomata in leaf mean	Width of stomata in leaf mean	Vascular bundle diameter in midrib of leaf mean
Control	297.3	34.1	21.1	135.7
Nanozinc	116.2	44.6	30.8	148.1

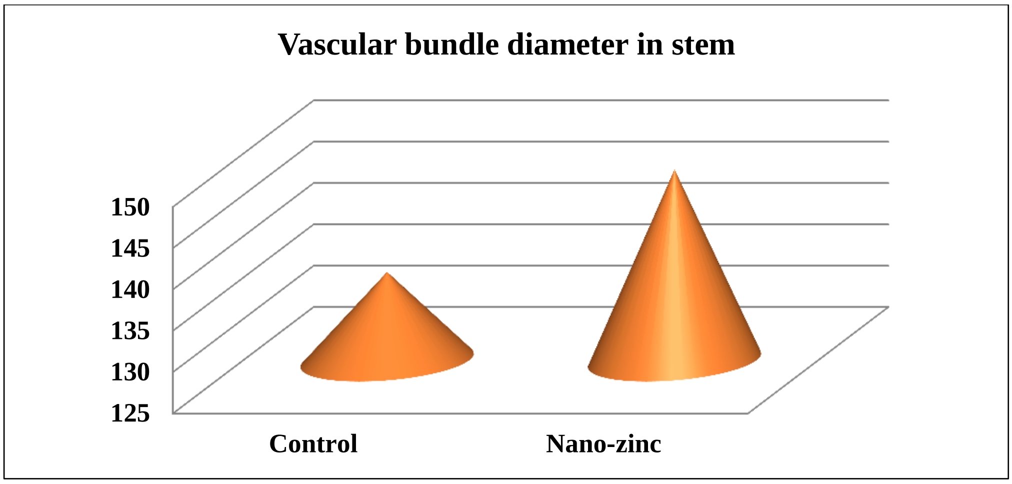

Figure 2. Diagram shows the compared treatments by Nano-zinc and control of Vascular bundle diameter in stem.

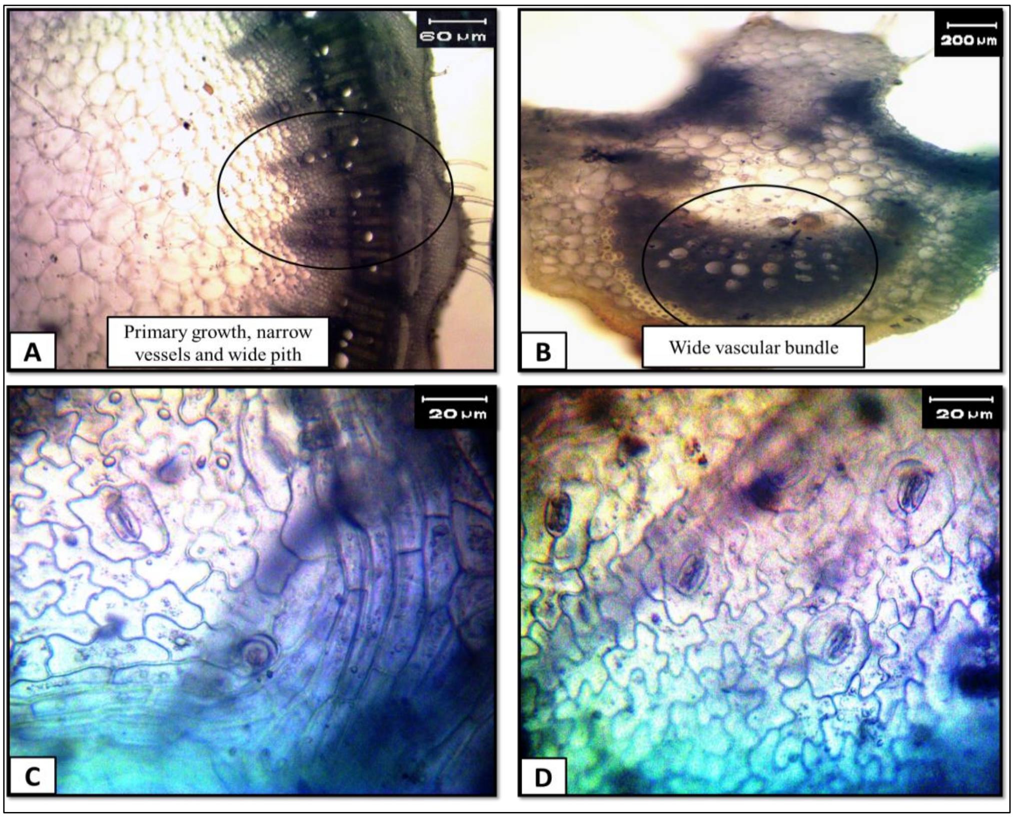

Figure 3. Nano-zinc treatment shows: **A-** Stem, **B-**Midrib of leaf, **C-** Adixial epidermis, **D-** Abxial epidermis.

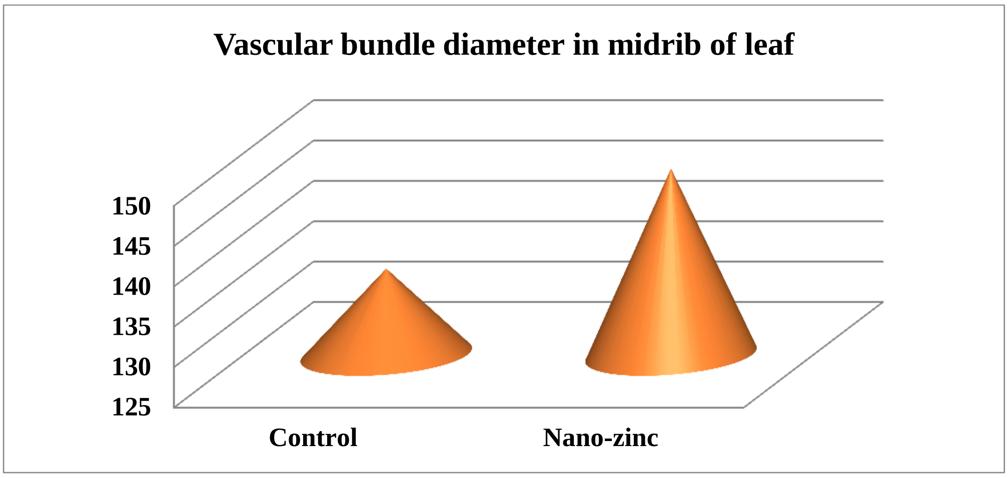

Figure 4. Diagram shows the compared treatments by Nano-zinc and control of Vascular bundle diameter in midrib of lead

4.Discussion

The results of the stem from the shape and forming to the cross-section tissue of cuticle, epidermis, glandular and eglandular hairs, cortex, and then the vascular bundles composed agree with (19, 20). It was referred that the midrib of leaf is crescent in shape and composed of cuticle, epidermis covered by glandular and eglandular hairs (21).

The results found that Nano zinc was the best; it helps keep the vascular bundle in an active stage to carry out the transport process and deliver the absorbed materials and nutrients to the rest of the plant parts. This is clear from the amount of starch storage present in the stem pith, which reflects positively on the process of making food in the leaf and storing excess of it in various plant tissues, including the parenchyma cells in the stem pith (22, 23), in contrast to control concentration, which accelerate the secondary growth of the stem, in which xylem vessels form in the aging stage and the formation of new vessels decreases, which it is reflected in the process of transport and delivery of nutrients to the rest of the plant, and this is reflected in the amount of starch stored in the pith of the stem (24, 25). Also the increase in the size of the vascular bundle in the midrib of leaf and the increase in the length and width of the stomata of the leaf reflects the increase in the efficiency of nutrient absorption from the stem and the increase in the process of photosynthesis, food production, and plant growth. The results agree with (26). It was mention that the focus studies now on recent advances in green nanotechnology, supplying details on reliable synthetic pathways towards the nanoparticles, which makes the topic of our current research of great importance to obtain high quality and efficient crops without any side effects or anatomical or morphological deformities on the treated plants (27).

Increasing the concentration of zinc in the leaves by increasing the level of its addition to the plant has led to an increase in the activity of enzymes related to the growth of vascular bundles and the faster withdrawal of nutrients and the development of flower formation, which led to an increase in the number of pods in the plant. This result is consistent with the results of a number of researchers who indicated that the addition of iron and zinc significantly increased the number of pods in the plant for mung and soybean crops (28, 29).

The version of Zn uptake and hyperaccumulation mechanisms in plants has grown greatly recently; some plants have created a range of physiological adaption plans to abide and hyperaccumulate Zn in their aboveground regions (30).

5.Conclusion

The water drought condition has negatively impacted the blossoming and development of dehydrated matter accumulation and yield of soybeans. Nevertheless, the harmful effects of deficiency stress were relieved when ZnO nanoparticles were applied as foliar to the leaves of soybeans. The foliar application of 0.75 gm.L-1 ZnO nanoparticles acted the best to fix the adverse effect of deficiency and improve the plant features (diameter of vascular bundles in stem and leaves, length and width of stomata of leaves). According to these results, it can be indicated that the foliar application of ZnO can amend the adverse impact of deficiency requirements and support the crop of soybeans.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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