

Response Of A Medicinal Plant Peganum Harmala To Iron Oxide Nanoparticles F_3O_4 (Nps)

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Abstract

Iron nanomaterials are widely present in the environment and their concentrations are increasing day by day. In many applications, iron nanomaterials are included, but the minimum and maximum concentrations that are not harmful to plants are not yet known. Effect of F_3O_4 (NPs) on Peganum harmala plants for the purpose of showing anatomical responses as effective biomarkers for increasing the concentration of nanomaterials in the ecosystem. Electron microscopy to determine the effect of different doses of F_3O_4 (NPs) ranging from (0-600 mg / L) were used. The cross-sections of leaves were compared in terms of epidermis, parenchyma cells, vascular cylinder, especially xylem and phloem vessels. Epidermal variations were studied stomata, guard cells and normal cells of the epidermis. The laboratories of the Department of Environmental Sciences and Pollution at the College of Science, University of Kufa for the year 2022 were used to conduct experiments. The anatomical results of the studied plants showed that the largest effect was on the epidermis of the plants, especially on the stomata system, where the stomata were closed and decreased in number, while the vascular system was less affected by the variation in the concentration of F_3O_4 (NPs) compared to the rest anatomical indicators. The severity effect was proportional to the increase in concentration. The results indicate that an insufficient supply of F_3O_4 (NPs) (150 mg / L) can be a plant growth stimulator and the results can be used in practical applications to increase production and improve crops as a fertilizer. Whereas, treatments with high concentrations inhibit antioxidants, causing a deficiency of growth activity in plants.

Keywords: Iron oxide nanoparticles, Epidermis, Vascular bundle, Parenchyma, and Xylem

INTRODUCTION

The plants can found in nature. They can accumulate nanoparticles at very high concentrations ,Nanomaterials (NPs), The particle size is less than or equal to 100 nanometers) have entered a wide applications [1,2,3]. Plants also make an important contribution to life from ancient times to the present. They play a significant role in agricultural systems as a product of food and fuel, and have an important social and economic role through their use in medicines, dyes, poisons, shelter, fiber, and religious and cultural ceremonies [4]. There is a strong relationship between plants and their iron content because plants need iron (Fe) in many enzymes and cellular metabolism as essential micronutrients. Lack of iron supply leads to poor production in the plant, and causes malnutrition in humans [5,6,7]. Despite this, if iron is found in the soil more than 70 to 400 mg / kg, which is the permissible limit, it causes serious problems for the plant as well as for the rest of the ecosystem through its transfer in the food chain [8]. The use of F_3O_4 (NPs) in industry is in many industries, especially solar cells, biosensors, cosmetics such

as sunscreens, etc. [9]. In agriculture, for example, in fertilize and protect the plant under conditions of environment stress [10]. All this led to the piling up of F_3O_4 (NPs) in the environment. The response of plants to NPs and especially to seed growth and anatomical responses has been studied recently in water, terrestrial and atmospheric environments [11]. Therefore, organisms, especially plants, are expected to be affected [12]. anatomical measuring one of important physiological trails to indicate the various stresses including temperature, heavy elements and drought affect the leaves of plants [14,15,16]. Therefore, the study focused on a statement of F_3O_4 (NPs) on The cross-sections of leaves were compared in terms of epidermis, parenchyma cells, vascular cylinder, especially xylem and phloem vessels. Epidermal variations were studied stomata, guard cells and normal cells of the epidermis. Is a new study to determine the critical concentrations of F_3O_4 (NPs) and an indicator of nano-contamination.

MATERIALS AND METHODS

The plants preparation

P. harmala plants Sterilization was carried out with sodium hypochlorite and washing with running water and then washed with sterile deionized water, then soaked in a suspension of F_3O_4 (NPs) at different concentrations (0, 150, 300 and 600 mg / L). Then put in glass jars, with (ten seeds per dish up on filter paper); Then, ten mL of deionized water as a reference treatment or various concentrations of NPs suspensions were mixed with Hoagland's solution. They were kept, after being closed with adhesive tape, in a grow cabin at 25 °C. After 72 h, 10 ml of F_3O_4 (NPs) suspension was added. After three weeks of growth, the plant pigments were measured.



AMAGE 1: The growth of studied plants with Hoagland's solution and F_3O_4 (NPs) suspension.

Anatomical Indices:

1. Cellular and Sub-Cellular Structural and Ultrastructural Observations:

The preparation of plant tissue samples for cellular structural and ultrastructural observation was performed according to the method developed by Fedorenko et al., (2018) for TEM observations. A 1 mm leaf sample was obtained from the middle of a fresh leaf directly taken from the experimental plants. The collected samples were fixed using 2.5% glutaraldehyde/0.1 M phosphate buffer solution (PBS) at room temperature for 2 h. The fixed samples were washed with PBS. After washing, the samples were incubated for 1 h in 1% OsO_4 /0.2 M PBS solution. The increasing concentration of ethanol and acetone (separately 50%, 70%, 96%, and 100%) were used for dehydration. The dehydrated samples were embedded in Epon-812 embedding medium. Semi-thin (about 1 m thick) and ultrathin (about 100 nm thick) sections were prepared by a microtome (Leica EM UC6, Leica, Wetzlar, Germany). The semi-thin sections were stained with 1% toluidine blue and examined under the SEM (Tecnai G12, Tecnai G12 spirit biotwin, FEI Company, Czech Republic).

2. Measurement of Stomatal Aperture:

Fresh leaf samples were obtained and prepared for scanning electron microscopy (SEM) to observe the stomatal aperture. The leaf samples were exposed to sunlight for 30 min and then frozen at 45 °C. The adaxial surface was sprayed with a gold/palladium target using a Mini Sputter Coater SC7620 vacuum deposition unit (Quorum Technologies, Laughton, East Sussex, UK) with a 30 s spraying mode at 18 mA. The samples were analyzed using an EVO-40 XVP SEM, Carl Zeiss, Oberkochen, Germany at an accelerating voltage of 10–15 kV. The phenotypic differences of the upper and lower epidermis were compared for all studied plants. As well as for various treatments.

Nanoparticles Characterization

Ready-made Fe_3O_4 nanoparticles were USA with a specification of 95% + 50 nm size. The aqueous suspension was examined after drying by electron microscopy (TEM) with a magnification of 100,000 - 320,000 to obtain digital images of particle size and morphology. 2 mg of Fe_3O_4 (NPs) was taken, then stirred the Fe_3O_4 (NPs) and sonicated for 4 hours in an ultrasonic device. Then Fe_3O_4 (NPs) suspension was deposited on (100 carbon film-covered grids) which are copper grids. TEM, dried, and examined under TEM. The globular shaped particles predominantly, with a size of between 15 and 40 nm, primary individual (Fig. A) and aggregate (Fig. B).

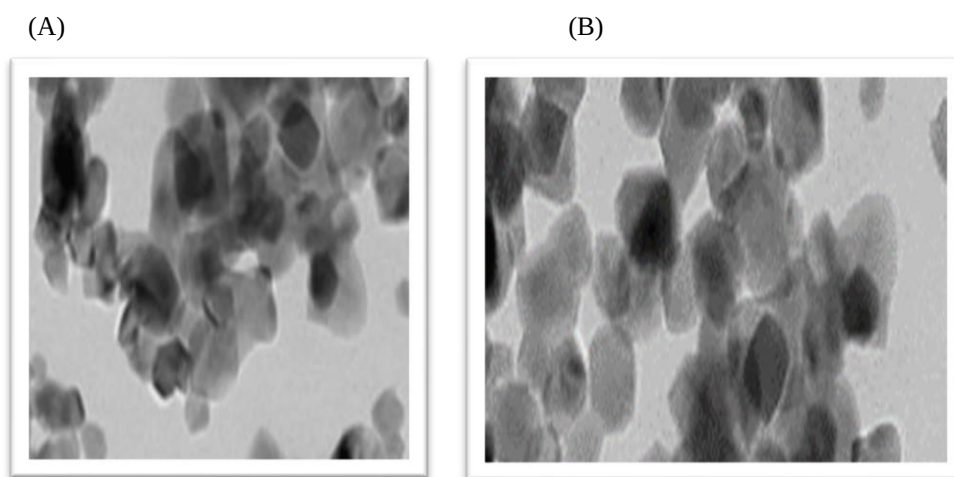


Image 2. transmission electron microscope image of Fe_3O_4 (NPs) at magnification of (A)= $3 \times 10^5 \times$ (aggregate) and (B)= 10×10^4 (Individual primary)

Statistical analysis results

The experiment was performed from four replicates in a completely random template design, MS Excel was used and Statistically significant was determined with a value of $p \leq 0.05$ as per Hoshmand, 1993[20].

RESULTS AND DISCUSSION

Response of Peganum harmala leave cross section to Fe_3O_4 (NPs) treatments

The Scanning electron microscope (SEM) photograph analysis of the studied Peganum harmala leave cross section revealed an anatomical structure typical of Dicotyledonous in the control variant (plate 1). The structure of the leaf tissue was characterized by an ordered organization and the cell's uniform localization in the chlorenchyma of the leaf. The division of mesophyll cells into palisade and spongy parenchyma was remotely traced. The cells of the parenchyma are located in several rows between the upper and lower epidermal layers, which were characterized by the round form. The insignificant proportion of tightly contacting cells and the presence of an extensive intercellular space was established

for the above-mentioned cells of parenchyma spatial organization. The vascular bundles consist of about four sectors, the xylem, phloem, parenchyma and bundle sheath (plates 1- 4). However, there were anatomical differences in the dimensions of the bundles in different treatments. These differences selected for comparison (plate 1-4).

Response of *Peganum harmala* leave cross section

The (SEM) photograph of *Peganum harmala* plants leave cross section revealed in the control variant (plate 1). The structure of the leaf tissue was characterized by an uniform localization in the parenchyma of the leaf. The cells of the parenchyma were characterized by the round form. The vascular bundles consist of about four sectors, the xylem, phloem, parenchyma and bundle sheath (plate 1).

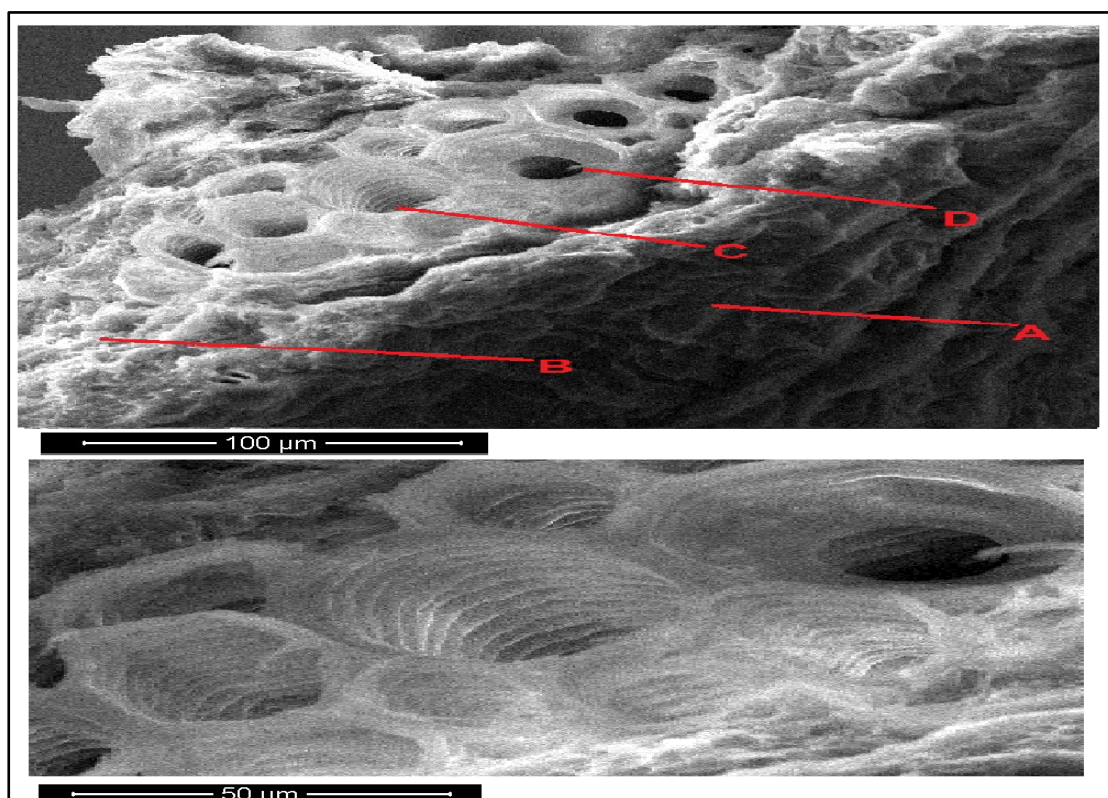


Plate 1: Scanning electron microscope images of *Peganum harmala* leave cross section under control treatments. A- epidermis , B- parenchyma, C- phloem, D- xylem.

Treatment of 150 mg/L showed a noticeable increase in the leaf cross section, which is a result of the increase in the layers of parenchyma. The vascular bindle increased in number, especially the vessels of xylem and phloem (Plate 2).

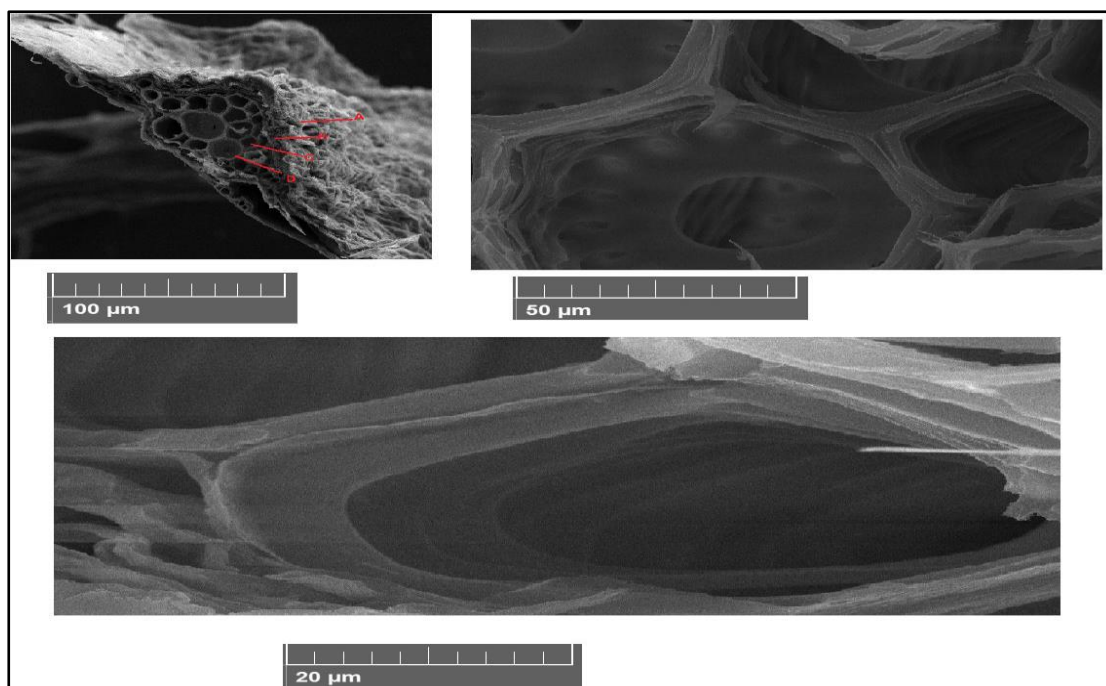


Plate 2: Scanning electron microscope images of *Peganum harmala* leaf cross section with 150 mg/l treatments. A- epidermis , B- parenchyma, C- phloem, D- xylem.

Peganum harmala leaf cross section with 300 mg/l treatments showed a decrease in the parenchyma cells size. The vascular bundle decreased in number, especially the vessels of xylem (Plate 3).

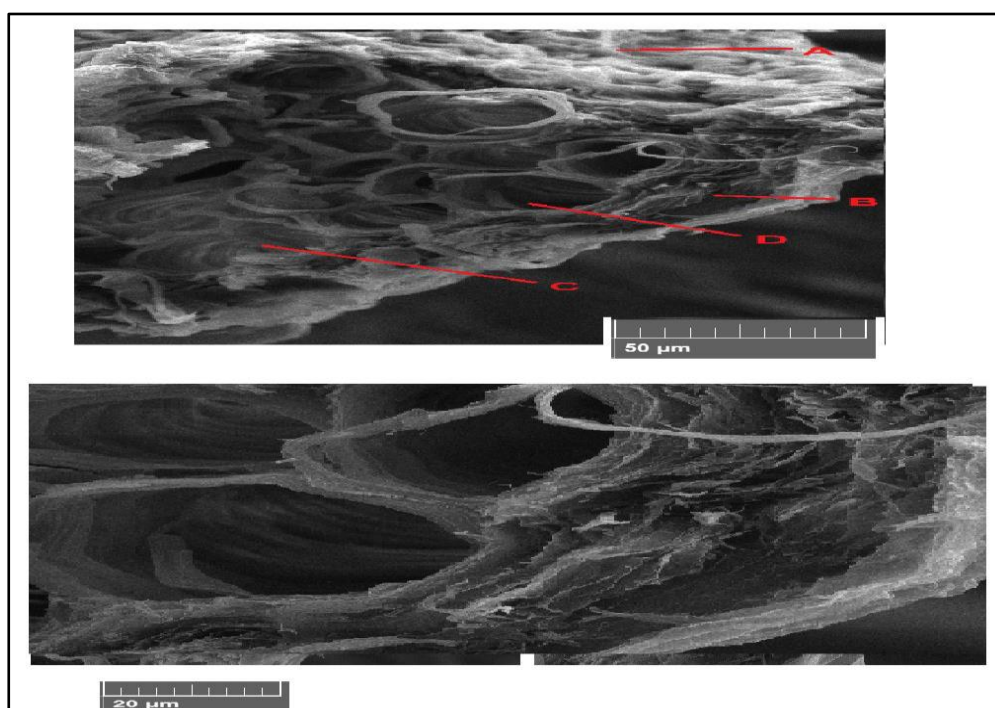


Plate 3: Scanning electron microscope images of *Peganum harmala* leaf cross section with 300 mg/l treatments. A- epidermis , B- parenchyma, C- phloem, D- xylem.

at the end of the experiment showed decay in the parenchyma tissue, vascular bundle and increase epidermis shrivel of *Peganum harmala* leaf cross section with 600 mg/l treatments.

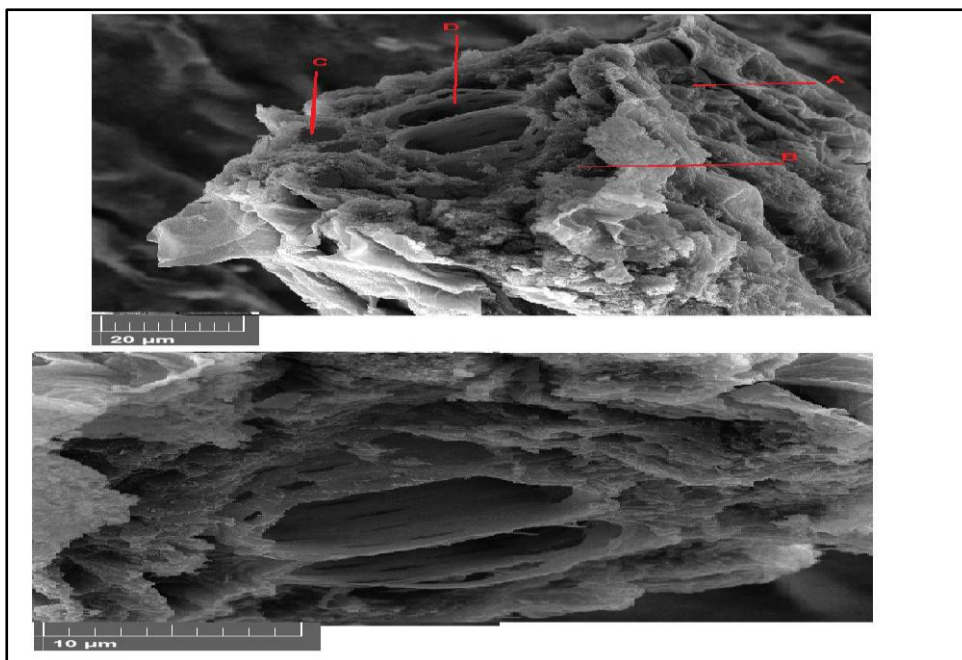


Plate 4: Scanning electron microscope images of *Peganum harmala* leaf cross section with 600 mg/l treatments. A- epidermis , B- parenchyma, C- phloem, D- xylem.

Response of *Peganum harmala* leaf stomata to F_3O_4 (NPs) treatments

(SEM) images of *Peganum harmala* leave longitudinal section under various treatments showed in plate (5-8). The control treatments showed epidermal cells are arranged in parallel rows and the stomata are arranged in a symmetrical shape. The stomata pores were open and the guard cells equal in shape and well swollen (plate5).

The longitudinal section with 150 mg/l treatments of F_3O_4 (NPs) appeared ordinary epidermal cells shrunk and the cell walls are thicker. The stomata are open and the stomatal pores is long. The guard cells are symmetrically shaped (plate 6). The treatments with 300 mg/l of F_3O_4 (NPs) showed different response comparable to the control where ordinary cells of the epidermis appeared irregular in shape. The cell walls are unequal in thickness. The stomata are recessed into dense folds. The orifice is slightly open. Guard cells of unequal thickness (plate 7).

The plate8 appeared a clear deposition of nanomaterials appeared on the surface of the epidermis, and the epidermal cells appeared wrinkled, elongated and narrow, as they were clearly reduced in size. The opening of the stoma is irregular. The guard cells are small in size (plate8).

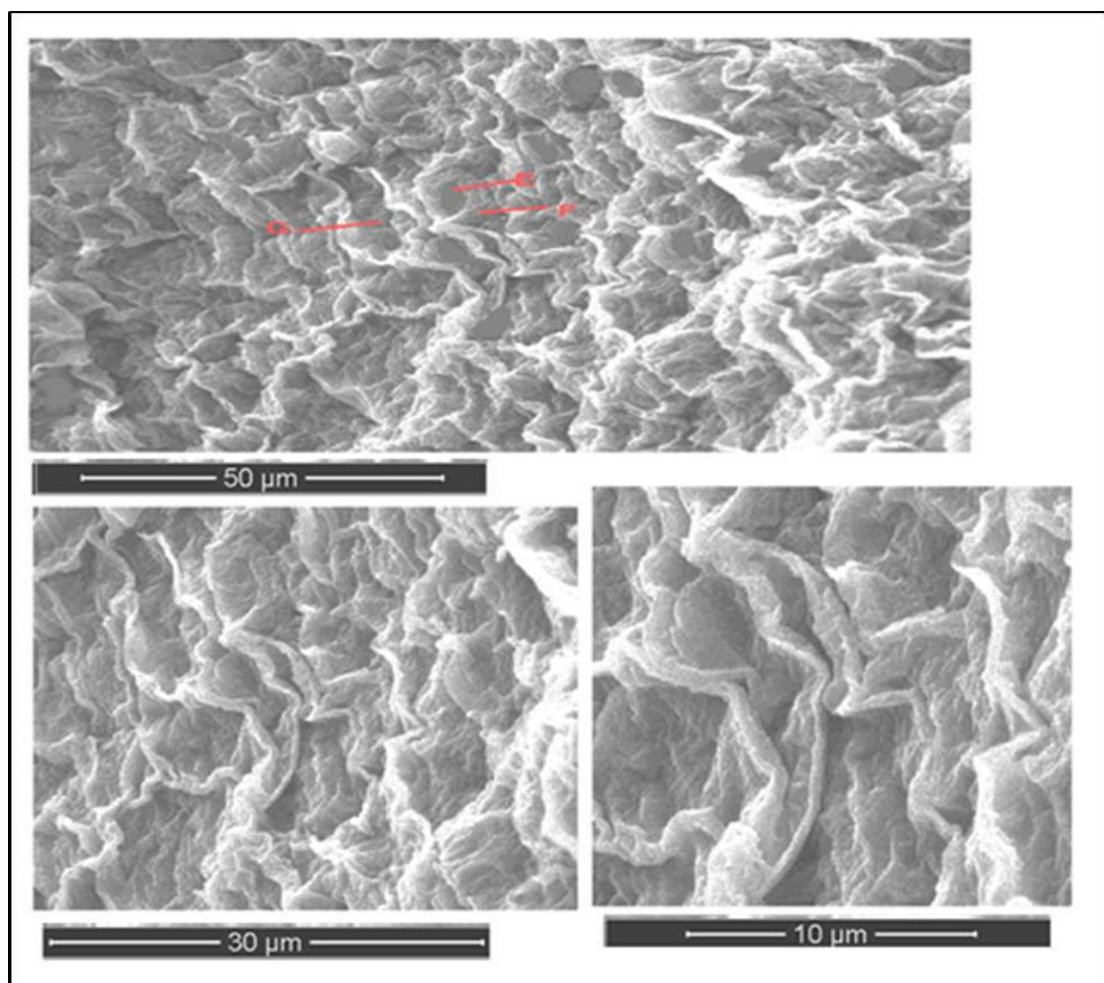


Plate 5: Scanning electron microscope images of *Peganum harmala* leaf longitudinal section under control treatments. E- stomata pore , F- guard cell, G- ordinary cell.

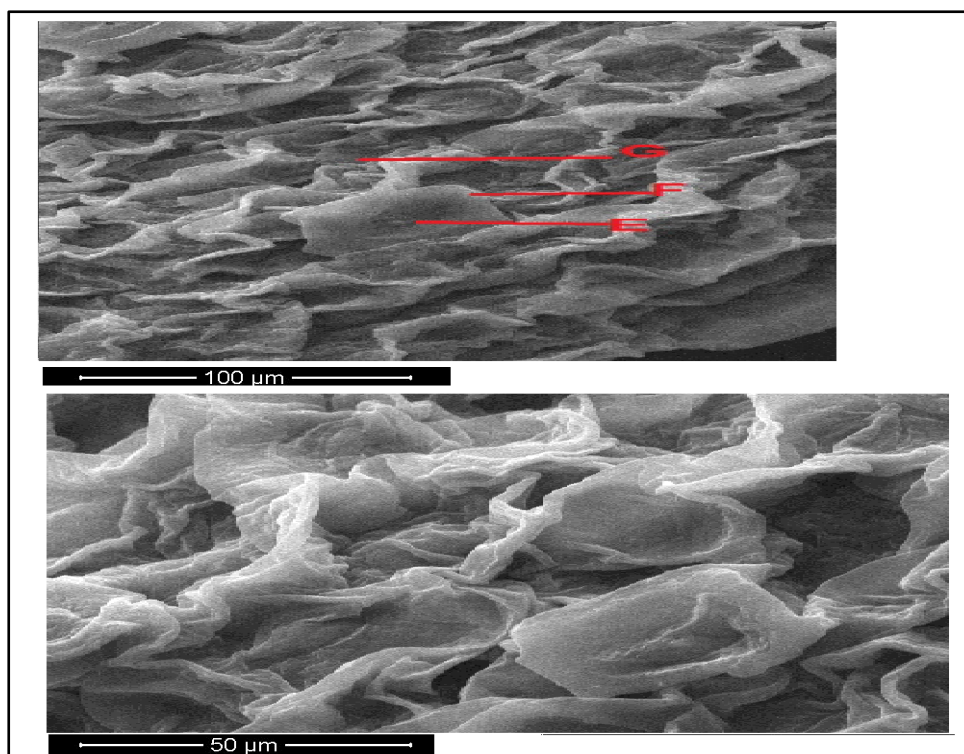


Plate 6: Scanning electron microscope images of Pegalum harmala leaf longitudinal section with 150 mg/l treatments. E- stomata pore , F- guard cell, G- ordinary cell.

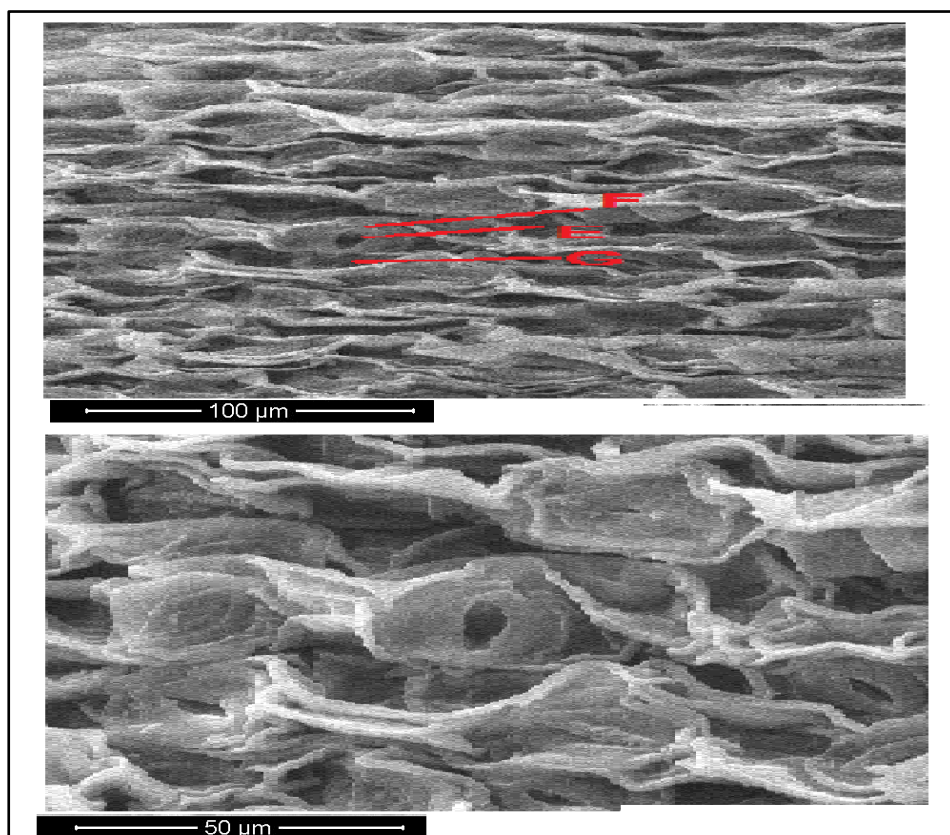


Plate 7: Scanning electron microscope images of Pegalum harmala leaf longitudinal section with 300 mg/l treatments. E- stomata pore , F- guard cell, G- ordinary cell.

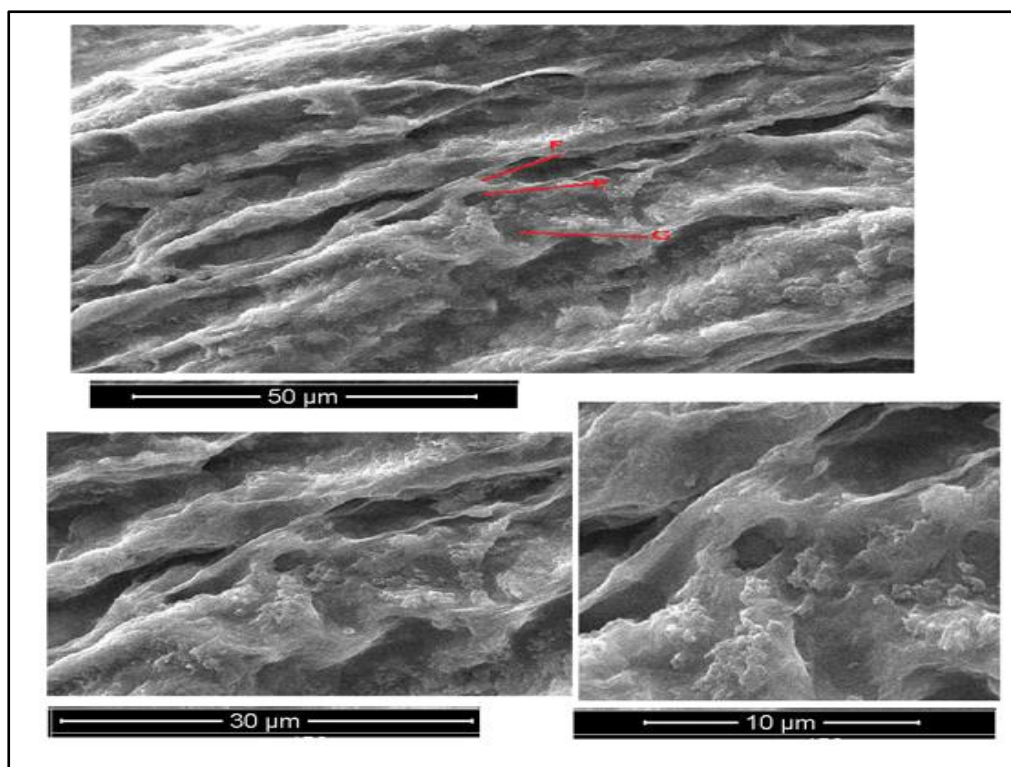


Plate 8: Scanning electron microscope images of *Peganum harmala* leaf longitudinal section with 600 mg/l treatments. E- stomata pore , F- guard cell, G- ordinary cell.

CONCLUSIONS

This study indicated that iron nanoparticles have a good stimulating effect and give stimulating results for seed growth, but if at low doses. The results showed a decrease in germination activity at high doses of F_3O_4 (NPs) in all studied plants. This corresponds to the Scanning electron microscope images of *Peganum harmala* leaf longitudinal and cross section in the leaves. The most important results related to the dependence of the response on the concentration dose of F_3O_4 (NPs). Therefore, the anatomical response of the plant and its metabolic activity depends on the proportion of doses with the effectiveness of the tested plants, as plants differ in response and determining the optimal dose depends on the type of plant. The seed is a potential fertilizer to enhance productivity in stressed, environments. The proper use of F_3O_4 (NPs) will also be beneficial in increasing the production quality of crops for human nutrition [21].

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