Investigation of the improvement in morphological and biochemical properties of soybean plants treated with hydroxyapatite nanoparticles

A. F. Zahran [1,*], Kh. M. Alashry [2], A. B. Abd-EL Razik [3], A. Khattab [1]

[1] *Agricultural Biochemistry Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt*
[2] *Soil and Water Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.*
[3] *Genetics Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.*

[*] Corresponding author E-mail: ahmedzahran201540@gmail.com (A. Zahran)

ABSTRACT:

Our work amid to synthesis nano-hydroxyapatite (HANPs) and nano hydroxyapatite hybrid loaded urea (NHANPs) using chemical precipitation reaction technique to create a novel design of recent soil fertilizers able to maximize availability of plant nutrients for uptake by plants while minimizing their loss through leaching or surface runoff in the soil called slow-release. To examine those nano-fertilizers, design a pot test turned into performed for the duration of the summer season 2021 on soybean plants growth. The pot test blanketed 16 treatments which have been mixtures of three factors. The first factor was (HANPs) with soil addition for four concentrations, i.e. 250 kg fed^{-1} (Monocalcium phosphate as positive control), 50 kg (HANPs) fed^{-1}, 25 kg (HANPs) fed^{-1} and 12.5 kg (HANPs) fed^{-1}. The second factor was (NHANPs) with soil addition for four concentrations, i.e. 150 kg fed^{-1} (Ammonium sulphate as positive control), 50 kg (NHANPs) fed^{-1}, 25 kg (NHANPs) fed^{-1}, and 12.5 kg (NHANPs) fed^{-1}. Data reflected a significant upgrade in all growth traits studied. Treatment (25 kg HANPs+25 kg NHANPs) and treatment (12.5 kg HANPs+12.5 kg NHANPs) surpassed all positive control treatments in root and shoot fresh weight and number of leaves each plant which reflect the great result of these chemical substances. In conclusion, the combined application of nano fertilizers might be recommended to maximize soybean growth and productivity.

Keywords: Chemical precipitation; Soybean; Nano fertilizers; Hydroxyl apatite nanoparticles; hydroxyapatite hybrid loaded urea; slow release.

INTRODUCTION

Mined phosphate rock is increasingly taken into consideration as a useful strategic resource whose extraction may want to grow to be significantly restrained in the future because of continuous consumption, and there are no alternatives for phosphorus in agriculture. Utilization of phosphorus from the implemented industrial phosphorus fertilizers by plants could be very low because of its immobilization and complicated chemical reactions in soils. The performance of implemented phosphorus fertilizer is believed to be as little as 20%, relying on physical and chemical properties. This has brought about a look for greater strategies for increasing crop manufacturing in low phosphorus soil (Shenoy and Kalgudi 2005). Phosphorus use efficiency may be progressed through optimizing land use, preventing erosion, preserving soil quality, enhancing fertilizer recommendations and fertilizer placement techniques, improving crop genotypes, incentivizing mycorrhizas (Schr€oder *et al.* 2011), and the use of manures and biochar (Gunes et al. 2014). In addition to this, the use of artificial nano-hydroxyapatite [Ca_{10} (PO_4) 6$(OH)_2$] (HANPs).

HANPs may be a promising way to upgrade phosphorus fertilizer use efficiency (Mehmet *et al*. 2018). The present-day literature on HANPs is especially focused on its biomedical implementations, but potential agricultural implementations have not been accurately addressed (Kottegoda *et al.* 2011). HANPs was used traditionally as phosphorus fertilizer; however, it has low solubility. Therefore, there is also an increased opportunity for phosphorus solubility to be executed through a nanoparticle's formulation. Furthermore, from a scientific perspective, the HANPs' reactive surface functional groups present abundant chances for surface modification through immobilization of strategic chemicals for the creation of nanohybrids with multifunctional qualities. One of the most promising options for phosphorus element delivery in agricultural applications is HANPs ($Ca_{10}(PO_4)_6(OH)_2$) nanoparticles. Wet chemical precipitation of HANPs nanoparticles is the simplest method available for synthesis with a high yield.

Hybrid nanomaterials have attracted scientific attention owing to combinations of nanocomponents that give rise to multifunctional properties because of synergistic effects from interfacial interactions.

HANPs and their hybrids have been extensively studied because of their importance in material science, biology, and medicine. The main source of nitrogen fertilizer is urea [CO $(NH_2)_2$], which contains 46% N by weight. Although this highly nitrogen concentration in urea, ammonia volatilizes before it can be effectively absorbed by the plants because of the early breakdown of urea in the soil brought on by water, volatilization, and urease enzymes. This poses a serious threat to future food security and presents a significant challenge to global agriculture. In this regard, urea-modified hydroxyapatite nanoparticles were employed as a slow-release fertilizer by Kottegoda *et al.* (2011). This nanohybrid's gradual urea release slows down the rate at which urea breaks down in soil, increasing plants' ability to use nitrogen agronomically. Liu and Lal (2014) confirmed nitrogen HANPs as an alternative nitrogen and phosphorus fertilizer, and they suggested that NHANPs can potentially upgrade soybean grown in peat-perlite mixtures.

Soybeans are an essential beginning of legume protein for cooking and animal feed worldwide. It is anticipated to enhance an important crop in Africa (Sinclair *et al.,* 2014). Soybean resides in a singular position in science and farming, apart from being a crop with vast uses. Soybeans are of age in almost all parts of the planet for human use, manufacturing, and animal feed (Boydak *et al*., 2002). Soybean plays a main role in providing protein and oil wanted by human beings (Agarwal, 2007; Shi and Cai 2010). Its protein has excellent potential as a major beginning of digestive protein. The oil presented from soybean sources is highly eatable and holds no cholesterol (Essa and Al-Ani 2001). The aim of this research is to evaluate the response of soybean plants to some synthetic nano-minerals fertilizers.

MATERIALS AND METHODS

Experimental site

Through 2021, summer season a pot trail was carried out at the El-Nada Research and Experimental Station Farm (30°27′ N, 30°93′ E), at Abu Ghalib, Giza Governorate, Egypt, to study the influences of wet synthesis chemical precipitation reaction technique of treatments, on growth of soybean plants (*glycine max,* Merrill C.V. Giza 111). The soil was sandy, and its properties are shown in Table 1.

Experimental treatments

The experiment included 16 treatments which were the combinations of Hydroxyl apatite nanoparticles (HANPs), and Nitrogen Loaded Hydroxyapatite Nanoparticles (NHANPs). First, soil additions of four concentrations, i.e. 250 kg fed^{-1} (Monocalcium phosphate as positive control) (P1), 50 kg Hydroxyl apatite nanoparticles per feddan (P2), 25 kg (HANPs) per feddan (P3) and 12.5 kg (HANPs) per feddan (P4). Additionally, soil additions of four concentrations, i.e. 150 kg fed^{-1} (Ammonium sulphate as positive control) (N1), 50 kg (NHANPs) per feddan (N2), 25 kg (NHANPs) per feddan (N3), and 12.5 kg (NHANPs) (N4).

Experimental practices and design:

The treatments were arranged in completely randomized design and planted in pots on 25 th May 2021 with three replicates. Seeds were inoculated with *Bradyrhizopium Jabonicum* before sowing directly. Soybean (C.V. Giza 111) seeds ((acquire from, Field Crops Research Institute, ARC) were broadcasted at a rate of 30 kg fad^{-1}, after irrigation. All different urged enlightening practices were adopted.

Preparation Experimental substrates

Preparation and characterization of hydroxyapatite nanoparticles (**HANPs**).

Water solutions of calcium hydroxide (Ca $(OH)_2$) and ortho-phosphoric acid (H_3PO_4, 85%), both of analytical grade, were used as reactants for the preparation of HANPs. HANPs powder was synthesized by 1L of an aqueous suspension of H_3PO_4 (0.6M) slowly dropwise to a 1L of an aqueous suspension of Ca $(OH)_2$ (1M) under forcibly stirring for about 2 hours at room temperature. Concentrated NaOH was added till a final pH of 10.5 was achieved. The reaction takes place according to the following equation. Paz *et al* (2012).

H_3PO_4 + 10 Ca $(OH)_2$ → Ca_{10} $(PO4)_6$ $(OH)_2$ + 18 H_2O.

Preparation and characterization of NHANPs.

Seventy-five kg of solid urea was mixed with a suspension containing Ca $(OH)_2$ (9. 65 kg, 75 L) and stirred for 45 minutes. To this mixture, H_3PO_4 (0. 6 M, 5. 05 L) was added slowly, drop by drop, leading to a composite that had a urea to HANPs ratio of 6:1. This composite was then stirred with mechanical agitation for an additional 2 hours. The resulting HANPs were dried using a flash drying method at a temperature of 60 °C, creating a urea-HA nanohybrid. According to

Kottegoda *et al* (2017) the presence of urea in the solution facilitates for its coating on HANPs as the nucleation occurs.

Characterization of HANPs and NHANPs

The HANPs and NHANPs solutions obtained were washed using distilled water and dried in oven at 80°C for 24 hours and characterized with x-ray diffraction (XRD), scanning electron microscope (SEM) and transmission electron microscope (TEM) as proved in figure 1 and 2

Sampling and assessments

Growth parameters

After 40 days of sowing each pot, five plants were randomly selected to examine the following growth characters: -

Root fresh weight (gram/plant),

Shoot fresh weight (gram/plant),

Number of leaves per plant.

Seed chemical composition

Total nitrogen percentage: -

Total nitrogen in seeds was measured using a changed version of the micro Kjeldahl method detailed in A. O. A. C. (1995). To find the amount of crude protein, the total nitrogen was multiplied by 6. 25. After that, the protein percentage was determined.

Seed oil percentage: -

To determine the percentage of oil, petroleum either was primarily used for extraction in a Soxhlet apparatus, following the guidelines provided by A. O. A. C. in 1995.

Seed macro elements content

To analyze the levels of nitrogen, phosphorus, and calcium in the seeds (%), we took 1 g of soybean seeds, ground them up, and mixed them with 10 mL of a concentrated acid solution made from $HClO_4$ and HNO_3 in a 4:9 ratio. This mixture was placed in flasks and heated on a hot plate for 240 minutes until a colorless residue formed. Next, we determined the nitrogen content using the macro-Kjeldahl method based on A. O. A. C. (1995) and measured P, K, Ca, Fe, Zn, Mn, and Cu using a flame photometer following (Jackson, 1973).

Statistical analysis

Data was subjected to analysis of variance (ANOVA) according to (Gomez and Gomez, 1984), using COSTATC software. To evaluate the differences among averages, the least significant difference (LSD) test was applied at a probability level of 0. 05%.

RESULTS AND DISCUSSIN

Morphological and Physiochemical Characterization of HANPs and NHANPs

SEM (Fig.1A and B), and TEM (Fig.1C and D) images show the morphological statement of HANPs and (B) NHANPs, which HANPs appear spherical, smooth, and non-aggregated, while the NHANPs appeared needle shaped. This may be due to the successful loading of urea onto HANPs. Furthermore, the physicochemical analyses using X-rays also showed the crystalline structure of HANPs and NHANPs, as shown in Fig.2 A and B

Effect of HANPs on Morphological and Biochemical analysis of soybean plants and seeds.

Effect of HANPs on normal growth of soybean plants

Data presented in Table 2 and Fig.3A shows that soybean growth parameters (root fresh weight, shoot fresh weight and numbers of leaves) were significantly influenced by treating with HANPs. In this respect, 50 kg HANPs per feddan (P2) had the highest values in root fresh weight, shoot fresh weight and numbers of leaves. In sequence, 25 kg HANPs per feddan (P3) came in second order in all growth parameters. Moreover, 12.5 kg HANPs per feddan (P4) came in third order, surpassing the positive control (p1) in all growth parameters. These results are close to the results mentioned by Liu and Zhao (2013) and Liu and Lal (2014).

Effect of HANPs on quality traits of soybean seeds

Data tabulated in Table (-2-) revealed that HANPs significantly increased oil percentage of soybean seeds. The highest percentage of oil was given by treating soybean plants with 50 kg HANPs per feddan (P2) while recommended dose of phosphorus (P1) was the inferior in this respect. In sequence, 25 kg HANPs per feddan (P3) came in second order in seed oil percentage. It is remarkable that the treatment 12.5 kg HANPs per feddan (P4) showed non-significant result when compared with recommended treatment (P1) which reflects a positive result. On the other hand, treating soybean plants with HANPs did not enough to reach the 5% level of significance in seed protein percentage. Our results similar to (Liu and Lai, 2015) and Mehmet *et al* (2018)

Effect of HANPs on nitrogen, phosphorus and calcium accumulation of soybean seeds.

phosphorus and calcium accumulation in seeds were remarkably affected by treating soybean plants with HANPs. Although decreasing HANPs dose to 12.5 kg per feddan (P4) but this treatment had no significance effect in compared with recommended treatment (highly phosphorus and calcium fertilizers P1) on both phosphorus and calcium accumulation in seeds which reflect positive result, while P2 and P3 treatments scored the highest accumulation of phosphorus and calcium in soybean seeds surpassing statistically in contrast with positive control (P1). On the other hand, a non-significant result in nitrogen accumulation was achieved by treating soybean plants with HANPs, as presented in Table (2). These results drew attention from some investigators by (Golezani and Lotfi, 2012) and Mahmoud *et al.* 2013.

Hydroxyapatite is considered a promising example of a nano-fertilizer based on nanoparticles of compounds rich in phosphate and then calcium, which is one of the essential nutrients for plants. Traditionally, HANPs serve as a fertilizer that provides phosphorus, although it has a low rate of solubility. Data revealed a higher value affected by HANPs that may be attributed to a greater potential achieved by the solubility of phosphorus and calcium through nano-formulation. Furthermore, surface-reactive functional groups on hydroxyapatite nanoparticles provide several options for surface modification by immobilizing key molecules to form nano-hybrids with multifunctional capabilities (Carmona *et al.,* 2021). In addition, the three-dimensional structure of hydroxyapatite nanoparticles gives great flexibility in accepting mixtures of ions through the process of rotation or substitution of calcium atoms. Thus, the properties and applications used by different hydroxyapatite nanoparticles can be controlled, such as catalysis (Paz *et al.,* 2012), adsorption of proteins, ion exchangers, and biomaterials (Pirvulescua *et al.,* 2014). It's clear from results that all plants treated with HANPs produced the highest numbers on growth, quality traits (%protein and % oil) and macronutrients (nitrogen, phosphorus and calcium) accumulations in seed; results varied depending on the additive concentrations, this means high absorption or uptake by plants treated with HANPs. Liu *et al* (2013) attributed this to two reasons: The first one was soluble phosphates can be removed from the soil solution more readily when the pH of the soil solution changes, which formed soluble phosphates into precipitations of iron phosphate, manganese phosphate, aluminum phosphate and tricalcium phosphate non soluble forms. In contrast, HANPs tend to stay stable in suspension and are generally less influenced by the pH of the solution, as well as by the presence of other ions or solid materials. The second one, a solution with soluble phosphorus, can leach from the soil column more quickly compared to the HANPs solution, due to the greater viscosity of the HANPs. As a result, more phosphorus remains in the growing medium, allowing plant roots to absorb it when HANPs are present.

Effect of NHANPs on Morphological and Biochemical analysis of soybean plants and seeds

Effect of NHANPs on the growth of soybean plants.

Data presented in Table (-3-) and Fig.3B illustrates the significant effect of NHANPs on soybean growth attributes (root fresh weight, shoot fresh weight and numbers of leaves). In this context, 50 kg NHANPsper feddan (N2) was the superior treatment compare with all treatments in root fresh weight, shoot fresh weight and numbers of leaves. Furthermore, 25 kg NHANPsper feddan (N3) and 12.5 kg NHANPs per feddan (N4) showed in descending order in all growth parameters outnumbering the recommended treatment (N1), as shown in Fig3. These trends are in good accordance with those obtained by (Kottegoda *et al.* 2017).

Effect of NHANPs on quality traits of soybean seeds

It's noticed from data in Table (-3-) that NHANPs significantly upgraded seeds oil and protein percentages of soybean. The 50 kg NHANPs per feddan (N2) was the potent practice for producing the maximal seeds oil and protein percentages while recommended dose of (N1) was the inferior in this respect. Herein, 25 kg NHANPs per feddan (N3) came in second order in seed protein percentage. It is remarkable that the treatment 12.5 kg NHANPs per feddan (N4) did not enough to reach the 5% level of significance in seed oil and protein percentage when compared with recommended treatment (N1) which reflects a positive result. These results are similar recorded by Paz *et al* (2012).

Effect of NHANPs on nitrogen, phosphorus and calcium accumulation of soybean seeds

As shown in table (3), nitrogen, phosphorus and calcium accumulation in seeds were remarkably affected by treating soybean plants with NHANPs. Although decreasing NHANPs dose to 12.5 kg per feddan (N4) but this treatment had not statistically significance effect in compared with recommended treatment (highly nitrogen fertilizers N1) on nitrogen, phosphorus and calcium accumulation in seeds which reflect positive result, while 50 kg NHANPs per feddan (N2) treatment scored the highest accumulations of nitrogen, phosphorus and calcium in soybean seeds outnumbering statistically in contrast with positive control (N1). Concerning 25 kg NHANPs per feddan (N3) treatment result obtained in the second order in nitrogen, phosphorus and calcium accumulation. These results drew attention by some investigators as Paz *et al* (2012). The early breakdown of urea in the soil happens because of water, volatilization, and the activity of urease enzymes. This process releases ammonia before plants can properly absorb it. This presents a significant issue for agriculture worldwide and puts future food security at risk. Thus, the purpose of this study is to use the chemically modify the surface of hydroxyapatite with urea particles, which are the most widely used sources because they contain nitrogenous nutrients, which have the ability to dissolve in water and then combine or mix with soil components and then absorb moisture in the soil, which leads to a slow and sustainable release of nitrogen as a result of diffusion. Microbial decomposition or spray on the surface of the plant depends on the weak link or interaction between hydroxyapatite nanoparticles and urea particles in this hybrid, which is responsible for the slow excretion of urea from this compound for the slow and sustainable release of macronutrients that can be made available to the soil and plants. Plant nutrients at a slower rate. In this respect, the availability of NHANPs upgraded the ability of plants to absorb more nitrogen, phosphorus and calcium, which was reflected in high values of growth parameters, quality traits (%protein and % oil) and macronutrients (nitrogen, phosphorus and calcium) accumulations in seeds. (Kottegoda *et al.* 2017).

Effect of interaction between HANPs and NHANPs.

Effect of interaction between HANPs and NHANPs (NHA) on root fresh weight of soybean plants.

As shown in Fig.4, the interactions between HANPs and NHANPs had a significant effect on root fresh weight. In this context, (N1 P3 or P4) (N2 P2), (N2 P3), (N3 P2 or P3) and (N4 P2 or P3) were the most effective combinations for producing highest root fresh weight compared with all combinations. It should not forget (N1 P4) and (N2 P4) as promising combinations. These trends are in good accordance with those obtained by Paz *et al* (2012) and Kottegoda *et al.* 2017.

Effect of interaction between HANPs and NHANPs on shoot fresh weight of soybean seeds.

Fig.5 revealed the interactions between HANPs and NHANPs. A significant increment on shoot fresh weight was obtained by combinations (N1 P3 or P4) (N2 P2 or P3), (N3 P2 or P3) and (N4 P2 or P3). It should not forget (N1 P4) and (N2 P4) as hopeful combinations. These trends are in good accordance with those obtained by Paz *et al* (2012) and Kottegoda *et al.* 2017.

Effect of interaction between HANPs and NHANPs) on the number of leaves per plant of soybean plants.

The interactions between HANPs and NHANPs had a significant increment in the number of leaves per plant. Combinations (N1 P3 or P4) (N2 P2 or P3), (N3 P2 or P3) and (N4 P3) surpassing all combinations. Fig.6. These trends are in good accordance with those obtained by Paz *et al* (2012) and Kottegoda *et al.* 2017.

Effect of interaction between HANPs and NHANPs on seed protein percentage of soybean seeds

It's noticed from Fig.7 that all combinations of (N2) and (N3) achieved extreme significance in contrast with all combinations. It should not forget (N4 P2) and (N4 P4) as promising combinations. These trends are in good accordance with those obtained by Paz *et al* (2012) and Kottegoda *et al.* 2017.

Effect of interaction between HANPs and NHANPs on seed oil percentage of soybean seeds.

Fig 8 indicated that combinations (N1 P2 or P3) (N2 P2 or P3), (N3 P2 or P3) and (N4 P2 or P3) gave the highest values of seed oil. It cannot be overlooked that combinations (N1 P4) and (N4 P4) were hopeful combinations. These trends are in good accordance with those obtained by Paz *et al* (2012) and Kottegoda *et al.* 2017.

Effect of interaction between HANPs and NHANPs on % nitrogen accumulation of soybean seeds

It's noticed from Fig. 9 that all combinations of (N2) and (N3) scored the highest accumulation of nitrogen in soybean seeds in contrast with all combinations. It should not forget (N4 P2) and (N4 P4) as promising combinations. These trends are in good accordance with those obtained by Paz *et al* (2012) and Kottegoda *et al.* 2017.

Effect of interaction between HANPs and NHANPs on phosphorus accumulation of soybean seeds.

Fig. 10 revealed the interactions between HANPs and NHANPs. It's noticed from this figure that all combinations of P2 and P3 produced the maximum phosphorus accumulation in soybean seeds outnumbered all combinations. These trends are in good accordance with those obtained by Paz *et al* (2012) and Kottegoda *et al.* 2017.

Effect of interaction between HANPs and NHANPs on calcium accumulation of soybean seeds.

It's remarkable from Fig. 11 that all interactions containing HANPs special P2 and P3 had an extremely significant upgrade in calcium accumulation of soybean seeds beyond the excellence of all combinations. These trends are in good accordance with those obtained by Paz *et al* (2012) and Kottegoda *et al.* 2017.

The above-mentioned results related to growth, quality traits, and nutrient seed accumulation affected by additions of HANPs and NHANPs can be interpreted and discussed as follows: First, the increments in soybean growth, quality traits (% protein and % oil), and nitrogen, phosphorus, and calcium accumulations in seed with increasing concentrations of nitrogen could be owing to the operative role of nitrogen as an essential constituent of chlorophyll on dry matter accumulation. Nitrogen fertilizer effects on the production of carbohydrates by affecting the mean leaf area available to capture sunlight and absorb CO_2, upgrading the efficiency of the photosynthesis process David *et al*. (1999); Sobh *et al*. (2000) and Saudy *et al*. (2009). Additionally, nitrogen turns into amino acids in plants. These amino acids serve as both building blocks and precursors for proteins (Rai, 2002), which are essential for promoting cell growth and increasing the levels of pigments such as chlorophyll and carotenoids (Bahari *et al*., 2013). They consist of both acidic and basic groups that function as buffers, helping to keep a suitable pH level inside the plant cell (Davies, 1982). Amino acids enhance the photosynthesis process. Furthermore, the release of vitamins, amino acids, auxin, and the process of fixing nitrogen from the atmosphere by *Azotobacter* are direct factors that support root growth and overall plant development (Akbari *et al*., 2007). Furthermore, phosphorus serves as an essential nutrient as a part of several key plant structure compounds and acts as a catalyst in numerous vital biochemical reactions within plants. Phosphorus is the most significant nutrient for its capacity as energy transfer and storage in plants. We can consider adenosine diphosphate and triphosphate (ADP and ATP) the most significant types of phosphates that are associated with energy transfer. It is also entre in several structural components like sugar phosphates, phosphor proteins, phospholipids, coenzymes, nucleic acids, and metabolic substrates in plants. Providing phosphorus at the initial phase of crop growth is essential for enhancing reproductive structures. Some specific growth parameters were linked with phosphorus like augmented stalk and stem strength, promoting root development, enhanced flower formation and seed production, greater uniformity and earlier ripening of crops, improved nitrogen-fixing ability in legumes, better quality of crops, and higher resistance to plant diseases. (Preetha and Balakrishnan, 2017). Additionally, Calcium (Ca) is among the three secondary nutrients that plants need for proper growth, alongside magnesium (Mg) and sulfur (S). This element plays several vital roles in plants: it aids in the metabolic processes involved in nutrient uptake; it serves as a messenger within metabolic activities associated with growth and development, including cell division, differentiation, growth direction, and elongation. Additionally, calcium is critical for fortifying cell wall structure, as it forms calcium pectate, which provides stability to cell walls and connects cells. Calcium is also involved in enzymatic and hormonal activities; it supports plants in coping with heat stress by enhancing the function of stomata and aiding in the production of heat shock proteins. Furthermore, it contributes to disease resistance and influences the quality of fruits. Calcium has a part to play in stomatal regulation (Marschner, 1995; Mengel and Kirkby, 2001). According to Arvin *et al.* (2005), increasing calcium levels in plants boosts the resistance of plant tissue to bacterial pathogens and improves the structure of cell walls and membranes. When it comes to flower fertilization, calcium facilitates attraction, close-range communication, cell merging, signaling, and pollen tube development through the movement of calcium ions and hydrogen ions (H+), which drives the growth of the pollen tube. Following the fusion of gametes, the first observed event is an increase in cytosolic calcium levels, which is then followed by a rise in the rate of cell division, highlighting its significance in the reproductive growth of plants. (Seidel *et al.*, 2015) and (Fioreze *et al.*, 2018).

CONCLUSION

The results of this research confirmed that HANPs can stay stable in suspension, showing less sensitivity to the pH of the solution, other ions present, or solid materials. Consequently, providing plants with phosphorus and calcium in different plant stages reflects growth and productivity. Hence, hydroxyapatite nanoparticles are considered a promising example of a nano-fertilizer based on nanoparticles of compounds rich in phosphate and then calcium. On the other hand, because of the early breakdown of urea in the soil happens because of water, volatilization, and the activity of urease enzymes. This process releases ammonia before plants can properly absorb it. Therefore, a new hybrid nano fertilizer was modified to overcome this problem. NHANPs hybrid, which leads to a slow and sustainable release of nitrogen because of the weak link or interaction between HANPs and NHANPs hybrid, ensuring that nitrogen can be more available to the soil and plants at critical stages, which is reflected in growth consequently productivity.

REFERENCES

Agarwal, A.K., 2007. Bio-fulels (Alcohols &Biodisel) application as fuels for internal composition engines. Progress in Energy and Combustion Science 33: 233-71.

Akbari, G.A., Arab, S.M., Alikhani, H.A., Allahdadi, I., Arzanesh, M.H., 2007. Isolation and selection of indigenous Azospirillum spp. and the IAA of superior strains effects on wheat roots. *World J. Agric. Sci.*, 3(4): 523-529.

AOAC. 1995. Official Methods of Analysis. 14th Ed. Benjamin Franklin Station. Washington, D. C. U.S.A.: 490-510.

Arvin, M., Habib, A., Donnelly, J., 2005. Effect of calcium concentrations in medium on microtuberization of potato (*Solanum tuberosum L.). Iranian J. Biotech*. 3:3.

Bahari, A., Pirdashti, H., Yaghubi, M., 2013. The effects of amino acid fertilizers spraying on photosynthetic pigments and antioxidant enzymes of wheat (*Triticum aestivum* L.) under salinity stress. *International Journal of Agronomy and Plant Production,* 4 (4): 787-793.

Boydak, E., Alpaslan, M., Hayta, M., Gercek, S., Simsek, M., 2002. Seed composition of soybeans grown in the Harran region of Turkey as affected by row spacing and irrigation. *J. Agric. Food Chem*. 50, 4718–4720.

Carmona, F.J., Sasso, G., Rodríguez, G., Pii, Y., López, J., Guagliardi, A., Norberto, M., 2021. Urea-functionalized amorphous calcium phosphate nanofertilizers: optimizing the synthetic strategy towards environmental sustainability and manufacturing costs. *Scientific Reports*. 11 (1): p. 3419.

David, M., Wallach, D., Maynard, J.M., 1999. Models of yield grain protein and residual mineral nitrogen responses to applied nitrogen for winter wheat. *Agric. J.,* 91: 377-385.

Davies, D.D., 1982. Physiological aspects of protein turn over. *Encycl. Plant Physiol.,* 45: 481–487.

Essa, T.A., Al-Ani, D.H., 2001. Effect of salt stress on the performance of six soybean genotypes. *Pakistan Journal of Biology Science* 4: 175-177.

Fioreze, S.L., Tochetto, C., 2018. Effects of calcium supply on soybean plants. *Com. Sci., Bom Jesus,* 9(2): 219-225.

Golezani, K.G., Lotfi, R., 2012. Response of soybean cultivars to water stress at reproductive stages. *Int. J. of Plant, Ani. and Enviro. Sci*. 2 (2):198- 202.

Gomez, K.A., Gomez, A.A., 1984. Statistical procedures for Agricultural Research. John willey and sons. *Inc New York, U.S.A As hour and Talooth* 83.

Gunes, A., Inal, A., Taskin, M.B., Sahin, O., Kaya, E.C., Atakol, A., 2014. Effect of phosphorus-enriched biochar and poultry manure on growth and mineral composition of lettuce (Lactuca sativa L. cv.) grown in alkaline soil. *Soil Use and Management* 30:182–188.

Jackson, M., 1973. Soil Chemical Analysis; Pentice Hall of India Pvt. Ltd.: New Delhi, India, Volume 498, pp. 151–154.

Kottegoda, N., Munaweera, I., Madusanka, N., Karunarante, V., 2011. A green slow-release fertilizer composition based on urea-modified hydroxyapatite nanoparticles encapsuled wood. *Current Science* 101:73–78.

Kottegoda, N., Sandaruwan, C., Priyadarshana, G., Siriwardhana, A., Rathnayake, U., Arachchige, D., Kumarasinghe, A., Dahanayake, D., Karunaratne, V., Amaratunga, G., 2017. Urea-Hydroxyapatite Nanohybrids for Slow Release of Nitrogen.

ACS Nano 11, 1214–1221. Doi: 10.1021/acsnano.6b07781

Liu, R., Lal, R., 2015. Potentials of engineered nanoparticles as fertilizers for increasing agronomic productions. *A review. Science of the Total Environment* 514, 131–139.

Liu, R., Lal, R., 2014. Synthetic apatite nanoparticles as a phosphorus fertilizer for soybean (Glycine max). *Scientific Reports* 4:5686. doi:10.1038/srep05686

Liu, R., Zhao, D., 2013. Synthesis and characterization of a new class of stabilized apatite nanoparticles and applying the particles to in situ Pb immobilization in a fire-range soils. *Chemosphere* 91 594–601.

Mahmoud, G.O., Almatboly, M.A., Safina, S.A., 2013. Effect of irrigation intervals and fertilization systems on soybean seed yield and its quality. *J. Plant Production, Mansoura Univ.,* Vol. 4 (7).

Marschner, H., 1995. Mineral nutrition of higher plants. 2nd ed. Academic Press. London.

Mehmet, B.T., Sahina, O., Taskina, H., Atakol, O., Inal, A., Gunes, A., 2018. Effect of synthetic nano-hydroxyapatite as an alternative phosphorus source on growth and phosphorus nutrition of lettuce (*Lactuca sativa L.*) plant. *Journal of Plant Nutrition* 47:9, 1513-1526.

Mengel, K., Kirkby, E.A., 2001. Principles of Plant Nutrition. International Potash Institute, Bern, Switzerland.

Paz, A., Guadarrama, D., López, M., González, J., Brizuela, N., Aragón, J., 2012. A comparative study of hydroxyapatite nanoparticles synthesized by different routes. *Química Nova*. 35 (9): p. 1724-1727.

Pirvulescua, A., Salaa, F., Boldea, M., 2014. Variation of chlorophyll content in sunflower under the influence of magnetic nanofluids. *In Proceedings of the International Conference of Numerical Analysis and Applied Mathematics (ICNAAM-2014), Rhodes, Greece,* 22–28 September 2014.

Preetha, P.S., Balakrishnan, N., 2017. A Review of Nano Fertilizers and Their Use and Functions in Soil. *Int. J. Curr. Microbio. App. Sci* 6(12): 3117-3133.

Rai, V.K., 2002. Role of amino acids in plant responses to stress. *Biol. Plant,* 45: 471–478.

Saudy, H.S., El-Habbal, M.S., Ashmawy, F., Soliman, E.M., Abbas, Iman, K.h., 2009. Using chlorophyll meter for predicting wheat nitrogen requirements. *Annals Agric. Sci., Moshtohor,* 46 (4): 299-308.

Schr€oder, J.J., Smith, A.L., Cordell, D., Rosemarin, A., 2011. Improved phosphorus use efficiency in agriculture: A key requirement for its sustainable use. *Chemosphere* 84:822–831. Doi: 10.1016/j.chemosphere.2011.01.065.

Seidel, E.P., Egewarth, W.A., Piano, J.T.V., Egwarth, J., 2015. Effect of foliar application rates of calcium and Boron on yield and yield attributes of soybean (*Glycine max*). *Res. Afri. J. of Agri*. 10:170-173.

Shenoy, V.V., Kalagudi, G.M., 2005. Enhancing plant phosphorus use efficiency for sustainable cropping. *Biotechnology Advances* 23:501–513. Doi: 10.1016/j.biotechadv.2005.01.004.

Shi, G., Cai, Q., 2010. Zinc tolerance and accumulation in eight oil crops. *Journal of Plant Nutrition* 33: 982-997.

Sinclair, T.R., Marrou, H., Soltani, A., Valdez, V., 2014. Soybean production in Africa. Glob. *Food Biol*. Doi: 10.1016/j.fgs.20131.

Sobh, M.M., Sharshar, M.S., El-Said, Soad, A., 2000. Response of wheat plants to nitrogen and potassium application in salt affected soil. *J. Product*, 5: 83-97.

Table 1. Soil properties of the Research and Experimental Station at Shalakan.

Soil depth (cm)	Mechanical analysis %			Chemical properties			
	Clay	Silt	Sand	Organic matter %	pH	EC dSm^{-1}	$CaCo_3$ (%)
0–30	13.3	9.2	77.5	0.65	7.92	3.35	2.1
	Available macronutrients (mg kg^{-1} soil)						
	N			P		K	
	24.5			4.9		183	
	Soluble cations and anions (mg 100g $soil^{-1}$)						
	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
	0.0	10.5	14.7	9.4	7.8	5.6	20.9	0.3

Table 2. Effect of hydroxyapatite nanoparticles (HA) on growth parameters, quality traits (protein % and oil %) and elements accumulation (nitrogen, phosphorus and calcium) of soybeans.

Traits / Treatment	Root Fresh Weight (g)	Shoot Fresh Weight (g)	Number of Leaves/plant	% Protein	% Oil	% Nitrogen	Phosphor mg/100g seed	Calcium mg/100g seed
P1	2.55	4.70	4.25	41.05	18.75	6.75	398.50	1.88
P2	5.65	7.08	5.41	41.42	20.47	6.63	484.75	2.79
P3	7.02	8.90	7.06	41.40	20.00	6.63	454.75	2.35
P4	4.49	6.25	5.00	41.47	19.02	6.63	399.50	1.93
LSD	0.37	0.57	0.30	N.S	0.77	N.S	17.22	0.18

Table 3. Effect of urea-hydroxyapatite nanoparticles (HA) on growth parameters, quality traits (protein % and oil %) and elements accumulation (nitrogen, phosphorus and calcium) of soybeans.

Traits / Treatment	Root Fresh Weight (g)	Shoot Fresh Weight (g)	Number of Leaves/plant	% Protein	% Oil	% Nitrogen	Phosphor mg/100g seed	Calcium mg/100g seed
N1	4.56	6.00	5.29	39.42	19.50	6.31	427.50	2.17
N2	5.80	7.33	5.72	43.92	20.60	7.03	456.50	2.49
N3	4.49	6.85	5.54	42.10	19.92	6.73	436.75	2.40
N4	4.86	6.74	5.16	39.90	19.22	6.39	416.75	2.35
LSD	0.37	0.57	0.30	1.77	0.77	0.28	17.22	0.18

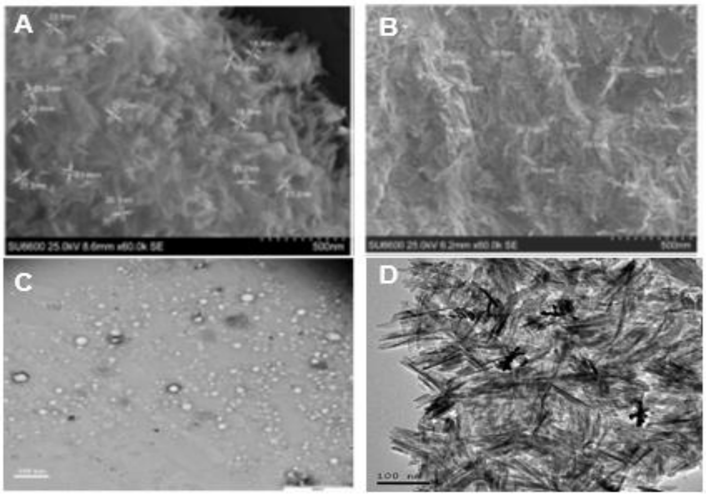

Figure 1. SEM images of synthesized of (A) HANPs and (B) NHANPs (Scale bar 500 nm), TEM images of synthesized of (C) HANPs and (D) NHANPs (Scale bar 100 nm)

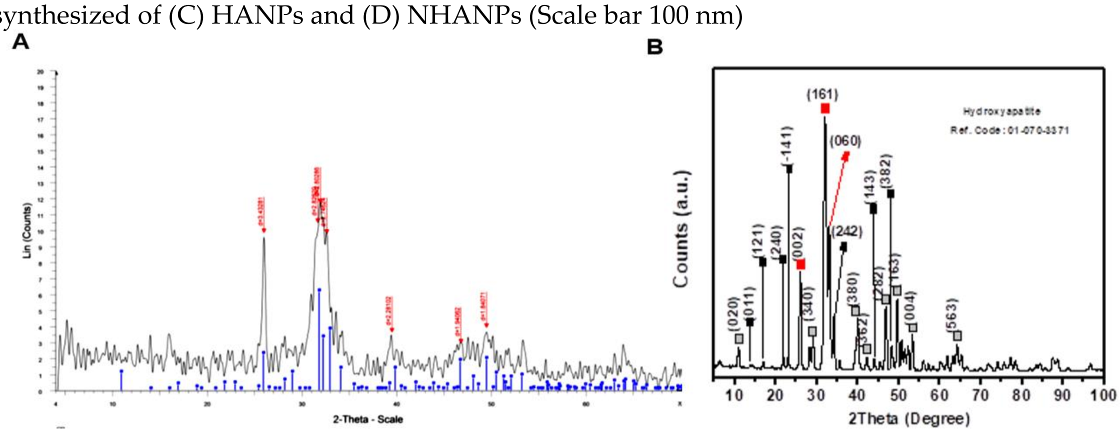

Figure 2. X-ray diffraction patterns of (A) HANPs and (B) NHANPs

Figure 3. Effect of (A) HANPs and (B) NHANPs treatments on growth of soybean plants.

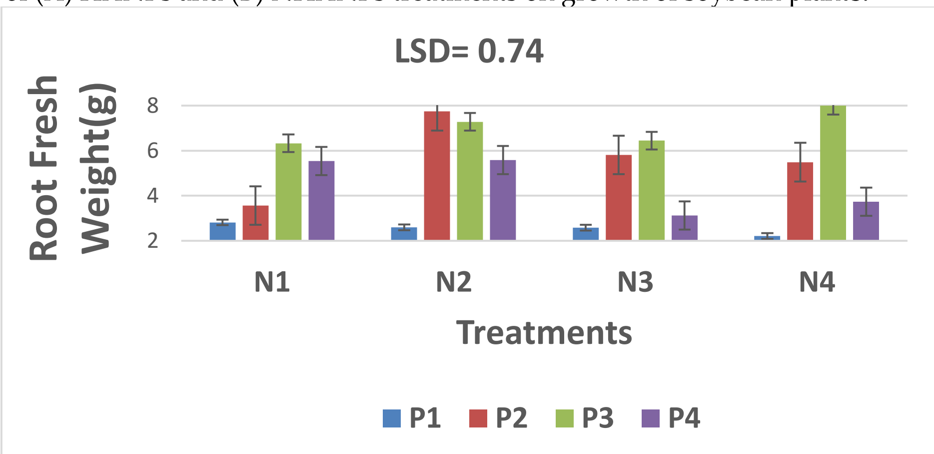

Figure 4. Interaction between HANPs (P) and NHANPs (N) on root fresh weight (g)

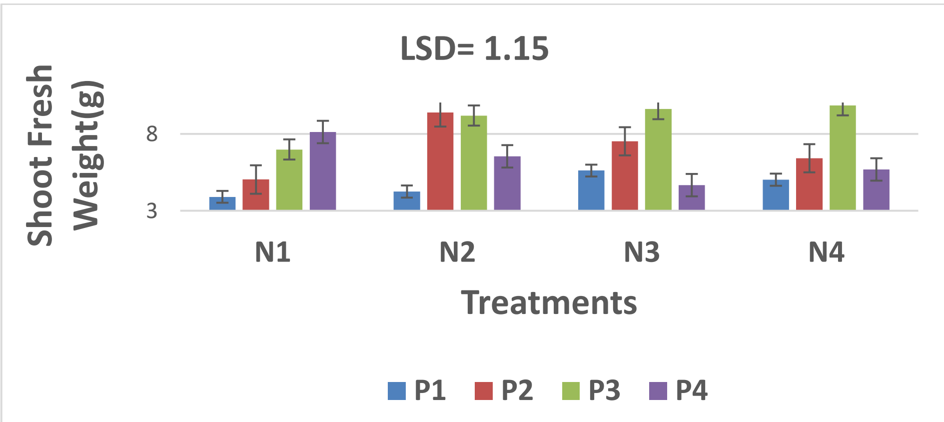

Figure 5. Interaction between HANPs (P) and NHANPs (N) on shoot fresh weight (g)

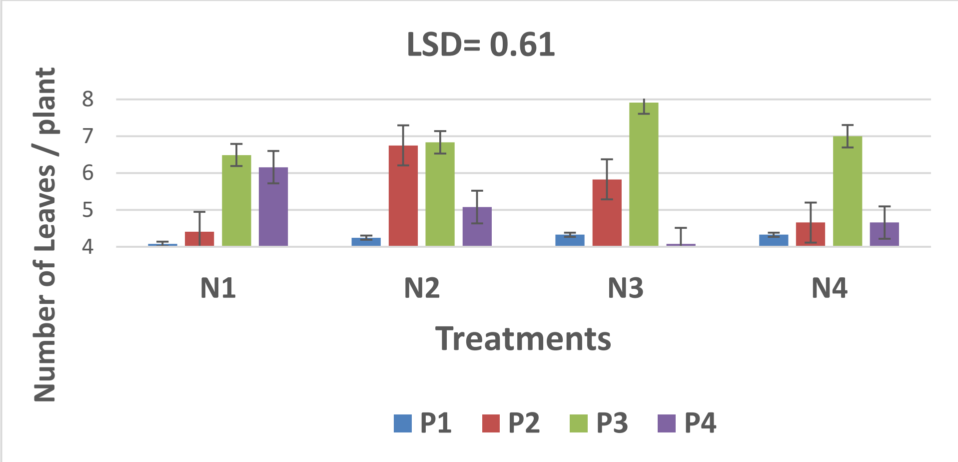

Figure 6. Interaction between HANPs (P) and NHANPs (N) on number of leaves

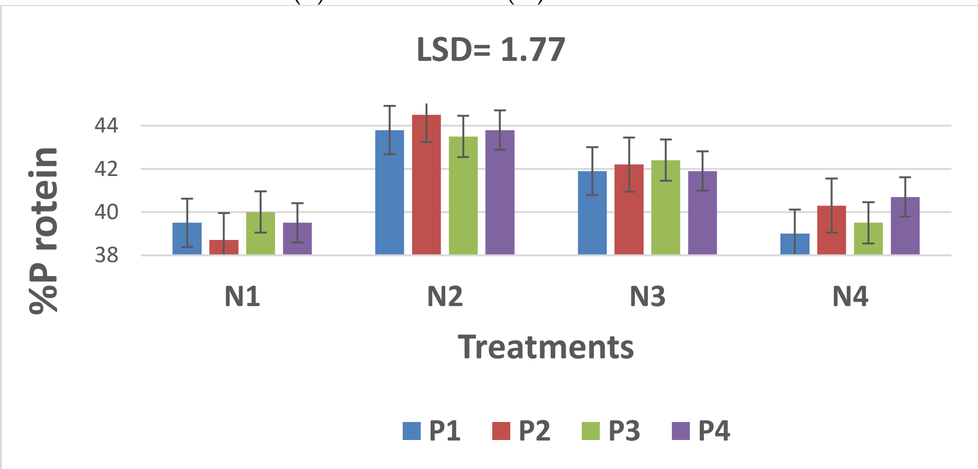

Figure 7. Interaction between HANPs (P) and NHANPs (N) on seed protein percentage

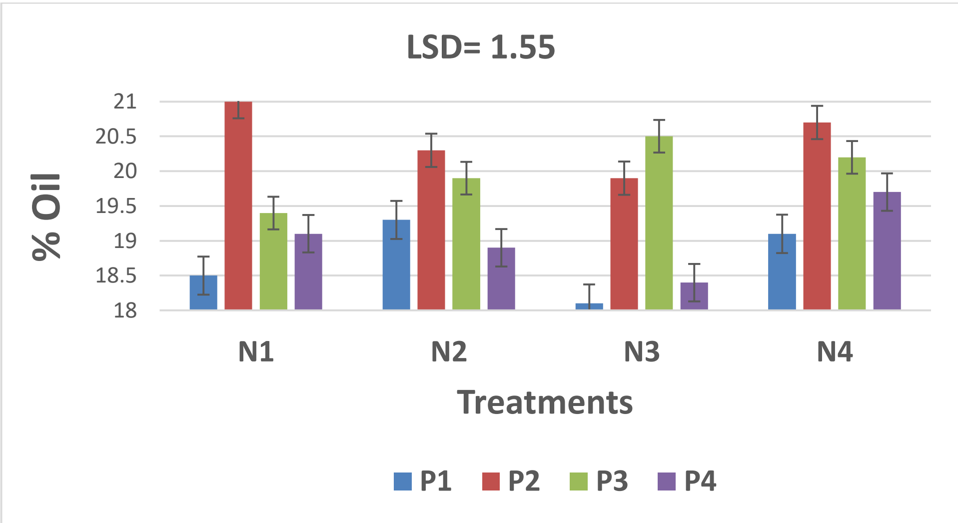

Figure 8. Interaction between HANPs (P) and NHANPs (N) on seed oil percentage

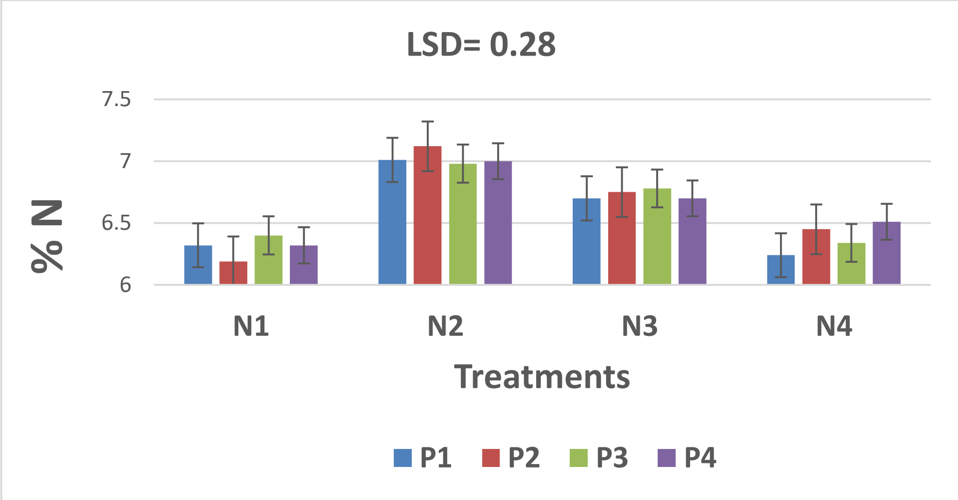

Figure 9. Interaction between HANPs (P) and NHANPs on seed nitrogen accumulation

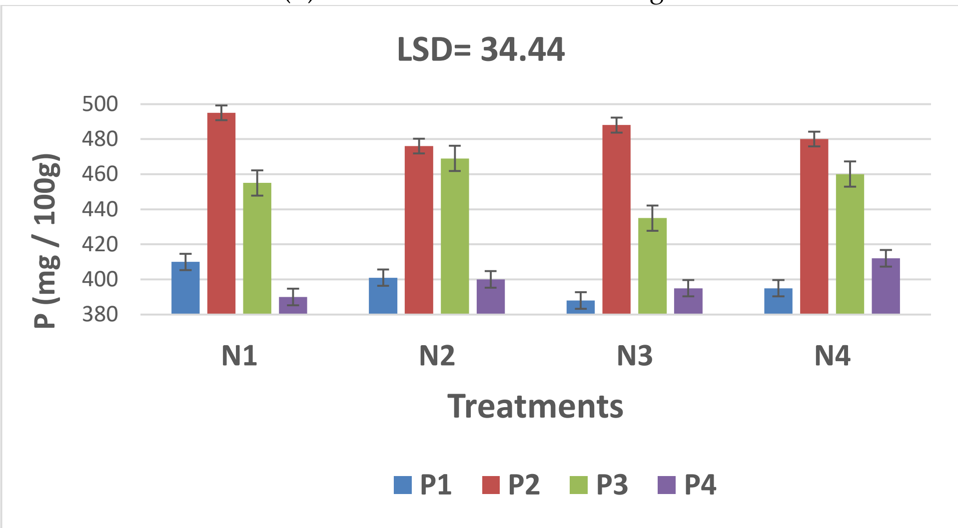

Figure 10: Interaction between HANPs (P) and NHANPs on seed phosphorus accumulation

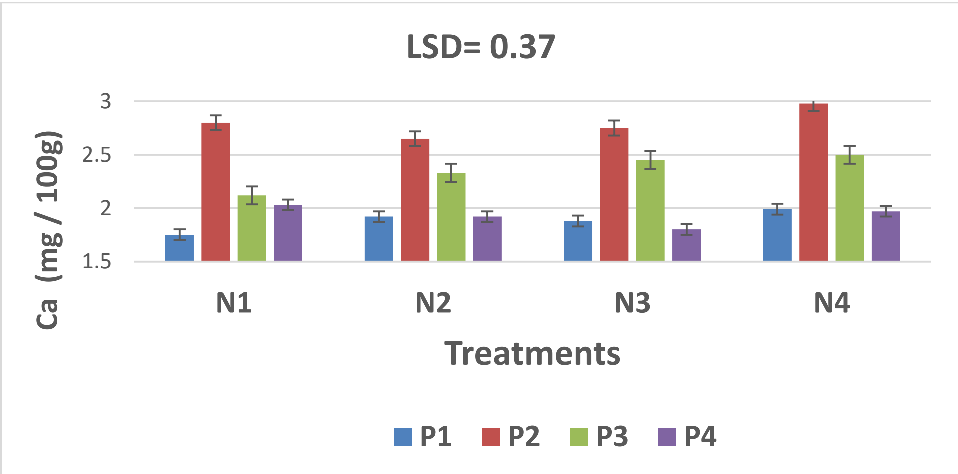

Figure 11: Interaction between HANPs (P) and NHANPs on seed calcium accumulation

دراسة تحسين الخواص المورفولوجية والبيوكيائية لنباتات فول الصويا المعالجة بجسيمات نانوية من الهيدروكسي أباتيت

أحمد فكري زهران [1,*] ,خالد محمد العشري [2] ,أشرف بكري عبد الرازق [3] ,أحمد خطاب [1]

[1]قسم الكمياء الحيويه الزراعيه, كلية الزراعه, جامعة الأزهر, القاهرة, مصر.

[2]قسم علوم الأراضي والمياه ,كلية الزراعه, جامعة الأزهر , القاهرة , مصر.

[3]قسم الوراثة, كلية الزراعة, جامعة عين شمس, القاهره, مصر.

*البريد الإليكتروني للباحث الرئيسي:ahmedzahran201540@gmail.com

الملخص بالعربي

إستخدمت تقنية تفاعل الترسيب الكميائي الرطب لجسيمات الهيدروكسي أباتيت النانويه في إبتكار أنواع جديدة من الأسمدة الحديثة للتربة مثل جسيمات الهيدروكسي أباتيت النانوية وجسيمات الهيدروكسي أباتيت المحملة باليوريا القادرة علي زيادة توافر و إمتصاص المغذيات النباتية مع تقليل فقدها من خلال الرشح أو الجريان السطحي فيما يسمي بالإطلاق البطئ للمغذيات. ولتقيم هذه الأسمدة الجديدة أجريت تجربة أصص في فصل الصيف موسم 2021 علي نمو نباتات فول الصويا . تضمنت التجربة64 معاملة تجمع بين ثلاث عوامل .العامل الأول هوثلاثة تركيزات للتربة من جسيمات الهيدروكسي أباتيت النانوية (12.5/25/50) كجم / فدان. مع إضافة تركيزرابع من فوسفات أحادي الكالسيوم بتركيز 250 كجم / فدان كعنصر تحكم إيجابي. أما العامل الثاني فهو جسيمات الهيدروكسي أباتيت النانوية المحملة باليوريا بثلاثة تركيزات للتربة (12.5/25/50) كجم /فدان مع إضافة تركيزرابع من كبريتات الأمونيوم بتركيز 150كجم/فدان كعنصر تحكم إيجابي. وقد عكست البيانات تحسن ملحوظ في جميع صفات النمو المدروسة وقد تفوقت المعاملة 12.5/25 كجم من جسيمات الهيدروكسي أباتيت النانوية والمعاملة 12.5/25 من جسيمات الهيدروكسي أباتيت المحمله باليوريا علي جميع معاملات التحكم الإيجابي من حيث وزن الجذور الطازج وعدد الأوراق لكل نبات مما يعكس نتائج إيجابية لهذه المادة الكيميائية . في الختام يوصي بالإستخدام المشترك للأسمدة النانوية لزيادة النمو وإنتاجية نبات فول الصويا.

الكلمات الاسترشادية : الترسيب الكميائي,فول الصويا,الأسمدة النانوية,جسيمات الهيدروكسي أباتيت النانوية,جسيمات الهيدروكسي أباتيت المحملة باليوريا النانوية , الإطلاق البطئ.