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Combined Effect of Nanoscale Nutrients (Zinc, Calcium, and Silica) on Growth and Yield of Groundnut (*Arachis hypogaea* L.)

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Abstract: It has been estimated that by 2050, the world's population could exceed 9 billion. Increased population results in hampering food availability. Nanoscale nutrients have been considered new entrants to modern agriculture. Herein, we are the first to report on the effects of combined application(at field scale) of nanoscale nutrients of zinc (N-ZnO), calcium (N-CaO), and silica (N-SiO_2) on the growth and yield of groundnut. The sol-gel method was used to prepare the nanoscale nutrients and was characterized using the techniques such as scanning electron microscopy (SEM), and dynamic light scattering. Significant Pod yield (2934.44Kg/ha) was recorded with the combined application of nanoscale nutrients @ 350 ppm and was 18 % over bulk nutrients (2472.78 kg/ha). Increased pod yield may be ascribed to an increase in SCMR (8.5% higher over bulk nutrients), leaf area, total dry matter besides peg to pod ratio (4.7% over bulk nutrients), and test weight (27% over bulk nutrients). Overall, the better performance of groundnut has been accredited for the combined application of nanoscale nutrients @ 350 ppm. The obtained results point out that the application of nanoscale nutrients in agriculture for enhanced and sustained is promising.

Keywords: nanotechnology; Groundnut; growth; yield.

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1. Introduction

Groundnut (*Arachis hypogea* L.) is one of the important oilseed crops. Worldwide, groundnut is cultivated in 29.82 million hectares with a total production of 50.63 million metric tonnes and average productivity of 1.7 metric t ha^{-1} [1].To meet the growing demand of food for increasing population, production and productivity need to be increased. Despite using different agronomic practices, nutrient management practice is considered one of the major strategies for increasing groundnut productivity. Numerous studies reported the changes in plant morpho-physiological and yield attributes against to availability of nutrients [2].

Although 17 elements are currently known to increase plant growth and yield, the application of calcium, zinc, and silicon are known to be more important for groundnut. Ca plays a crucial role in cell division and regulation of hormonal activity. Groundnut plants need Ca at different phenophases from the first appearance of pegs to the formation of fruit and till the maturation of pods and increases in both yield and quality [3]. Zn is a key structural element in protein synthesis, the precursor of auxin, a growth-promoting hormone, and plays a key role as a cofactor in several physiological processes of plants. Although silicon is abundant, most sources of silicon are insoluble. Plants absorb orthosilicic acid form, i.e., $Si(OH)_4$, an uncharged molecule through roots, by an active and passive mechanism. $Si(OH)_4$ is irreversibly precipitated as amorphous silica (SiO_2–nH_2O; also referred to as ‘opal’, silica gel, or phytoliths in higher plants). Ortho silicic acid (H_4SiO_4) is converted into insoluble SiO_2 through polymerization, mostly deposited beneath the leaf cuticle and in cell walls [4].

Currently, nanoscale nutrients comprising beneficial elements for plant nutrition have been fascinating in modern agriculture. Owing to large surface area to volume ratio, high reactivity, tunable pore size and particle morphology, the diversified nanomaterials enhance the uptake through leaves and other plant parts besides minimizing loss by runoff, evaporation, hydrolysis, etc. The technology is also suggested to enhance the absorptivity of nutrients (micro & macro) of plants.

Nano Calcium application increased plant growth and yield in Peanut [5], Rice [6], Snapbean [7], Sweet pepper [8], etc. Furthermore, Nano Zinc application also increased yield in different crops, viz., groundnut [9], Sunflower [10], Flax [11], Mustard [12], etc. Numerous studies reported foliar application of nano-silica decreased disease incidence and enhanced resistance to pests and pathogens in a wide range of monocot and dicot plants [13,14].

However, available reports on the combined application of multiple nano nutrient elements are scanty. Therefore, the present study was carried out on a field scale to understand the combined effect of Nanoscale nutrients (Zinc, Calcium, and Silica) on the growth and yield of Groundnut (*Arachis hypogaea* L).

2. Materials and Methods

2.1. Preparation of nanoscale nutrients.

N-CaO was prepared by the method of Naseeruddin *et al*. [15]. N-CaO particles were prepared using the sol-gel method, where calcium nitrate (1%) was mixed with sodium citrate tri-basic dehydrate (0.05%) and stirred for 3 hours at 600^{o}C. Then, the solution was filtered using Whatman filter paper and dried at 1000^{o}C for 6 hours. The collected powder was used for further characterization.

ZnO was synthesized by the Sol-gel method [15,16]—modified oxalate decomposition technique used N-ZnO particles preparation. Zinc oxalate was prepared by mixing 0.2 M of zinc acetate and 0.2 M of oxalic acid. The resulted precipitate was collected and rinsed with double deionized water and air-dried. The oxalate was then grounded and decomposed in the air by placing it in a preheated furnace at 500^{o}C for 45 minutes.

Nanoscale Silica was prepared by the method described by the Stober method [17]. Briefly, Tetraethyl orthosilicate (TEOS) and CH_3COOH (acetic acid-glacial) were mixed using a magnetic stirrer for 30 min and rinsed with double-deionized water (DI-water), and allowed to dry in air a room temperature. Then the mixture was made into a fine powder and decomposed in the air by keeping it in a preheated muffle furnace for 90 min at 600 °C.

2.2. Characterization of nanoscale nutrients.

The hydrodynamic diameter (size) and zeta potential of the nanoparticles were measured by using the dynamic light scattering technique (Nanopartica, HORIBA, SZ-100). The surface morphology and shape of the nanocrystals were characterized by scanning electron microscopy (using Hitachi SEM (TM 3000)).

2.3. Field experiment.

2.3.1. Experimental details.

Ten treatments of three replications were laid out in randomized block design during the year 2020 at the Regional agricultural research station, Tirupati. Groundnut variety Dharani is used in the experimental study. The soil composition of the experimental study area was found to be Sandy loams with a pH range of 6.9, organic carbon 0.19%, EC: 0.12 (dS m^{-1}), Available N: 226 kg/ha, Available P_2O_5: 25.7 kg/ha, Available K_2O: 270 kg/ha.

The treatment details are T1: 50 ppm (N-CaO + N-ZnO + N-SiO_2), T2: 100 ppm (N-CaO + N-ZnO + N-SiO_2) T3: 200 ppm (N-CaO + N-ZnO + N-SiO_2), T4: 400 ppm (N-CaO + N-ZnO + N-SiO_2), T5: 800 ppm (N-CaO + N-ZnO + N-SiO_2), T6: 1000 ppm (N-CaO + N-ZnO + N-SiO_2), T7: 1500ppm (N-CaO + N-ZnO + N-SiO_2), T8: 2000 ppm (N-CaO + N-ZnO + N-SiO_2), T9: Bulk ($ZnSO_4$ @2000 ppm + $Ca(NO_3)_2$, @1000 ppm + TEOS @ 1000 ppm), T10 : Control.

The treatments, i.e., a combination of 3 nano oxides at various concentrations, were imposed at 30 DAS and 50 DAS as a foliar spray. The recommended dose of fertilizers (RDF) is 20 N: 40 P_2O_5: 50 K_2O. All treatments were given the recommended dose of fertilizers. Proper agronomic and plant protection management was done to all the treated plants for their maximum growth expression.

2.4. Data Collection and statistical analysis.

2.4.1. Growth attributes.

2.4.1.1. Plant height (cm).

The height of the groundnut plant was measured and expressed in cm from the base of the plant to the tip of the terminal bud at harvest from five plants randomly in each net plot area.

2.4.1.2. Number of branches (No.).

Five Groundnut plants were taken, the number of branches per plant was counted, and the average was expressed in number.

2.4.1.3. Leaf area (cm^2 $plant^{-1}$).

Leaf area was calculated for the three selected plants by destructive analysis at 60 DAS. It was measured using Leaf Area Meter (Li-COR model LI 3000), and the average was expressed as leaf area per plant in cm^2.

2.4.1.4. SPAD chlorophyll meter reading (SCMR).

The SPAD chlorophyll meter reading (SCMR) was measured with SPAD chlorophyll meter at 60 DAS (three groundnut plants).

2.4.1.5. Total dry matter (g plant^{-1}).

Five groundnut plants were collected and were sun-dried initially and later dried in a hot air oven at 60°C to a constant weight and expressed as g plant^{-1}.

2.4.1.6. Nodules (No.).

Five plants were collected at harvest, and a number of nodules per plant were counted and the average was expressed in number.

2.4.2. Yield and yield attributes.

2.4.2.1 Flower to Peg ratio.

The total number of flowers that converted into pegs was counted and expressed as a flower to peg percentage [18].

2.4.2.2. Peg to Pod ratio.

The percentage of the number of pegs that transformed into filled pods was counted and expressed as a peg to pod percentage [18].

2.4.2.3. Number of filled Pods.

For the five plants at harvest, the number of filled was counted, and the average value was expressed as several filled pods plant^{-1}.

2.4.2.4. Test weight.

A hundred kernels were collected from a composite sample of kernel yield from each net plot area, weighed, and expressed in gm.

2.4.2.5. Pod yield (kg ha^{-1}).

Groundnut pods were taken from haulms, sun-dried until they reached a consistent weight, and then expressed in kilograms per hectare.

2.4.2.6. Haulm Yield (kg ha^{-1}).

After removing the pods from the haulms, the plants were sun-dried thoroughly until a constant weight was achieved, and haulm output was recorded (kg ha^{-1}).

2.4.3. Statistical analysis.

The treatments were maintained in 3 replications each, and all the data was analyzed using OPSTAT. Data was represented in Mean ± Standard deviation followed by one-way ANOVA using Duncan's multiple range test (DMRT) using the latest version of SPSS.

3. Results and Discussion

3.1. Dynamic light scattering experiments.

The sizes of synthesized nanoparticles (Fig. 1) are perfectly within the nanoscale range, i.e., N-CaO (78.4 nm), N-ZnO (54.6 nm), and N-SiO_2 (69.8 nm). The zeta potentials (Figures 1b, 1d, 1f respectively) of the 3 nanoscale nutrients N-CaO (66.2 mV), N-ZnO (32.5 mV), and N-SiO_2 (-31.4 mV) infer the strong dispersion stability of the nanoparticles.

However, N-CaO performed better in zeta potential value, reflecting excellent stability.

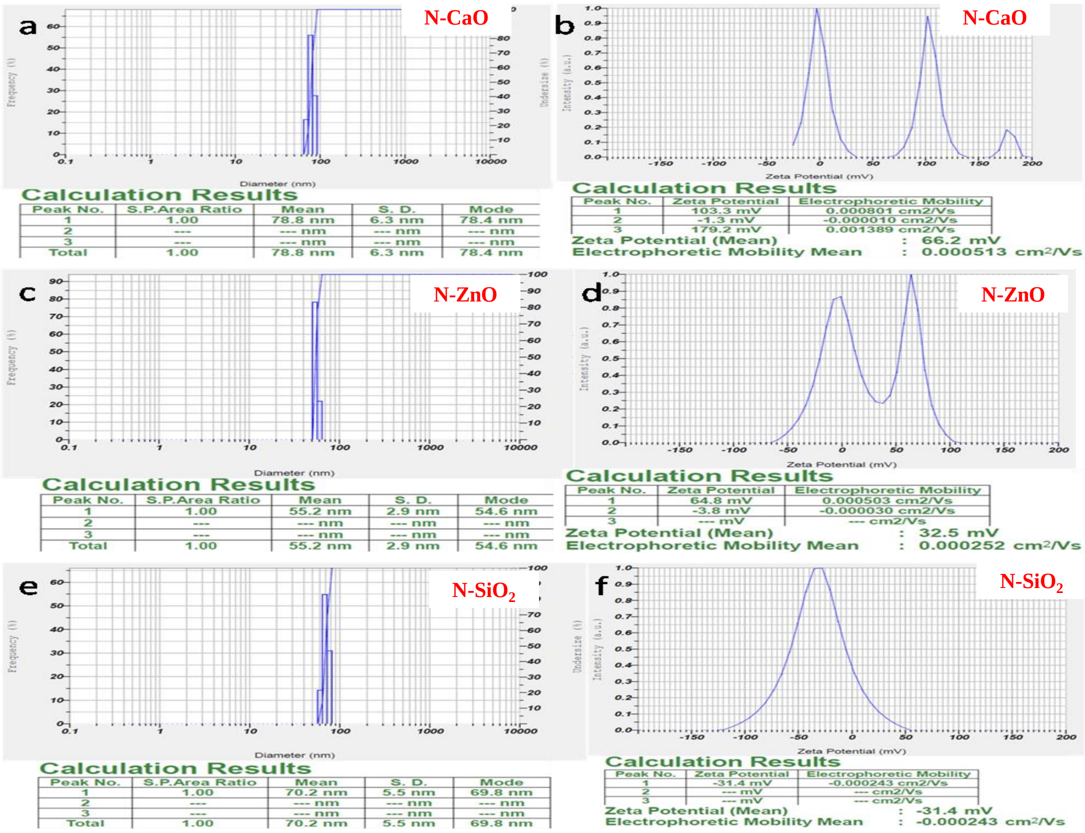

Figure 1. Microgram representing particle size distribution pattern of Nanoparticles: a-Size of N-CaO; the b-Zeta potential of N-CaO; c-Size of N-ZnO; the d-Zeta potential of N-ZnO; e-Size of N- SiO_2; f- Zeta potential of SiO_2.

3.2. Scanning electron microscopy.

The morphological features of CaO nanoparticles predict the agglomeration of the particles with uniformity and reveal their porosity. The particles showed different shapes with irregular surface lattice (Fig. 2). The morphology of SiO_2 particles is crystalline with porous nature and is within the nanoscale dimensions. Agglomeration has been visible as a consequence of the interaction (electrostatic/van der Waals) between particles of smaller size [19,20].

Scanning electron microscopic studies (SEM) of N-ZnO revealed the agglomeration of spherical particles with nano size and uniform distribution. The larger size of the particles was probably due to van der Waals clusters of smaller entities and magnetic interactions among the particles. The results are in good concurrence with Sohail *et al*. [21].

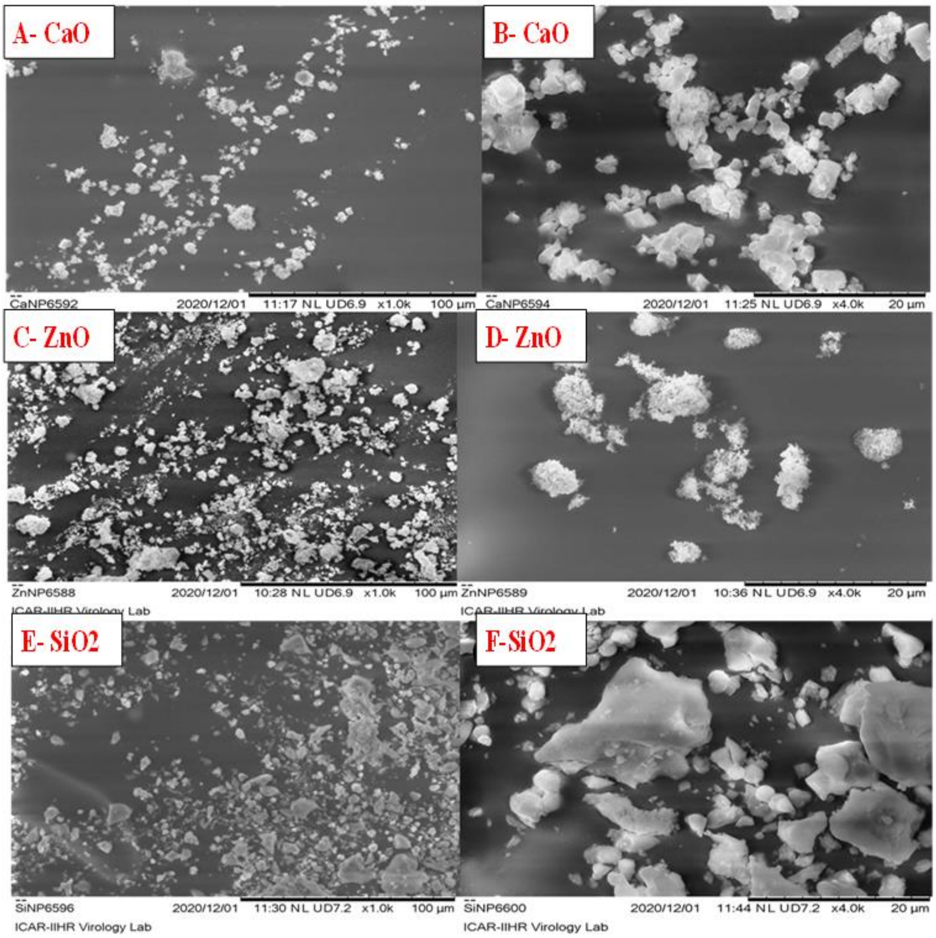

Figure 2. SEM micrograph of Nanoparticles: A, B -Surface morphology of N-CaO in x1.0 k and x4.0k resolution, respectively; C,D- surface morphology of N-ZnO in x 1.0 k and x 4.0k resolutions, respectively; E, F- surface morphology of N-SiO_2 in x1.0 k and x4.0k resolutions respectively.

3.3. Growth attributes.

The combined application of N-CaO, N-ZnO, and N-SiO_2 on groundnut plants (Fig. 3) showed an increase in growth among tested concentrations compared with the bulk and control plants in whatever concentration was used. The enhanced growth of plants could be ascribed to the capability of nanoscale particles (CaO, ZnO, and SiO_2) entry through the stomata due to their small particle sizes [22].

Figure 3. Effect of combined nanoscale nutrients (N-CaO + N-ZnO + N-SiO_2) on Groundnut plants.

These nanoparticles may be transported through the symplastic pathway [23] or apoplastic pathway to the metabolic sites [24]. As Calcium, Zinc is an essential macro and micronutrient and could have acted as a starter fertilizer for plants' metabolic activities. On the other hand, Silicon (Si) application helped in enhancing plant growth besides abiotic stress tolerance [25].

Plant height increased on treating with various nanoscale nutrient concentrations (N-CaO + N-ZnO + N-SiO_2) (Fig. 4A), but a more obvious increase was observed at 400 ppm (43.33 cm) over bulk (4.50 cm) and control (28.70 cm). This could be ascribed particularly to the application of nanoscale Zn. Zn is a precursor of tryptophan amino acid [26] and auxin, a growth-promoting hormone. Auxin is responsible for cell division and leaf expansion [26]. Nano CaO impacts plant development by modulating auxin accumulation, transport, and signaling and the expression of many auxin-related genes [27-31]. Thus, helping in the maintenance and development of meristematic tissues. Foliar application of nano-silica insoluble SiO_2 through polymerization mostly deposits beneath the leaf cuticle and in cell walls [4]. Similarly, Ohet *et al.* [32] reported activation of cell division and cell elongation in crops could be major metabolic events that induced faster plant growth. Further, reports are available on the increased plant height, biochemical, and yield in the absence and presence of compost, along with the spraying of nano ZnO [11].

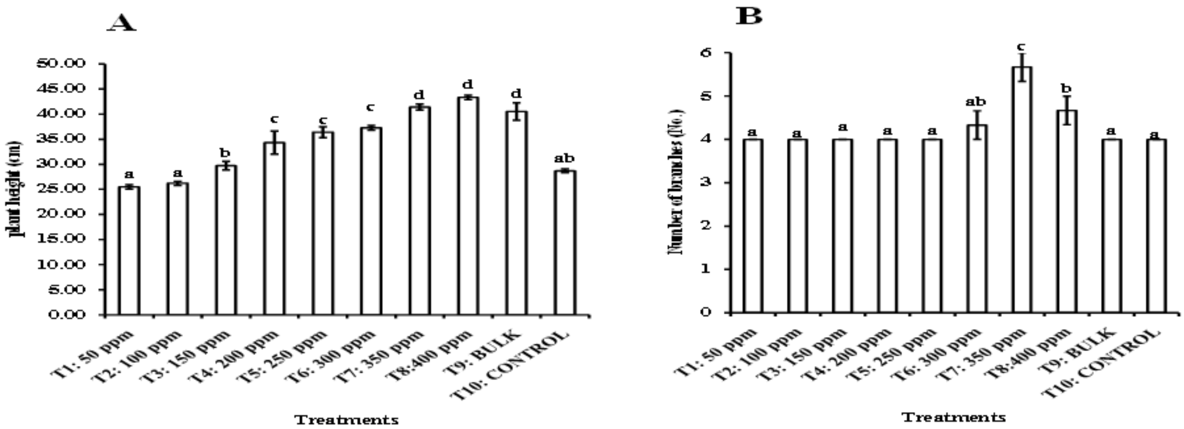

Figure 4. Effect of combined nanoscale nutrients (N-CaO + N-ZnO + N-SiO_2) on (A) plant Height (cm) and (B) Number of Branches.

The number of branches (Fig. 4B) was significantly higher at 350 ppm (5.67) over bulk (4.0) and control (4.0). Leaf area (Fig. 5A) and SCMR content (Fig.5B) recorded significantly higher at nanoscale nutrient concentrations (N-CaO + N-ZnO + N-SiO_2) @ 350 ppm (1443.42 cm^2/plant, 49.12) over bulk (1331.35 cm^2/plant, 45.06) and control (1058.86 cm^2/plant, 40.95). An increase in leaf area could be assigned to auxin, which is responsible for leaf expansion [27]. ZnO NPs foliar spray improved chlorophyll contents in wheat [30]. Calcium is often involved in the processes of increasing the antioxidant enzymatic activity and stabilization of the cell membrane structures and lowering the hydrogen peroxide generation and the, lipid peroxidation [31] and cell wall [27]. Toki and Halloul [32] also reported that Zinc nanoparticles significantly increased leaf area and chlorophyll content in wheat over bulk Zn.

Alongside the increase in leaf area, SCMR, and the number of branches, nanoscale nutrient concentrations (N-CaO + N-ZnO + N-SiO_2) were significantly higher than total dry matter (Fig. 6A) was, recorded at 350 ppm (26.5 g/plant) over bulk (21.6 g/plant) and control (19.92 g/plant). Higher plant height and leaf area in nanoscale-treated plants resulted in increased dry matter production. N-CaP significantly increased the shoot and dry root weights, the nutrient content in the shoot and root, and yield and nutrient concentration in Snap bean [7]. A number of nodules (Fig.6B) were significantly higher at 350 ppm (42) over bulk (36) and control (35). Calcium-treated Soybeans increased the dry weight of stems, leaves, roots, and grain yield [37]. Calcite nanoparticles significantly increased sweet pepper growth compared to calcite-based foliar fertilizer [8].

Application of SiO_2 through external foliar treatments has increased the resistance to pests and diseases of plant species [13]. In this respect, Ca and Zinc have been used as a source of fertilizer. In comparison, Si accumulation below the cuticle acts as a mechanical barrier to entry of pests and diseases.

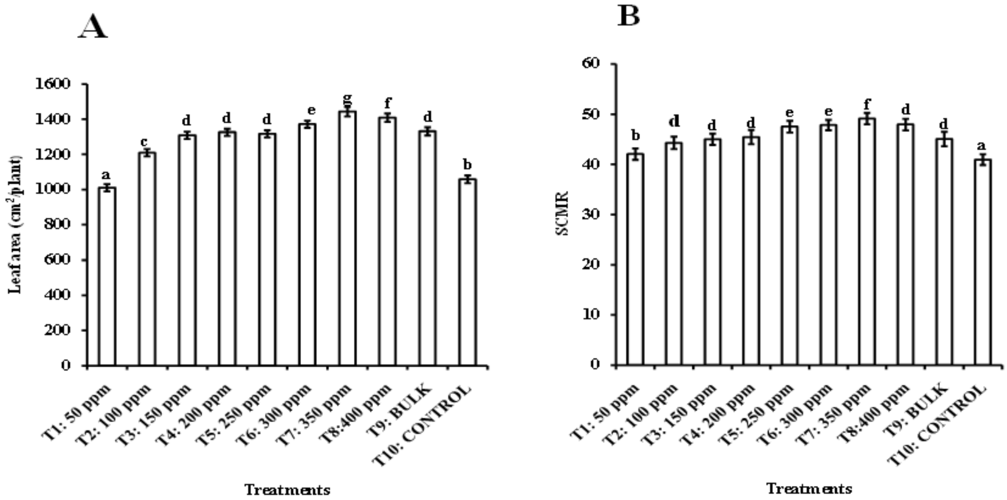

Figure 5. Effect of combined nanoscale nutrients (N-CaO + N-ZnO + N-SiO_2) on (A) Leaf Area (cm^2/plant) and (B) SCMR.

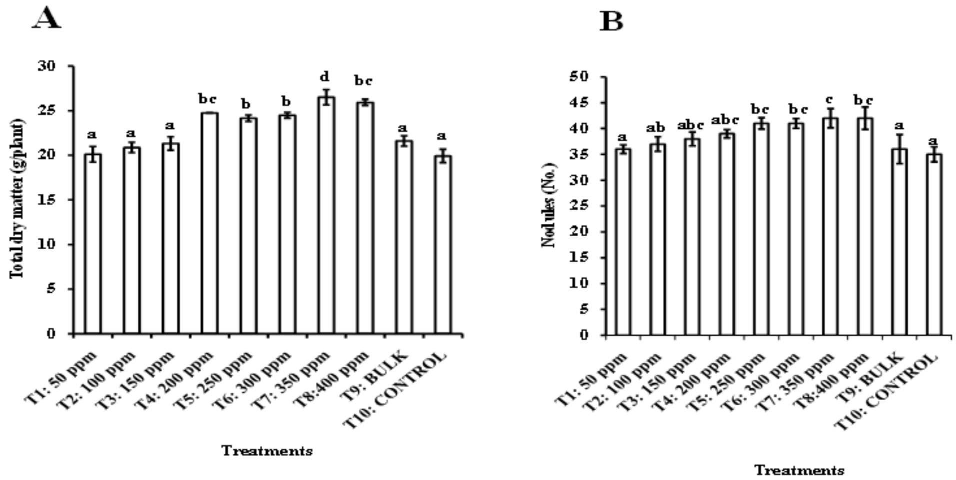

Figure 6. Effect of combined nanoscale nutrients (N-CaO + N-ZnO + N-SiO_2) on (A) total dry matter (g/plant) and (B) Number of nodules.

3.4. Yield and yield attributes.

The data on yield and yield attributes were given in Table1; recorded significant differences in groundnut on treating with combined nano nutrients (N-CaO + N-ZnO + N-SiO_2) among tested nanoparticle concentrations compared with the bulk and control plants. Combined nanoscale nutrients application at 350 ppm resulted in increased flower to peg ratio (50.11), peg to pod ratio (54.51), number of filled (13.5), and over bulk (44.82, 52.04, 12.37) and control (40.19, 39.27, 9.67) plants respectively. Calcium deficiency is the main cause of decreased peg to pod ratio in control over treated plants (bulk and nanoscale).

Significant unfilled pods were recorded in control (4.47) compared to bulk (2.47) and 350 ppm (0.33) nano treated (N-CaO + N-ZnO + N-SiO_2) plants. Calcium is very important for peanut embryo and pod development [34]. Groundnut needs 90 % Ca^{2+} during the pod formation stage. Lack of Calcium leads to embryo abortion, unfilled pod formation, and reduced peg to pod ratio.

Test weight recorded significantly higher at nanoscale nutrient concentrations (N-CaO + N-ZnO + N-SiO_2) @ 350 ppm (38.94 g) over bulk (30.66) and control (30.5). Ca helps in peanut cell integrity, and zinc has involved in the synthesis of auxin biosynthesis. Auxin is necessary for grain size and starch accumulation. Nano calcium application enables the transmission of nutrients from the aerial peg soil to the shell, with little influence on kernels.

Combined spraying nanoparticles (N-CaO + N-ZnO + N-SiO_2) increased pod yield whatever concentration was used, but more obviously at 350 ppm (2934.44 Kg/ha), which was significantly higher over bulk (2472.78 kg/ha) and control (1800.00 kg/ha). Haulm yield increased significantly at 350 ppm (4887.00 kg/ha) over bulk (4654.67 kg/ha) and control (4032.00 kg/ha). The biological yield was increased due to increased leaf area and more plant height. An increase in pod yield can be ascribed to increased growth attributes viz., leaf area and total dry matter, yield attributes viz., peg to pod ratio, the number of filled pods, and test weight. Similar results were reported by Prasad *et al*. [9] when ZnO NPs were applied to peanut crops. The application of zinc nanoparticles helps in hormonal synthesis, cell elongation, and plant growth [35-37]. Application of N-SiO_2 acts as a viable option to reduce biotic and abiotic stress in the crop growing period and helps increase plants' growth and yield [38-41].

However, applying calcium has its own limitations as it exhibits antagonistic properties against potassium and which reflects in the lowering of potassium levels and thereby affecting the yield and quality of the agricultural crops. In this study, an increase in combined nanoscale concentrations up to 350 ppm showed a positive increase in the growth and yield of peanuts. Gradual increase in a concentration above 350 ppm, there was a decrease in growth and yield.

Table 1. Effect of combined nanoscale nutrients (N-CaO + N-ZnO + N-SiO_2) on yield and yield attributes of groundnut.

Treatments	Filled pods (No.)	Unfilled Pods (No.)	Flower To peg (%)	Peg to Pod ratio (%)	Test Weight (g)	Pod yield (Kg/ha)	Haulm yield (Kg/ha)
T1: 50 ppm	8	4	42.40	44.67	24.95	1954.00	4374.00
T2: 100 ppm	9	3	41.28	48.82	30.43	2144.44	4573.33
T3: 150 ppm	9	3	48.00	43.72	31.69	2263.33	4564.33
T4: 200 ppm	11	3	50.11	48.46	31.78	2375.61	4616.67
T5: 250 ppm	9	2	42.15	49.78	31.98	2646.67	4673.00
T6: 300 ppm	11	2	47.37	49.47	34.89	2833.33	4751.67
T7: 350 ppm	14	1	50.11	54.51	38.94	2934.44	4887.00
T8:400 ppm	12	1	46.30	52.04	37.38	2826.67	4835.33
T9: Bulk	10	2	44.82	39.27	30.66	2472.78	4654.67
T10:Control	8	4	40.19	31.81	30.50	1800.00	4032.00
SEM	0.17	0.57	2.09	2.07	1.16	9.58	1.87
CD (0.05)	0.49	1.71	6.26	6.19	2.45	29.64	3.63

4. Conclusions

Foliar application (@ 30 & 50 DAS) of combined nanoscale nutrients @ 350 ppm (N-CaO + N-ZnO + N-SiO_2) proved to improve the growth and yield of groundnut. Physiological attributes like SCMR, leaf area, total dry matter, and yield attributes like a flower to peg ratio, peg to pod ratio, a number of filled pods, and test weight was also recorded to be significantly higher at 350 ppm. Future studies are needed to grow various varieties and crops at the field level under varying agro-climatic conditions to evaluate the efficacy and cost-effectiveness of foliar application of combined nanoscale nutrients.

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Conflicts of Interest

The authors declare no conflict of interest.

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