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 Sulphate-supplemented NPK nanofertilizer and its effect on maize growth

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Abstract

Sulphate-supplemented nitrogen, phosphorus and potassium (NPKS) fertilizer was nanoformulated through ionic gelation of chitosan (CS) and tripolyphosphate (TPP) at pH value of 5.5 to afford a series of nanofertilizers. The CS-TPP nanoparticles and CS-TPP-NPKS nanofertilizers were engineered using different dose of CS (0.125, 0.25, 0.5 and 1%) and NPKS fertilizer (20, 40 and 60 ppm). These nanoparticles were characterized through particle size distribution, zeta potential, SEM, XRD, FTIR, EDX and ICP-MS. The results revealed CS-TPP-NPKS particles with size range of 220–530 nm, polydispersity index (PDI) value between 0.2–0.5 and positive zeta potential. A preliminary evaluation of these nanoparticles along with inorganic NPK and NPKS fertilizers were conducted under greenhouse condition using maize (Zea mays L.) as a test crop. Findings from this study showed that inorganic NPKS (20:7:3:0.5) fertilizer, CS-TPP nanoparticles, and formulated NPKS nanofertilizers gave higher maize plants growth increases than NPK fertilizer and control treatments. The tallest plant (37.73 cm) and highest number of leaves (9) were recorded with the CS-TPP-NPKS nanofertilizer treatment obtained using 1% CS/NPKS (40 ppm). On the other hand, application of the nanoformulated derivative with 0.25% CS/NPKS (20 ppm) produced maize plants with superior chlorophyll content with 12.71 chlorophyll index value. A highly varied magnitude of the coefficient of variation in nutrient contents and uptake was recorded among the treatments. These results revealed that the incorporation of sulphur nutrient into NPK fertilizer and its transformation to nanoparticles have the potential characteristics for effective and productive growth of maize and sustainable agricultural activities.

1. Introduction

The United Nations projected that the global population is rapidly increasing every year, and by 2050 this should be around 9.2 billion [1]. However, report from Food and Agriculture Organization [2] indicates serious global concern regarding future capacity to produce sufficient agricultural products to sustain livelihoods and satisfy the needs of the ever growing number. This can be achieved by increasing agricultural productivity and maintaining good soil quality and land [3]. To this end, the use agricultural inputs such as inorganic nitrogen, phosphorus and potassium (NPK) fertilizers has been reported to increase crop productivity by approximately 60% [4]. However, the major concern with the use of inorganic fertilizers is that they have been reported to promote environmental problems such as soil salinity, degradation and water pollution [5–7]. Global warming, on the other hand, has been anticipated to impact on global food production owing to the frequent excessive heat and water scarcity [8]. The latter is known to decrease the photosynthetic carbon assimilation in plant through inhibition of the Calvin cycle enzymes activity [9]. Furthermore, sunlight irradiation of plants with
The low molecular weight chitosan polymer oxygen species, responsible for the oxidative stress in plants [10]. Remarkably, regulation of the level of reactive oxygen species can be effected via antioxidant mechanism.

Maize is one of the most essential cereal crops for food security worldwide and has been reported to exhibit an increased sulphur dependency [11]. Sulphur assimilation in plants occurs in the form of sulphate ions (SO4^2-) that are very less available in the soil. Sulphur deficiency in soil has regularly been articulated in the agricultural sector and this was found to be responsible for the decreased protein biosynthesis and chlorophyll content, thereby limiting the crop production [12, 13]. In that regard, it is obvious that sulphate-integrated NPK fertilizer can serve as a valuable strategy to improve maize nutrition for increase growth and productivity. Basically, adequate sulphur concentration for ideal development of plants has been reported to vary between 0.1 and 0.5% of dry weight [14]. However, owing to the water-solubility nature of sulphate, there is a possibility for leaching losses to take place. Nanotechnology has attracted much scientific and industrial interest as one of the key enabling approach that has a potential to revolutionize agronomic practices by improving fertilizer use and nutrients absorption in plants [15]. Consequently, this technology has also been found to alleviate agricultural issues such as eutrophication [16]. Nanofertilizers are characterized by a large surface area, an improved infiltration capability and a slow release of encapsulated nutrients [17, 18]. Nontoxic chitosan is one of the most prominent and biocompatible polymers that has been used as a carrier in various applications for the delivery of active ingredients due to its excellent film-forming ability and cost-effectiveness [4]. For example, Heba et al reported the use of nanosize chitosan-NPK fertilizer for the wheat plants enhanced growth [19]. Likewise, Ha and co-workers described the controlled slow discharge of NPK from loaded chitosan nanoparticles for the increased development of coffee plants [20]. It is worth noting that the application of chitosan biopolymer in maize cultivation has also been found to induce water deficit tolerance and stimulate the antioxidant activity [21]. The current study aimed to formulate the pre-fabricated sulphate-containing NPK fertilizer (NPKS) into chitosan nanoparticles and investigate the effect of the NPKS-loaded nanofertilizer on maize growth and nutrients uptake under greenhouse.

2. Materials and methods

2.1. Chitosan nanoparticles and nano NPKS fertilizer preparation

The low molecular weight chitosan polymer (50 kDa) with deacetylation degree of 95% and tripolyphosphate (TPP) as the cross-linker were obtained from Sigma Aldrich, Johannesbug, South Africa. The inorganic NPK 20:7:3 fertilizer and maize used in this study were purchased from a local Hinterland agricultural inputs marketing store. The magnesium sulphate MgSO4 used as a source of sulphur was purchased from Merck, Johannesbug, South Africa. All experiments for the preparation of chitosan nanoparticles and NPKS-loaded nanofertilizers were carried out using deionized water.

A series of chitosan nanoparticles (CS-TPP NPs) were fabricated utilizing an adapted ionic gelation procedure described by Gan et al [22]. Initially, the chitosan solutions containing 0.125%, 0.25%, 0.5% and 1% were prepared by dissolving chitosan powder 0.125, 0.25, 0.5 and 1 g, respectively in acetic acid 0.35% 100 ml and set aside under stirring (900 rpm) overnight at room temperature. The pH of the solutions was adjusted to 5.5 with 0.5 M NaOH solution. Subsequently, an aqueous solution of TPP 0.25% (w/v) was prepared and added dropwise to the chitosan solutions under stirring for 60 min at room temperature at the chitosan/TPP volume ratio of 30 ml/30 ml. Next, the resultant solutions were dried at 50 °C for 24 h. The occurrence of CS-TPP NPs was evidenced with the transformation to an opalescent solution. This takes place through electrostatic inter and intra-molecular linkages between the negatively charged phosphate group of TPP and the positively charged amino group of CS. On the other hand, the NPKS fertilizer was prepared by incorporating through crushing 0.5% of sulfate salt as the source sulphur into the acquired NPK 20:7:3 fertilizer. The latter represents one of the commercially available inorganic NPK fertilizers grade recommended for maize production in South Africa. The NPKS-loaded nanofertilizers (CS-TPP-NPKS NPs) were formulated by adding 30 ml solution of NPKS fertilizer into 30 ml of CS-TPP emulsion under magnetic stirring for 4 h at room temperature. The following doses of NPKS were adopted: (i) 20 ppm; (ii) 40 ppm and (iii) 60 ppm.

2.2. Morphological, structural characterization and slow-release behavior of nano NPKS fertilizer

The size and surface charge of the nanoformulations were determined by dynamic light scattering using a Malvern Zetasizer Nano-series (Malvern Instruments, United Kingdom). The formulations were filtered using a PTFE 0.2 μL pore size membrane to remove possible dust particles before analysis. For the crystallinity phase of the inorganic NPKS fertilizer and nanofertilized counterpart, X-ray diffraction analysis was performed using the Shimadzu 6000 diffractometer with Cu-Kα radiation source at 0.154 nm wavelength and variable slits of 40 kV/30 mA over 2θ values ranging from 2 to 800. The scanning speed was maintained at 50 min⁻¹. FTIR
spectra were obtained using a Perkin Elmer spectrophotometer (USA) in the range of 400 to 4000 cm\(^{-1}\) at 4 cm\(^{-1}\). The morphological characteristics were determined using an Auriga Cobra focused-ion beam microscope (JEOL-JSM 7500 F, USA). The sulphur loading percentage in CS-TPP-NPKS NPs was determined using an Agilent 7500 CE inductively coupled plasma-mass spectrometer (ICP-MS) fitted with CRC (Collision Reaction Cell) technology for interference removal. After digestion in HNO\(_3\), the samples introduction was accomplished through a Micromist-type nebulizer and standard quarts spray chamber. The instrument was optimized using a solution containing Li, Y, Ce and Tl (1 ppb) for normal low-oxide/low interference levels (≤1.5%) while upholding high sensitivity across the mass range. The following instrumental parameters were set: Forward power 1550 W, plasma gas flow rate 15 l min\(^{-1}\), nebulizer gas flow 1.2 l min\(^{-1}\), sampling depth 8 mm and spray chamber temperature 2 °C. The instrument was calibrated using ULTRASPEC\(^{®}\) certified custom mixed multi-element stock standard for quantitative results.

To ascertain the NPKS encapsulation efficiency (EE) and loading efficiency (LE) of the prepared nanofertilizer, the colloidal dispersion obtained from the stirring mixture of NPKS fertilizer (40 ppm) and CS-TPP emulsion (1%CS) was centrifuged at 6 000 rpm for 30 min. The supernatant was kept aside and the residue was further washed and filtered. The filtrate was then added to the supernatant solution. The uncoated mass of each nutrient (N, P, K, or S) was determined from the concentration of nutrient, obtained by ICP-MS analysis, and the total volume of liquid. Furthermore, the yielded nano NPKS fertilizer was freeze-dried to determine its total weight. The EE and LE values were calculated using the following equations:

\[
LE(\%) = \frac{W_0}{W} \times 100 \quad EE(\%) = \frac{W_0}{W_1} \times 100
\]

Where \(W_0\) is the weight of NPKS envelopment in the chitosan nanoparticles, \(W\) is the weight of chitosan nanoparticles and \(W_1\) is the amount of NPKS added to the nanoparticle suspension.

The slow-release behavior of nano NPKS fertilizer was tested in aqueous media at pH 7.8. The N, P, K, and S release was measured at room temperature by ICP-MS. A 100 mg portion of dried CS-TPP NPs loaded with NPKS fertilizer was immersed in 20 ml of distilled water. At definite time intervals (12, 24, 48, 72, 96, 120, 144, and 168 h), 2 ml release medium was collected and analyzed.

2.3. Soil sample analysis
Approximately 150 g of soil sample prior to trial initiation and scooped sample from each pot upon termination of the trial were air-dried, sieved and analyzed for pH, resistance, and organic C, total N, P, K, and SO\(_4\)-S contents. The pH of the soil was measured in a soil-water suspension (1:2.5) using a pH meter [23]. Total N content in the soil samples was determined calorimetrically following sulfuric acid mixed with salicylate and copper and potassium digestion salts (CuSO\(_4\), K\(_2\)SO\(_4\)) and selenium (SeO\(_2\)) as catalysts using Kjeldahl procedure [24]. The soil extractable P was determined calorimetrically using Bray P1 procedure as described by Bray and Kurtz procedure [25]. The organic C was analyzed using dichromate oxidation and titration with ammonium ferrous sulfate [26]. Extractable K was evaluated using 1 M ammonium acetate, and the concentration was quantified by atomic absorption spectrophotometry. The total sulphur content in soil samples measured as S-SO\(_4\) was determined with a 1:2.5 acidic ammonium acetate extracting solution using the turbidimetric method [27] and the concentration quantified using atomic absorption spectrophotometry at 420 nm. The hydrometer method was employed to determine the particle size distribution after which the soil textural class was established using the textural triangle. The results of pre-planting soil physical and chemical properties (table 1) revealed loam textural classification with low extractable phosphorus and S-SO\(_4\) concentration, which were below the threshold level of 8–15 mg kg\(^{-1}\) and 9 mg kg\(^{-1}\), respectively for maize production [28, 29].

2.4. Greenhouse experiment
The experiment was performed in the greenhouse at the North-West University experimental farm, Molelwane (25° 47' 404’S, 025° 37’ 292’E, 1298.08 m above sea level). The study consisted of NPK, NPKS, CS-TPP NPs, CS-TPP-NPKS NPs as treatments and a control where nothing was added. Each of the 0.25% and 1% CS treatments except control had varying concentrations of 20, 40 and 60 ppm of the nutrient constituents. These were placed in black polythene bags containing 5.5 kg soil collected from an uncultivated portion on the field at the farm. Prior to weighing of air-dried soil sample into the planting pots, roots, debris, stones and other unwanted materials were removed by sieving. The soil-filled pots with proper treatment labelling in three replicates were arranged in a completely randomized design, irrigated with 500 ml tap water and left overnight for equilibration. Four maize seeds were sown in each pot at 5 cm depth but thinned down to three plants per pot at 2-weeks after seedling emergence with the plants watered twice a week using 200 ml tap water. Application of 1.0 g of the treatments was done three weeks after the trial setup. The maize plant growth was assessed at 2-weekly intervals by measuring the plant height and stem diameter using a tape and Vernier calliper. Counting
of the number of leaves per plant and chlorophyll content measurement using chlorophyll meter CCM–200 plus model were also done. Statistix program (Version 10.0.0.9) was used to perform analysis of variance (ANOVA) on the experimental data.

Maize plants were harvested at 10 weeks after planting (WAP) for the above ground biomass from the surface using a sharp scissor and put into brown paper bags. Harvested samples were then dried in an oven at 70 °C for 72 h to constant weight and recorded as dry matter yield (g pot⁻¹). Dried maize shoot sample from each treatment pot was separately milled and kept for laboratory determinations. Two gram milled plant samples were digested using a mixture of 15% H₂SO₄ and sodium sulphate salt (96% Na₂SO₄) with selenium oxides (SeO₂) as a catalyst. Total N content in 2 g tri-acid wet digested samples on heated Kjedahl block at 500 °C was determined following reaction with salicylate and sodium hypochlorite solution with nitroprusside added as a catalyst [30]. Total N concentration in digest was read on an automated segmented flow analyzer (Seal AA3) at 660 nm after blue colour development following sample reaction with salicylate and sodium hypochlorite solution. Phosphorus and potassium determination in samples were measured according to Obalebo et al method [31]. Phosphorus concentration in plant digest was measured calorimetrically by spectrophotometry at 442 nm while potassium concentration was read on atomic absorption spectrophotometer at a wavelength of 766.5 nm. Total sulphur content in the plant was determined on milled plant samples using a LecoTrumac CNS analyzer. Nutrient uptake was estimated using the equation below.

\[
\text{Nutrient uptake (mg pot}^{-1}) = \% \text{Nutrient content} \times \text{dry matter yield (g pot}^{-1})
\]

### 3. Results and discussion

#### 3.1. Characterization of the prepared Chitosan nanoparticles and nano NPKS fertilizer

Table 2 showed the zeta potential, nanosize and polydispersity index (PDI) characteristics of the CS-TPP NPs at four different CS powder concentrations. The lower particles size and PDI values were obtained with 1% and 0.25% CS solutions; hence these were chosen as the optimized concentrations for further experimental characterization. The average particle size distribution of the CS-TPP NPs was 145.4 nm (PDI of 0.17) using 0.25% CS solution and 450.5 nm (PDI of 0.24) using 1% CS solution (figures 1(a) and (b)). Moreover, the respective surface charge was determined to be 14.7 mV and 19.9 mV as shown in figures 1(a’) and (b’). A lesser concentration of CS powder in solution led to a reduced magnitude of the zeta potential owing to relatively higher solution electrolyte concentration and suppressed Stern layer [32]. This outcome compares with the finding by Huang et al regarding the characterization of cross-linked low molecular weight CS, obtained in hydrogen peroxide [33].

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**Table 1. Physical and chemical properties of the soil used in the greenhouse study.**

| Soil properties | Value |
|----------------|-------|
| Clay           | 18%   |
| Silt           | 32%   |
| Sand           | 50%   |
| Textural class | Loam  |
| pH (H₂O)       | 7.81  |
| Resistance (ohms) | 690 |
| Organic C      | 1.08% |
| Total N        | 782 mg kg⁻¹ |
| Bray 1-P       | 2 mg kg⁻¹ |
| K              | 278 mg kg⁻¹ |
| SO₄-S          | 4 mg kg⁻¹ |

**Table 2. CS-TPP NPs at different concentrations of CS.**

| CS powder | Zeta potential (mV) | Size (nm) | PDI  |
|-----------|---------------------|-----------|------|
| 1%        | 19.9                | 450.5     | 0.24 |
| 0.5%      | 15.8                | 800.6     | 0.76 |
| 0.25%     | 14.7                | 145.4     | 0.17 |
| 0.125%    | 6.3                 | 716       | 0.66 |
The zeta potential, particle size distribution and PDI of the CS-TPP-NPKS NPs using different concentrations of NPKS fertilizer (20, 40 and 60 ppm) are outlined in Table 3. Generally, the zeta potential values of the nanofertilizers were found to be lower relative to that of the corresponding CS-TPP NPs. This indicates that the entrapped NPKS fertilizer suppresses the surface charge of the positively charged CS-TPP carrier. CS is presumably adopting a diffuse array resulting from the electrostatic repulsion of the $–\text{NH}_2$ groups \[34\]. The size distribution and PDI values of the nanofertilizers varied from 225 nm (PDI of 0.41) in 0.25% CS/NPKS (60 ppm) to 529 nm (PDI of 0.31) in 1% CS/NPKS (60 ppm). An increment in CS% resulted in inflated particles. Similarly, the use of 1% CS solution and upper concentration of NPKS fertilizer led to enlarged nanoparticles 255–529 nm. However, at 0.25% CS the upsurge in NPKS concentration had no significant effect on the CS-TPP-NPKS NPs size. It has been reported that suitable nanofertilizer size for application in agricultural was around 100–500 nm \[35\]. This suggests that the nanofertilizer CS-TPP-NPKS NPs prepared in this study have the prerequisite feature for agronomic application.

SEM pictures of the CS-TPP NPs and CS-TPP-NPKS NPs revealed discrete spherical-like particles as shown in figures 2(a) and (b). The observed slight aggregation is seemingly ascribed to positive zeta potential and high specific surface energy \[36\]. The EDX analysis of SEM image of CS-TPP-NPKS NPs prepared using 1% CS/NPKS (40 ppm) revealed the representative elements at energy values of 0.27 (C; 39.41 Weight%), 2.01 (P; 2.09 Weight%), 3.31 (K; 0.24 Weight%) and 2.31 KeV (S; 0.30 Weight%). Peak corresponding to nitrogen element was not spotted and this could be attributed to absorption at the beryllium window X-ray detector and the presence of carbon with large mass absorption coefficient \[37\]. ICP-MS analysis of this sample revealed a

**Table 3.** Characteristics of nano NPKS fertilizers (size, PDI and zeta potential) using various concentrations.

| CS-TPP-NPKS NPs at different conditions | Size (nm) | PDI  | ZP (mV) |
|-----------------------------------------|----------|------|---------|
| 0.25% CS/NPKS (20 ppm)                 | 246.0    | 0.37 | 7.02    |
| 1% CS/NPKS (20 ppm)                    | 255.9    | 0.22 | 5.19    |
| 0.25% CS/NPKS (40 ppm)                 | 241.0    | 0.39 | 9.42    |
| 1% CS/NPKS (40 ppm)                    | 326.0    | 0.32 | 18.8    |
| 0.25% CS/NPKS (60 ppm)                 | 225.0    | 0.41 | 14.9    |
| 1% CS/NPKS (60 ppm)                    | 529.8    | 0.31 | 18.0    |

**Figure 1.** Size distribution of the CS-TPP NPs using (a) 0.25% CS and (b) 1% CS solutions. Zeta potential of the CS-TPP NPs using (a’) 0.25% CS and (b’) 1% CS solutions.
sulphur weight% loading of 0.32. In addition, the N concentration was determined using ICP-MS, and the N weight% was recorded to be 4.59. Both nitrogen and sulphur application influence the cereal protein and amino acid composition. The recorded weight% of sulphur in the as-characterized CS-TPP-NPKS NPs was in the range of the optimum amounts of sulphur for maize development. Auxiliary analysis of the SEM image of CS-TPP-NPKS NPs fabricated with 1% CS/NPKS (60 ppm) exhibited the following content C (45.21 Weight%), P (3.28 Weight%), K (0.52 Weight%) and S (0.52 Weight%). The N, P, K, and S nutrient encapsulation efficiency, obtained through calculation using 1% CS/NPKS (40 ppm) nanoparticles, was found to be 96.4, 95.7, 99.0, and 99.7%, respectively. The calculated total contents of N, P, K, and S nutrients in this formulated nanofertilizer were also assessed for the loading capacity. These were found in the amount of 0.36% N, 0.35% P, 0.30% K, and 0.37% S.

Figure 2(c) displays the XRD patterns of NPKS fertilizer and NPKS-loaded chitosan nanofertilizers. Though the former exhibited the typical diffractogram of a substance with totalcrystallinity (figure 2(c) inset), the latter revealed low intensity and broader peak at around $2\theta = 25^\circ$. In analogy with literature precedent on the development of nanoformulated zinc loaded fertilizer [36], this indicates an important interaction between NPKS fertilizer and the CS NPs in CS-TPP-NPKS NPs nanofertilizer, leading to the desired amorphous nature. Hence, the solubility of the prepared nanofertilizers was improved. It should be noted that the diffraction pattern of nanochitosan particles reveals a broad peak at $2\theta = 28^\circ$ [38].

Figure 2(d) displays the FTIR spectra of CS-TPP NPs and NPKS-loaded CS-TPP nanofertilizer. The FTIR spectrum of CS-TPP NPs exhibits the distinctive bands at 3244, 2870, 1612, 1540, 1055, 879 and 740–580 cm$^{-1}$, assigned to the O–H, asymmetrical C–H, amide C=O, amino N–H, phosphate P–O and O–P–O stretching vibrations, respectively [39, 40]. CS-TPP NPs loading with NPKS fertilizer led to relatively intense bands corresponding to O–H and N–H bending modes complemented with broadening of the band attributed to C–H stretching. This observation confirms a physical interaction between the NPKS nutrients and the cross-linked chitosan biopolymer. The occurrence of a band at 1398 cm$^{-1}$ supports and electrostatic interaction between the carboxylate groups (O=C–O$^-$) of CS-TPP NPs and the potassium cations of the incorporated NPKS fertilizer [40]. Furthermore, The absorption bands corresponding to phosphate vibrations were more intense in FTIR spectrum of NPKS-loaded CS-TPP nanofertilizer, comparative to those in spectrum of bare CS-TPP NPs. This characteristic reveals loading of other source of phosphorus nutrient. Similar observation could be evidenced with band ascribed to N–H stretch absorption at 1540 cm$^{-1}$. On the other hand, the band at 1251 cm$^{-1}$ was
attributed to the plane bending vibration of S–O–H, indication of the presence of sulphate nutrient within the CS-TPP-NPKS NPs nanofertilizers.

One of the most important features of the prepared sulphate-supplemented NPK nanofertilizer was its controlled-release potential. Figure 3 presented the in-vitro release profiles of N, P, K, and S from CS-TPP nanoparticles using the 1% CS/NPKS (40 ppm) nanofertilizer as model. Relative to NPKS fertilizers exhibiting 89.4, 91.7, 91.3, and 93.5% release of N, P, K, and S in 12 h, a slow and gradual behavior was experienced with the nanofertilizer. It could be observed that the 1% CS/NPKS (40 ppm) nanoparticles released only 6.3% N, 11.6% P, 15.1% K, and 3.4% S after 24 h. Moreover, the sum of nutrients release was found to be less than 25% after 48 h and not above 60% after a week.

3.2. Greenhouse analysis after treatment

3.2.1. Characterization of postharvest soil samples

The chemical characteristics of the soil after the greenhouse study are shown in table 4. The total N content ranged between 702 mg kg\(^{-1}\) for the pot treated using 1% CS/NPKS at 20 ppm concentration and 802 mg kg\(^{-1}\) for the pot treated with 0.25% CS/NPKS (60 ppm) nanofertilizer. The lowest P content of 3 mg kg\(^{-1}\) was recorded in pot treated with 0.25% CS/NPKS at 40 ppm concentration. However, pots that received 0.25% CS/NPKS (40 ppm), 1% CS/NPKS (40 ppm) and 0.25% CS/NPKS (60 ppm) nanofertilizers contained extractable P that were greater than the threshold level of 8 mg kg\(^{-1}\). The low values of extractable P content obtained in soil may contribute in the displayed reddish-purple colour of the leaves of the grown maize plants in the affected pot. The obtained soil sulphur content after crop harvest was above the threshold level of 9 mg kg\(^{-1}\) and the highest sulphur content of 30 mg kg\(^{-1}\) was obtained from the 1% CS/NPKS pot with 20 ppm nanofertilizer. The implication of these results is that application of the 0.25% CS/NPKS nanofertilizer with either 20 or 60 ppm concentration maintained adequate soil phosphorus and sulphur levels after crop harvest while the application of 1% CS/NPKS (40 ppm) nanofertilizer maintained both phosphorus and sulphur threshold level after crop harvest.

3.2.2. Effects of different treatments on maize growth and dry matter yield

At the onset of our study, the effect of the CS biopolymer was assessed via the treatment with procured NPK fertilizer, sulphate-supplemented NPK fertilizer and the cross-linked CS-TPP NPs obtained with different dose
of CS powder (figure 4). The results revealed a noteworthy effect of the sulphate-containing fertilizer NPKS (19 cm) and the polymeric CS-TPP NPs in enhancing the maize growth. An increase in the concentration of CS from 0.25 to 1% in nanoengineered CS-TPP NPs resulted in superior plant height (21 cm). This improvement has previously been argued to occur through boosted absorption of water by osmotic adjustment and enhanced antioxidative enzymes activities [41]. In this light, the 1% CS-TPP NPs was employed as standard control for the subsequent analyzes. The use of fabricated nano NPKS fertilizers with various nutrients concentrations had significant impacts ($P \leq 0.05$) on maize plant height (figure 5(A)), mean number of functional leaves per plant (figure 5(B)), stem diameter (figure 5(C)), chlorophyll content (figure 5(D)) and mean dry matter yield (table 5), comparative to the control. The plant height increased throughout the greenhouse trial period with nano NPKS fertilizer treatments. The highest plant height (37.73 cm) was recorded at 10 WAP from treatment using 1% CS-TPP-NPKS (40 ppm) NPs nanofertilizer (figure 5(A)). At constant level of CS (0.25%) and increased concentration of NPKS from 20 to 40 ppm for the formulation of CS-TPP-NPKS nanofertilizer, an increased plant height was witnessed at 8 WAP (figures 5(A) and (b)). However, further increase in NPKS amount beyond 40 ppm did not lead to a significant change in plant height (figures 5(A)(a) and (c)). Comparable trend was observed with CS-TPP-NPKS nanofertilizer fabricated using 1% CS/NPKS (20–60 ppm). Earlier studies have underscored the importance of maize plant height and stem diameter not only on yield but as key attributes for the improvement of cereal crops under drought stress [42].

The number of leaves per plant during the sampling period increased at 4, 6, 8 and 10 WAP (figure 5(B)). At 6 WAP the maximum number of leaves 7 was observed with CS-TPP-NPKS nanofertilized using 0.25% CS/NPKS (20 ppm) (figure 5(B)(a)) and 1% CS/NPKS (40 ppm) (figure 5(B)(b)). More importantly, at 10 WAP the latter afforded the maize plants with the highest number of leaves 9. These leaves exhibited reddish-purple (anthocyanin) pigments, probably as a result of cooler night temperatures and shading in chlorophyll content [43]. Nonetheless, the anthocyanin pigments have been found to act as protecting agent in plants by hosting antioxidant enzymes.

Assessment of the effect of formulated CS-TPP-NPKS NPs on the stem diameter of maize plant exposed a similar progression between the control treatment and treatment using 1% CS/NPKS (20, 40 and 60 ppm) (figures 5(C)(a)–(c)). Though the use of 0.25% CS/NPKS (20 ppm) produced maize plants with the thickest stem diameter 12.33 mm at 10 WAP, the use of the derivatives with increased amount of NPKS (40 ppm and 60 ppm) led to the maize plants with thinner stem diameter (figures 5(C)(b) and (c)), comparative to the control treatment and the treatment with 1% CS/NPKS (20, 40 and 60 ppm).

The chlorophyll content from the control, 0.25% CS/NPKS (20 ppm) (figure 5(D)(a)) and 0.25% CS/NPKS (60 ppm) (figure 5(D)(c)) treated pots increased throughout the sampling period. Chlorophyll is the plant pigment that gives an indication about the photosynthetic regime and its activity is influenced by the availability of the nutrients. The highest recorded leaf chlorophyll content (12.17 CCI) for the maize plants was at 10 WAP from 0.25% CS/NPKS (20 ppm) treatment (figure 5(D)(a)) followed by 11.10 CCI, value obtained at 6 WAP for the 0.25% CS/NPKS (40 ppm) and 1% CS/NPKS (40 ppm) treatments (figure 5(D)(b)). Therefore, at constant NPKS level of 40 ppm, a change in chitosan polymer amount had comparable effect on the leaf chlorophyll content possibly suggesting a balanced N and S nutrients content at the 40 ppm dose of NPKS in CS-TPP-NPKS nanofertilizer. These have been reported to demonstrate synergistic effects for better translocation of

![Figure 4. Effect of CS-TPP NPs application on maize growth 4 weeks after planting.](image-url)
photosynthates in maize plants growth \cite{12,44}. The sudden decrease in chlorophyll content experienced with the 0.25\% CS/NPKS (40 ppm) (figure 5(D)(b)), 1\% CS/NPKS (40 ppm) and 1\% CS/NPKS (60 ppm) (figure 5(D)(c)) at 8 and 10 WAP results from increased formation of the anthocyanin pigments, as established above. Likewise, CS-TPP-NPKS NPs formulation had a significant effect ($P \leq 0.05$) on leaf area as depicted in figure 5(E). Application of 1\% CS/NPKS (40 ppm) fertilizer afforded the highest leaf area of 429.6, 488.9 and 568.6 cm$^2$ after 4, 6 and 8 WAP, respectively. Relative to treatment with 1\% CS/NPKS (20 ppm), an increased NPKS dose to 40\% led to improved leaf area (figures 5(E)(a) and (b)). This is presumably attributed to increased S content which plays key role in carbohydrate and protein metabolism by activating several enzymes engaged in photosynthesis \cite{45}. An increased leaf area entails superior light interception. At 10 WAP, a decrease in leaf area for all treatments was observed and this was due to lesser number of leaves per plant.

Figure 5. (A) Plant height; (B) number of leaves; (C) stem diameter; (D) chlorophyll content; (E) leaf area using CS-TPP-NPKS nanofertilizers fabricated using (a) NPKS (20 ppm), (b) NPKS (40 ppm) and (c) NPKS (60 ppm) with a significant difference ($P \leq 0.05$).
Table 5. Effect of nano NPKS fertilizers at different concentrations on dry matter yield.

| Treatment                  | Dry matter yield (g pot⁻¹) |
|----------------------------|-----------------------------|
| Control                    | 10.67 Mb                     |
| 0.25% CS/NPKS (20 ppm)     | 10.37 ac                    |
| 1% CS/NPKS (20 ppm)        | 10.24 ac                    |
| 0.25% CS/NPKS (40 ppm)     | 9.69 d                      |
| 1% CS/NPKS (40 ppm)        | 10.31 ac                    |
| 0.25% CS/NPKS (60 ppm)     | 9.90 dc                     |
| 1% CS/NPKS (60 ppm)        | 10.06 bc                    |

Means with the same letter(s) (a/b/c) in the same column are not significantly different (P ≤ 0.05) according to Duncan’s Multiple Range Test.

Table 6. Maize shoot nutrients content and uptake as affected by various mineral and nano treatments.

| Treatments            | Nutrient content (%) | Nutrient uptake (mg g⁻¹) |
|-----------------------|----------------------|--------------------------|
|                       | N    | P    | K    | S    | N    | P    | K    | S    |
| Unamended soil        | 1.06 | 0.07 | 2.83 | 0.07 | 10.92| 0.72 | 29.15| 0.72 |
| 0.25% CS/NPKS (20 ppm)| 1.17 | 0.10 | 3.22 | 0.07 | 12.13| 1.04 | 33.39| 0.73 |
| 1% CS/NPKS (20 ppm)   | 1.25 | 0.09 | 2.97 | nd   | 12.80| 0.92 | 30.41| nd   |
| 0.25% CS/NPKS (40 ppm)| 1.40 | 0.09 | 3.89 | nd   | 13.57| 0.87 | 37.69| nd   |
| 1% CS/NPKS (40 ppm)   | 1.32 | 0.11 | 3.14 | 0.07 | 13.71| 1.14 | 32.62| 0.72 |
| 0.25% CS/NPKS (60 ppm)| 1.57 | 0.09 | 3.83 | 0.11 | 15.54| 0.89 | 37.92| 1.09 |
| 1% CS/NPKS (60 ppm)   | 1.31 | 0.09 | 3.44 | 0.09 | 13.18| 0.91 | 34.61| 0.91 |
| CS-TPP NPs            | 1.67 | 0.10 | 3.70 | 0.13 | 17.82| 1.07 | 39.48| 1.39 |
| NPK                   | 1.86 | 0.15 | 3.54 | 0.11 | 20.81| 1.68 | 39.61| 1.23 |
| NPKS                  | 1.70 | 0.11 | 2.96 | 0.09 | 21.51| 1.39 | 37.44| 1.14 |
| Mean                  | 1.43 | 0.10 | 3.35 | 0.09 | 15.20| 1.06 | 35.23| 0.99 |
| Coefficient of variation (%) | 18.0 | 21.1 | 11.4 | 24.3 | 24.1 | 26.7 | 10.6 | 26.1 |

Table 5 presents the mean dry matter yield of the maize plants obtained using different soil treatment after 10 WAP under greenhouse conditions. Though the control experiment exhibited the highest dry matter yield 10.67 g pot⁻¹, all the treatments revealed no substantial difference on yield. Nevertheless, the least mean dry matter yield of 9.69 g pot⁻¹ was obtained from the 0.25% CS/NPKS (40 ppm) treated pot. These data indicate that treatment with chitosan-containing nanofertilizers increases the total carbohydrate content that represents the main constituent of plants dry matter.

3.2.3. Postharvest plant tissue samples
The maize shoot nutrients content and uptake as influenced by various mineral fertilizer, and nanofertilizer treatments are shown in table 6. Total dry mass recorded from the treatment with NPK was 11.90 g while N concentration was 1.86%. Thus the computed total N uptake was 20.81 mg g⁻¹. The NPKS treatment, on the other hand, exhibited marginally higher N uptake value of 21.51 mg g⁻¹. The incorporation of sulphur into inorganic NPK had a beneficial effect on N uptake of the maize plant signifying the importance of sulphur in the nutrition of maize plants [42]. Comparing the inorganic fertilizer to the nano NPKS fertilizers, the CS-TPP NPs treatment (control for the nano NPKS fertilizer) produced maize plants with the highest N uptake of 17.82 mg g⁻¹ followed by 15.54 mg g⁻¹, value determined from the treatment with 0.25% CS/NPKS (60 ppm) derivative. For the S uptake, the CS-TPP NPs treatment gave the highest uptake of 1.39 mg g⁻¹, which was greater than that of NPK and NPKS treatments. The treatment with 1% CS/NPKS (40 ppm) nanofertilizer afforded the least S uptake (0.72 mg g⁻¹) and upper amount of P uptake. This observation suggests an adversarial effect of the 1% CS/NPKS (40 ppm) nanofertilizer on P and S nutrients in maize growth under the greenhouse conditions. The least P and K uptakes measured were 0.72 mg g⁻¹ and 29.15 mg g⁻¹, respectively both from the unamended soil. Quantifying nutrients uptake is essential criteria for optimizing fertilizer use and scheduling. Hence, the treatments with CS-TPP NPs and NPKS loaded NPs induced an upgrading of the N, P, K and S nutrition. The coefficient of variation in the measured nutrient contents as well as the estimated uptake among the treatments was beyond 20% for total P (21.1%) and S (24.3%) content; and N (24.1%), P (26.7%) and S (26.1%) uptake.
4. Conclusions

The XRD patterns of the synthesized CS-TPP-NPKS nanofertilizer confirmed its amorphous nature. The FTIR spectra analysis and ICP-MS technique exposed the encapsulation of N, P, K, and S nutrients within the prepared nanofertilizer and their interaction with the CS-TPP matrix. The average sizes of the suitable CS-TPP NPs were found to be 145 and 450 nm whereas the NPKS loaded CS-TPP NPs revealed worthy size ranging from 220–530 nm. These formulated nanofertilizer had commendable slow-release property, with the sum of nutrients release lower than 60% on the 7th day. The treatment with these nanoformulated sulphate-supplemented NPK fertilizers yielded maize plants with the maximum height, number of leaves, stem diameter and chlorophyll content at 10 weeks after planting, as compared to untreated, NPK fertilizer, NPKS fertilizer, and CS-TPP NPs treatments, under greenhouse conditions. However, the latter showed plants with superior dry matter yield. Moreover, the best plants growth was obtained with formulation 1% CS/NPKS (40 ppm). The magnitude of the coefficient of variation in the nutrient contents and uptake among the treatments was found to vary, but the highest value was recorded with the total phosphorus uptake. This study highlights the potential of the nanoformulated sulphur-containing NPK fertilizers on improving maize growth towards sustainable agriculture. Further investigation on the field application of these nanoengineered fertilizers for maize production is ongoing.

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Conflicts of interest

The authors declare no conflict of interest of any kind; the funders had no role in the design and implementation of this research and its data interpretation, nor in the decision to write and submit this manuscript.

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