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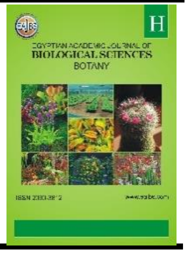

Growth, Productivity and Quality of Sweet Basal in relation to Minerals, Nanoparticles of Chitosan for NPK Fertilization

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ABSTRACT

Sweet basil (Ocimum basilicum L. var. Grand Vert) is an herbaceous plant that uses volatile oil and herbs in the market or as an ornamental plant. The experiment is divided into two parts, the first is the preparation of chitosan Nano-fertilizer as well as (CCS) and chitosan-NPK nano-fertilizer (CCS-NPK) as well as loaded on chitosan, and the second part is the application of the use of these different nano- fertilizers, which is recommended by NPK to study its effect on the morphological, chemical and oil properties and its chemical composition for sweet basil plants (*Ocimum basilicum* L. var Grand Vert). after planting it In the seasons 2017/2018 and 2018/2019 In the Experimental Field of a Medicinal and Aromatic Plants, EL- Qanater El-Khaieria Gezeret El-Shaeier, and the Medicinal and Aromatic dept Lab., (HRI), (ARC), Dokki, Giza, and Nanotechnology and Advanced Materials Central Lab., The study was the effect of Nano-fertilization and toxicity on the plant extract in order to ensure the safety of using nanoparticles. The results revealed that application of chitosan alone or as loaded on NPK or as NPs increased growth, yield, oil %, oil yield, the chemical structure of basil plants, and decreased toxicity of human hung cells, where the effective treatments were CS-NPK NF 400:50:300; CS-NPK NF 300:60:400 and Chitosan Nano 250 under the study conditions.

INTRODUCTION

Sweet basil (*Ocimum basilicum* L. var. Grand Vert) is a herbaceous plant belonging to Lamiaceae family, which has been grown for its volatile oil and herb for market or as an ornamental plant, Grand Vert is Variety cimum basilicum and Genovese well adapted to a wide range of growing conditions. It can grow up to 50 cm high However, it can easily reach more than a meter in length when kept for several years (when the climate allows it). A

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seedling is 15-20 cm high, has ovate-lanceolate leaves medium leaves, reaching 2-3 cm are pale to dark green, sometimes purplish purple in some varieties. The erect, branched stems have a square section like many Labiatae. They tend to become woody and bushy. The flowers, bilabiate, small and white, have the upper lip cut into four lobes. They are small in size and grouped in long tubular spikes, in the form of elongated clusters. The fine, oblong seeds are black. The Basil is an aromatic annual herb native to Asia with oval, shiny, dark and downy leaves that blossom at the end of summer. Very resistant to diseases. (USDA, 2008), Sweet basil is a rich source of natural plant chemicals, including monoterpenes, sesquiterpenes, and phenylpropanoids that possess antioxidant activities and proven human health benefits. (Shahram *et.al.*, 2011). the aroma profile and chemical compounds of sweet basil include high concentrations of linalool, other constituents include: 1,8-cineole, eugenol, and myrcene, among others 1,8-cineole, methyl chavicol, beta-carotene, vitamin C and A3 - caffeic acid and b - chemine (phenols), limonene (anti-cancer), methyl bisamate, myrcene, alpha-pinene, betapenin, cortin, rutin, alfa terpenine, tryptophan, (estragole) and methyl eugenol, In this species eugenol is synthesized from coniferyl acetate and NADPH (British, 1963), (Zheljazkov *et al.*, 2009), Some of these are useful as insect repellents, Insect repellent below. Also, of volatile oil antioxidants and every 100 gm has 22 calories containing (carbohydrates, fiber, protein and fats), salts of calcium, potassium, and iron (Andrea *et.al*., 2007).

The plant fertilization with the main essential elements needed by N, P and K These elements represent the main base for the composition affecting the growth and productivity of medicinal and aromatic plants and improving their vegetative growth and herbaceous and oily crops, amino acids, proteins, carbohydrates, fats, the formation of proteins, chlorophyll and pigments for nitrogen, phosphorus for root growth, flowers fruits, potassium roots, stems and cell walls (Corradini *et al*., 2010; Boroomand *et.al.*, 2012), (Youssef, *et al*., 2004), which assist in physiological and biological processes, metabolic processes, and the formation of (Corradini, et, al.,2010), Plant walls, cell division, DNA, and RNA. etc. (Devlin, 1975). Thus, obtaining a high yield of grass, increasing the amounts of active compounds and the formation of secondary metabolites (metabolites) in plants that produce EOS and phenolic compounds responsible for pollination and plant protection. Items (Nguyen *et al*., 2008).

At present, the trend of modern science in Nano-fertilizer has made a breakthrough in fertilization, the elements are used during foliar feeding. Two Long Ways (Coradini *et al*., 2010), Therefore, the encapsulation of nutrients inside nanoparticles, which are distinguished by their cationic polymeric properties, biodegradable and biological absorption, and their use in the method of small particles and polymers, led to optimal use in terms of use and reduction of waste as well as a significant reduction in the quantities used. Each 1 gram is approximately equivalent to using 100 kg of fertilizer in its natural state with its natural granule sizes such as Chitosan which is one of the most important polymers that are coated and coated for CS elements (Wu *et al.*, 2008), (Calvo *et al*., 1997).

Toxicity tests should also be taken care of to ensure that it is safe to use and that the plant is free from any harmful effects on humans when ingested, Nanotechnology is a tool that has shown many applications in the past decade, including agricultural application systems, have developed for use in fertilizers, pesticides, stimulants and other, more importantly, the application of this technique evaluates the negative aspects of its use due to the large number of nanosystems used in agriculture, especially in crops, and impact on morphogenesis, metabolism, genetic modification and the effect of the rest of them and their toxicity must be studied (Luis *et al*., 2020).

This investigation was conducted to study the effect of Chitosan (CCS), Chitosan nanoparticles, NPK nanoparticles and their combination and compared to recommended (control) on sweet basil plants (Ocimum basilicum L. var. Grand Vert) and the side effect of these materials (toxicity).

MATERIALS AND METHODS

The two seasons of field experiments were carried out at the Experimental Field Medicinal and Aromatic Plants, EL- Qanater El-Khaieria Gezeret El-Shaeier, and the Experimental Laboratory of Medicinal and Aromatic dept., Horticultural Research Institute (HRI), Agricultural Research Center (ARC), Dokki, Giza in two successive seasons of 2017/2018 and 2018/2019, to investigate the effect of some forms of Chitosan and NPK fertilizer such as, Chitosan Nano-fertilizer, NPK Nano-fertilizer) on sweet basil plants (*Ocimum basilicum* L. var. Grand Vert).

1- Plant Material and Procedures:

Basil seeds were obtained from the Medicinal and Aromatic Plants Research Department, Horticultural Research Institute (HRI), Agricultural Research Center (ARC), and it was planted in the experimental field in the medicinal and aromatic plants farm on the Barley Island in Qanater al-Khairiya In two seasons 2018-2019 and also 2019-2020, the seeds were sown on February 16, and seedlings were transplanted in late April in the early morning, for the study of the mineral, Nano and chitosan nanoparticles for NPK fertilization and their effect on growth, productivity and Quality of sweet basil

The experiment was conducted in three parts The first part is the manufacture of Nano-fertilizers, the second part is the use of these fertilizers in plant cultivation and making measurements that show the effect of spraying with nano-fertilizers on the production of grass crops, oil crops and their chemical composition and the third part is conducting a toxicity experiment on human lung cells to find out the toxicity and effect of nanoparticles on edible plants.

Fig. 1: Morphology of sweet basil plants (*Ocimum basilicum* 'Grand Vert') plants.

2-* The Used Fertilizer Types Are*:

2.1-Chemical Fertilizer:

The following fertilization treatments were used:

A-Sources of Mineral Fertilizers:

N source was ammonium nitrate (NH_4NO_3). The P source was phosphoric acid (H_3PO_4). The K source was potassium sulfate (K_2O). Control (untreated plants, Using only the basic recommended dose needs) about (100:45:75) unit (N:P: K) (ARC), Ministry of Agriculture and Land Reclamation (Egypt).

2-2 . Chitosan Fertilizer (Bio-Fertilizers):

source organic fertilizers were Pure laboratory chitosan (CCS), Chitosan CCS is one of the most important derivatives of chitin and the protein produced from the residues and wastes of marine crustaceans, which constitute 50-70% of the weight of the raw material, and therefore it is an important commercial component in scientific, agricultural and engineering applications.

Chitosan is used in agriculture, sewage treatment and tissue engineering applications due to its high stability, interactions, chelating properties, biodegradability and bio-absorption, so it is also bactericidal. (Gopa*l et al.,* 2019).

2.3. Nano Fertilizer: Preparation of Nano-chitosan Particles to Be Loaded with NPK Fertilizer:

2-3-A. Chitosan CS-PMAA Nanoparticles:

Chitosan is obtained by deacetylation reactions (free amino groups left by partial deacetylation- N-acetyl- D-glucosamine). It is a non-toxic, biocompatible and easily degradable biopolymer, with negative charges, which helps in positive interaction, with important biological activity, as it is anti-oxidant and anti-bacterial.

Chitosan nanoparticles (CCS) were prepared by ionic gelation method according to Calvo *et al.* (1997) with some modifications. The method utilizes the electrostatic interaction between the amine group of chitosan (Sigma-Aldrich, USA, molecular weight 50,000-190,000 Da, degree of deacetylation 75-85% and viscosity: 20-300 cP) and a negatively charged group of polyanion such as sodium tripolyphosphate (TPP) (Sigma-Aldrich, USA). DNA-free deionized water (Millipore, USA) was used for preparation and dilutions. CCS aqueous solution (0.2% w/v) was prepared by dissolving CCS in an acetic acid solution (1% v/v) at room temperature. Subsequently, TPP solution (0.06% w/v) was added dropwise to CCS solution under vigorous stirring for 30 min. The resulting chitosan particle suspension was centrifuged at 12000g for 30 min. The pellet resuspended in deionized water. The chitosan nanoparticles suspension was then freeze-dried before further use or analysis.

Characterization of Chitosan Nanoparticles:

The actual morphology of the as prepared CS nanoparticles was imaged by a High-Resolution Transmission Electron Microscope (HR-TEM) operating at an accelerating voltage of 200 kV (Tecnai G2, FEI, Netherlands). Diluted CS nanoparticles solution was ultra-sonicated for 5 min to reduce the particles aggregation. Using a micropipette, three drops from the sonicated solution were deposited on carbon coated-copper grid and left to dry at room temperature.HR-TEM images of the CS nanoparticles that were deposited on the grid were captures for morphological evaluation. Dynamic Light scattering (DLS) technique was utilized to estimate the average particle size distribution that was measured by zeta sizer (Malvern, ZS Nano, UK). The chemical structure of as prepared CS nanoparticles was assessed using X- ray Diffraction (XRD) technique. The corresponding XRD pattern was recorded in the scanning mode (X'pert PRO, PAN analytical, Netherlands) operated by a Cu K radiation tube (= 1.54 A ˚) at 40 kV and 30 mA. The obtained diffraction pattern was interpreted by the standard ICCD library installed in PDF4 software. All the Preparation and characterization processes were conducted at Nanotechnology and Advanced Materials Central Lab (NAMCL), Agricultural Research Center, Egypt.

2-3-B. NPK nanoparticles: Preparation and Characterization of Chitosan–NPK Nano-Fertilizer:

Chitosan (CS) (molecular weight 50,000-190,000 Da, degree of deacetylation 75-85% and viscosity: 20-300 cP), acetic acid, sodium tripolyphosphate (TPP), Tween 80, phosphoric acid (85%), potassium sulphate and ammonium nitrate. All the chemicals used in this study were used without further purification and were purchased from Sigma-Aldrich, USA chemical company. Chitosan-NPK Nano-fertilizer (CS-NPK NF) was prepared according to Corradini *et al*. (2010) with some modifications. Briefly, CS aqueous solution (0.2% w/v) was prepared by dissolving CS in acetic acid solution (1% v/v) at room temperature. The pH of the solutions was adjusted to 5.5 with 0.5 M NaOH solution. Subsequently, TPP solution (0.02% w/v) was added dropwise to CS solution under vigorous stirring for 30 min. NPK Nano-fertilizer was prepared by loading nitrogen (N), phosphorous (P) and potassium (K) into chitosan nanoparticles by dissolving different amounts of NPK into 100 ml of nanoparticle solution under homogenizing at 18000 rpm for 30 min in presence of Tween 80 at 25°C. The resulting solution to incorporate NPK into the nanoparticles presents this final concentration: i) 300, 400 and 500 ppm of N; ii) 40, 50 and 60 ppm of P; iii) 200, 300 and 400 ppm of K. Dynamic light scattering (DLS) measurement of size and Zeta Potential were undertaken using a Nano-zeta sizer (Malvern, ZS Nano, UK). The morphology of CS-NPK NF was imaged by a High-Resolution Transmission Electron Microscope (HR-TEM) operating at an accelerating voltage of 200 kV (Tecnai G2, FEI, Netherlands). Diluted CS-NPK NF solution was ultra-sonicated for 5 min to reduce the particles aggregation. Using micropipette, three drops from the sonicated solution were deposited on carbon coated-copper grid and left to dry at room temperature.HR-TEM images of the Chitosan–ammonium nitrate nanoparticles that were deposited on the grid were captured for morphological evaluation. Synthesis and characterization of Chitosan–ammonium nitrate nanoparticles were performed in Nanotechnology & Advanced Materials Central Laboratory (NAMCL), Agricultural Research Center (ARC), Giza, Egypt.

3-Toxicity:

The experiment was done on human hung cells, with the highest concentration of CS plant extract (T1) and CS NF fertilizer (T2, as well as the CS-NPK plant extract (T3) and CS-NPK NF fertilizer (T4), Cell Cytotoxicity assessment: Normal epithelial cells WI- 38 isolated from the lung were treated with different sources of fertilizer containing chitosan nanoparticles loaded with NPK or chitosan free (4000 ppm) of HMW chitosan and cytotoxicity, was measured after 24, h incubation by MTT assay, the experiment was carried out with both preparations and methods (Slater *et al*., 1963; Alley *et al.* (1994; van de Loosdrecht *et al*., 1988). In the main laboratory for science way of knowing the company, for scientific researche and consultations, Nasr City- Al Waha district, building 1-block 10-fourth floor

The experimental design was randomized complete block design (RCBD) in three replications and the treatments were randomly distributed as following as:

4-Treatments:

1- (Control) NPK 100: 45: 75 Kg. 2- Chitosan 1000 PPM
3- Chitosan 1500 PPM 4- Chitosan 2000 PPM
5- Nano Chitosan (CS NF) 1000 PPM (NPs,)
6- Nano Chitosan (CS NF) 250 PPM (NPs,)
7- Nano Chitosan (CS NF) 400 PPM (NPs,)
8- CS-NPK NF 300: 40: 200 PPM (NPs,)
9- CS-NPK NF 400: 50: 300 PPM (NPs,)
10- CS-NPK NF 500: 60: 400 PPM (NPs,)

Cultivation planting seedlings of sweet basil in a plot of 541.5 m3 , In basins with an area of three lines, the distance between the lines is 60 cm, and the distance between the banks is 25 cm. The number of tanks is 25 tanks during 2017/2018 and 2019/2020 seasons. And three cuts per season, fertilizer used is sprayed with its different concentrations and types in each cut.

5-Data recorded

1-Plant height (cm). 2-Number of branches/plants
3-Fresh weight /plant (g) 4-Dry weight /plant (g)
5-The yield of herb /plant (g) 6-Percentage of volatile oil (%)
7-Yield volatile oil / plant (ml.) 8-Chemical compounds on volatile oil (GLC)
9-Total chlorophyll

All collected data were submitted for analysis of variance using the described method by Gomez and Gomez (1984). All statistical analyses were carried out using the analysis of variance approach and the Cost at computer software program (CoStat, 2005). To compare the treatment means, the least significant difference (LSD at 0.05 level of probability) was utilized.

RESULTS AND DISCUSSION

The results in Tables (1, 2 and 3) revealed that various types of chitosan and NPK (bio and nanoparticles) had a significant effect on basil plant growth, yield, quality, and chemical characteristics during the 2017/2018 and 2018/2019 seasons.

The results in Table (1) cleared that there was a significant effect of the different treatments of chitosan and nano forms of chitosan and NPK on plant height (cm), fresh weight (FW) - g/plant, dry weight (DW)- g/plant and herb yield/plant at three cuts of basil plants, where the application of nano NPK fertilizer at the rate of 300:60:400 ppm recorded the highest values of plant height (cm), fresh weight /plant, dry weight/plant and herb yield/plant followed by application of nano- NPK 400:50:300 ppm, chitosan Nano at the rate of 250 ppm, in plant height, fresh weight, respectively during both seasons.

The presented results in Table (2) showed that there was a significant difference among the ten treatments, where CS-NPK NF 400:50:300 recorded the highest mean values of oil %, oil yield and chlorophyll content followed by CS-NPK NF 300:60:400 and Chitosan Nano 250 which gave the highest value of these traits as compared with the other treatments in most of the three cuts in both seasons.

An improvement in the features of this study by employing NPK in nanoparticles (NPs), owing to the function of NPK in many crops, where the addition of N is critical in improving vegetative development and hence increasing photosynthetic activity and so the accumulation of dry matte. N is also crucial in the molecular structure of critical biomolecules in photosynthesis and respiration, as is phosphorus in the formation and activation of coenzymes required for the function of many enzymes and the generation of amino acids that contribute to protein formation **(**Espinosa, 1999).Furthermore, P plays an important role in the formation of nucleic acids and energy compounds ATP and GTP, as well as its involvement in the synthesis of enzymatic collaborators such as NADP, which is required for many metabolic processes, and has a role in cell division and thus the development of roots, which aids in their expansion and increased nutrient absorption (Havlin *et al*., 2005).And Potassium (K) is primarily responsible for the enzymatic activity and protein stability, as well as the regulatory role in the mechanism of closing and opening stomata, which is favorably reflected in improving the utilization of photosynthesis and thus rising its growth due to the good balance of NPK (Shabala 2003).

The major role of fertilizer components in the increase in plant height, which is the result of nitrogen levels that promote the production of Auxins that encourage cell division and elongation of cells of the total vegetative plant, also has a direct impact on plant height because it is required to build the amino acid Tryptophan. It is the primary building element for indol acetic acid (IAA), the plant's principal hormone (AL-Asady and AL-Kikkhani, 2019). This might be owing to an increase in vegetative growth markers, which would boost photosynthetic efficiency by improving the effectiveness of the source and hence increase dry matter accumulation. The reason for the success of the nano NPK fertilizer treatment could be due to the role of nano fertilizer in increasing bioavailability, which relates to more efficient absorption of the added elements (Veronica *et al*., 2015), and enhance soil efficiency through the slow or controlled nutrient supply (Solanki *et al.,* 2015).

Nano-fertilizers also enhance the area available for various metabolic processes in the plant, increasing the rate of photosynthesis and producing more dry matter, which is reflected in the increased nutritional content of the plant NPK (Qureshi *et al*., 2018). These results are in agreement with those of Manikandan and Subramaian (2016), Kandil and Marie (2017), Abdel-Aziz *et al*. (2018 a and b), Burhan and Al-Hassan (2019), who found a substantial influence of nano fertilizer treatment on the aforementioned features. This could be due to the role of nano fertilizers in enhancing the solubility and dispersion or expansion of insoluble nutrients in the soil, as well as minimizing nutrient mineralization and soil stabilization, as well as its effect on increasing bioavailability as a result of enhanced soil biology effectiveness, leading to more efficient absorption of additives (Veronica *et al*., 2015).This assists in the maintenance of the plant's nutrient supply, which has a good influence on plant development (AL-Gym and Al-Asady 2020; Sharma *et al*., 2022).

The results in Table 3 and figure 2 showed that using chitosan 1000 increased pinene in cut1, B pinene in cut3; B- caryophlene in cut1, 2, and 3, while application of chitosan NPs 1000 increased methyle in cut1 and camphor in cut2, on the other hand, chitosan NPs 250 gave the highest values of pinene; camphor in cut2 content, as well as CS-NPK NF 300:60:400 increased pinene in cut 1 and 2; 1, 8 cineol in cut 2; and linalool in cut2; methyle B caryophlene of basil as compared with the others treatments. From these results, it can be concluded that application of chitosan alone or with NPK in mineral or NPs affected significantly chemical composition of basil.These results are in agreement with those reported by Corradini *et al*. (2010; Boroomand *et.al*. (2012) who indicated that these materials had significantly affected chemical composition of the plants. In addition, as compared to the control treatment, foliar application of Nano CS-NPK levels (Nano 10, 50, and 100 ppm) massively improved all growth and yield metrics, photosynthetic pigments, chemical components of potato tuber at harvest, and macronutrients in potato leaves and tubers. In this regard, 10% Nano CS-NPK was the most effective therapy when compared to the other two levels. Thus, using nano-fertilizers to accelerate plant growth, production, quality, and chemical composition might open up new avenues in agricultural practice (Elshamy *et al*., 2019).

Table 1. Effect of different types of chitosan and nanoparticles and NPK (recommended and nanoparticles) on growth and yield of basil (*Ocimum basilicum* L.) plants during 2017/2018 and 2018/2019 seasons.

Treatments (ppm)	Plant height (cm)			Fresh weight (FW) - g/plant			Dry weight (DW)- g/plant			Herb yield kg /fed		
	Season 2017/2018			Season 2017/2018			Season 2017/2018			Season 2017/2018		
	Cut1	Cut2	Cut3	Cut1	Cut2	Cut3	Cut1	Cut2	Cut3	Cut1	Cut2	Cut3
Control	54.88	62.78	61.78	59.52	100.31	115.61	24.65	30.17	27.56	1488.1	2507.7	2890.27
Chitosan 1000	61.89	67.67	65.55	153.99	216.45	189.11	32.60	39.58	36.94	3849.7	5411.3	4727.80
Chitosan 1500	65.33	69.78	66.33	213.18	246.94	233.61	35.67	43.91	39.39	5329.6	6173.6	5840.30
Chitosan 2000	71.89	75.67	74.44	295.45	343.04	319.27	73.17	76.69	75.79	7386.3	8576.0	7981.67
Chitosan Nano 1000	67.33	72.55	70.78	163.50	198.58	148.61	42.70	56.91	51.41	4087.4	4964.6	3715.27
Chitosan Nano 250	75.78	79.45	75.78	322.25	380.33	365.11	75.04	78.52	76.20	8056.2	9508.1	9127.77
Chitosan Nano 400	72.33	78.11	80.78	144.79	225.21	217.68	70.38	79.00	74.40	3619.7	5630.2	5441.93
CS-NPK NF 300:60:400	89.26	101.79	93.69	341.53	475.86	438.50	88.17	98.58	94.27	8538.3	11896.3	10962.33
CS-NPK NF 400:50:300	85.92	90.42	89.56	140.99	236.71	209.30	66.51	74.76	74.13	3524.8	5917.8	5229.17
CS-NPK NF 500:60:40	80.44	88.45	87.45	216.39	256.66	236.44	62.91	78.67	77.83	5409.7	6416.5	5911.10
LSD at 0.05	4.02	3.35	3.29	21.20	23.12	12.68	2.89	2.5	2.45	528.80	578.09	317.15
Treatments	Season 2018/2019			Season 2018/2019			Season 2018/2019			Season 2018/2019		
	Cut1	Cut2	Cut3	Cut1	Cut2	Cut3	Cut1	Cut2	Cut3	Cut1	Cut2	Cut3
Control	57.11	66.89	63.44	83.39	140.31	141.00	31.43	39.55	37.23	2084.73	3507.73	3525.00
Chitosan 1000	62.89	72.00	70.11	166.50	274.84	230.33	37.23	50.52	44.28	4162.50	6870.90	5758.33
Chitosan 1500	69.11	74.89	71.56	221.75	317.39	261.11	44.81	55.23	53.90	5543.87	7934.63	6527.77
Chitosan 2000	73.22	78.67	75.33	312.42	358.22	342.51	75.79	86.15	83.36	7810.60	8955.57	8562.67
Chitosan Nano 1000	70.11	74.78	71.45	184.70	250.25	233.38	57.62	62.85	61.06	4617.43	6256.37	5834.43
Chitosan Nano 250	76.00	84.45	81.89	363.64	417.73	393.50	82.17	91.12	88.61	9091.00	10443.33	9837.40
Chitosan Nano 400	73.78	69.55	80.89	159.65	282.99	266.72	71.95	81.23	79.17	3991.20	7074.87	6668.07
CS-NPK NF 300:60:400	94.26	111.00	97.02	383.71	543.92	528.82	86.81	103.73	100.14	9592.63	13597.67	13220.67
CS-NPK NF 400:50:300	87.78	93.54	90.52	222.84	398.60	384.06	72.22	75.34	72.17	5571.10	9965.03	9601.30
CS-NPK NF 500:60:40	84.11	90.22	82.11	237.45	448.29	433.89	66.67	73.22	68.94	5936.30	11207.67	10847.33
LSD at 0.05	2.81	4.3	4.05	30.02	18.15	11.23	3.28	3.33	3.15	750.54	435.91	289.61

Table 2. Effect of different types of chitosan and nanoparticles and NPK (recommended and nanoparticles) on volatile oil percentage and oil yield/plant chemical composition of Basil (*Ocimum basilicum* L.) plants during 2017/2018 and 2018/2019 seasons

Treatments (ppm)	Oil %			Oil yield ml. /plant			Total chlorophyll		
	Season 2017/2018			Season 2017/2018			Season 2017/2018		
	Cut1	Cut2	Cut3	Cut1	Cut2	Cut3	Cut1	Cut2	Cut3
Control	0.09	0.13	0.13	2.14	3.17	3.17	0.27	0.43	0.38
Chitosan 1000	0.19	0.24	0.21	4.72	6.03	5.17	0.33	0.44	0.41
Chitosan 1500	0.23	0.27	0.22	5.81	6.61	5.53	0.38	0.48	0.47
Chitosan 2000	0.25	0.27	0.24	6.14	6.75	5.92	0.46	0.52	0.48
Chitosan Nano 1000	0.23	0.25	0.23	5.78	6.22	5.72	0.46	0.49	0.47
Chitosan Nano 250	0.31	0.35	0.29	7.63	8.72	7.22	0.46	0.52	0.52
Chitosan Nano 400	0.26	0.30	0.27	6.61	7.58	6.83	0.45	0.49	0.48
CS-NPK NF 300:60:400	0.33	0.34	0.32	8.22	8.58	8.03	0.52	0.52	0.48
CS-NPK NF 400:50:300	0.35	0.36	0.33	8.86	9.00	8.31	0.49	0.52	0.47
CS-NPK NF 500:60:40	0.29	0.33	0.29	7.17	8.13	7.17	0.56	0.59	0.52
LSD at 0.05	0.03	0.02	0.02	0.24	0.22	0.14	0.04	0.03	0.03
Treatments	**Season 2018/2019**			**Season 2017/2018**			**Season 2018/2019**		
	Cut1	Cut2	Cut3	Cut1	Cut2	Cut3	Cut1	Cut2	Cut3
Control	0.13	0.19	0.16	3.38	4.81	3.97	0.40	0.44	0.40
Chitosan 1000	0.22	0.26	0.23	5.58	6.58	5.64	0.42	0.47	0.43
Chitosan 1500	0.26	0.29	0.27	6.58	7.24	6.70	0.47	0.53	0.49
Chitosan 2000	0.28	0.33	0.26	7.08	8.33	6.60	0.52	0.54	0.50
Chitosan Nano 1000	0.27	0.28	0.25	6.67	6.89	6.18	0.45	0.52	0.51
Chitosan Nano 250	0.34	0.37	0.34	8.59	9.24	8.53	0.48	0.54	0.54
Chitosan Nano 400	0.28	0.35	0.31	7.06	8.71	7.69	0.48	0.52	0.50
CS-NPK NF 300:60:400	0.36	0.37	0.35	8.89	9.38	8.86	0.51	0.56	0.53
CS-NPK NF 400:50:300	0.32	0.39	0.37	8.00	9.86	9.28	0.51	0.55	0.52
CS-NPK NF 500:60:40	0.33	0.35	0.34	8.28	8.92	8.40	0.57	0.61	0.54
LSD at 0.05	0.03	0.02	0.03	0.27	0.26	0.26	0.01	0.03	0.036

Table 3. Effect of different types of chitosan and nanoparticles and NPK (recommended and nanoparticles) on the chemical composition of Basil (*Ocimum basilicum* L.) plants during 2018/2019 seasons.

Treatments (ppm)	Pinene			B pinene			1,8cineol			linalool		
	Season 2017/2018											
	Cut1	Cut2	Cut3	Cut1	Cut2	Cut3	Cut1	Cut2	Cut3	Cut1	Cut2	Cut3
Control	0.661	0.370	0.620	0.547	0.150	0.460	6.094	4.160	6.190	43.348	45.340	48.660
Chitosan 1000	1.070	0.760	0.600	0.930	0.850	0.985	5.630	9.040	5.760	39.900	42.490	38.070
Chitosan 1500	0.480	0.810	0.659	0.630	0.270	0.431	4.700	6.790	5.490	42.980	42.140	44.110
Chitosan 2000	0.630	0.800	0.720	0.710	0.540	0.652	6.670	5.510	6.320	36.760	40.830	45.990
Chitosan Nano 1000	0.360	0.410	1.046	0.340	0.380	0.678	2.840	7.660	8.190	36.160	43.390	50.910
Chitosan Nano 250	0.630	1.000	0.810	0.440	0.830	0.936	5.180	11.550	8.450	47.410	47.240	37.030
Chitosan Nano 400	0.730	1.200	0.440	0.400	1.350	0.380	7.210	6.000	5.960	46.300	37.040	45.930
CS-NPK NF 300:60:400	0.940	1.400	0.570	1.220	0.270	0.354	4.430	9.600	5.040	35.860	44.180	40.600
CS-NPK NF 400:50:300	0.470	0.950	0.410	0.300	1.240	0.400	4.070	4.320	4.720	44.610	36.480	41.850
CS-NPK NF 500:60:40	0.680	0.610	0.730	0.500	0.270	0.697	6.010	7.090	9.530	46.630	37.710	44.530
Treatments	**Camphor**			**Terpineol**			**Methyle**			**B caryophlene**		
	Cut1	Cut2	Cut3	Cut1	Cut2	Cut3	Cut1	Cut2	Cut3	Cut1	Cut2	Cut3
Control	2.563	3.110	5.100	21.600	23.440	2.002	3.010	2.930	4.540	12.010	13.160	19.070
Chitosan 1000	2.480	8.050	3.500	6.800	6.320	2.660	6.410	2.660	7.080	17.230	18.780	19.930
Chitosan 1500	1.320	6.970	3.040	28.520	7.220	11.200	2.620	2.830	5.430	10.480	20.610	17.440
Chitosan 2000	2.830	2.430	6.130	34.630	29.600	2.034	2.250	2.440	4.510	8.190	10.250	19.570
Chitosan Nano 1000	2.030	5.270	3.240	38.240	5.900	1.370	3.700	2.380	2.930	8.340	25.360	19.620
Chitosan Nano 250	2.940	5.380	2.990	12.060	5.270	3.330	4.320	1.690	7.690	18.230	18.520	15.770
Chitosan Nano 400	4.840	4.070	3.160	18.790	6.500	1.670	3.920	2.080	4.330	8.260	20.430	25.950
CS-NPK NF 300:60:400	2.000	7.670	3.520	7.230	6.100	22.060	3.150	2.240	4.940	17.550	19.730	12.150
CS-NPK NF 400:50:300	1.820	3.840	4.850	23.370	7.310	2.410	3.520	3.140	6.930	12.680	17.860	19.830
CS-NPK NF 500:60:40	1.950	2.920	4.720	4.960	33.940	1.814	3.870	1.780	5.530	28.430	9.510	19.540

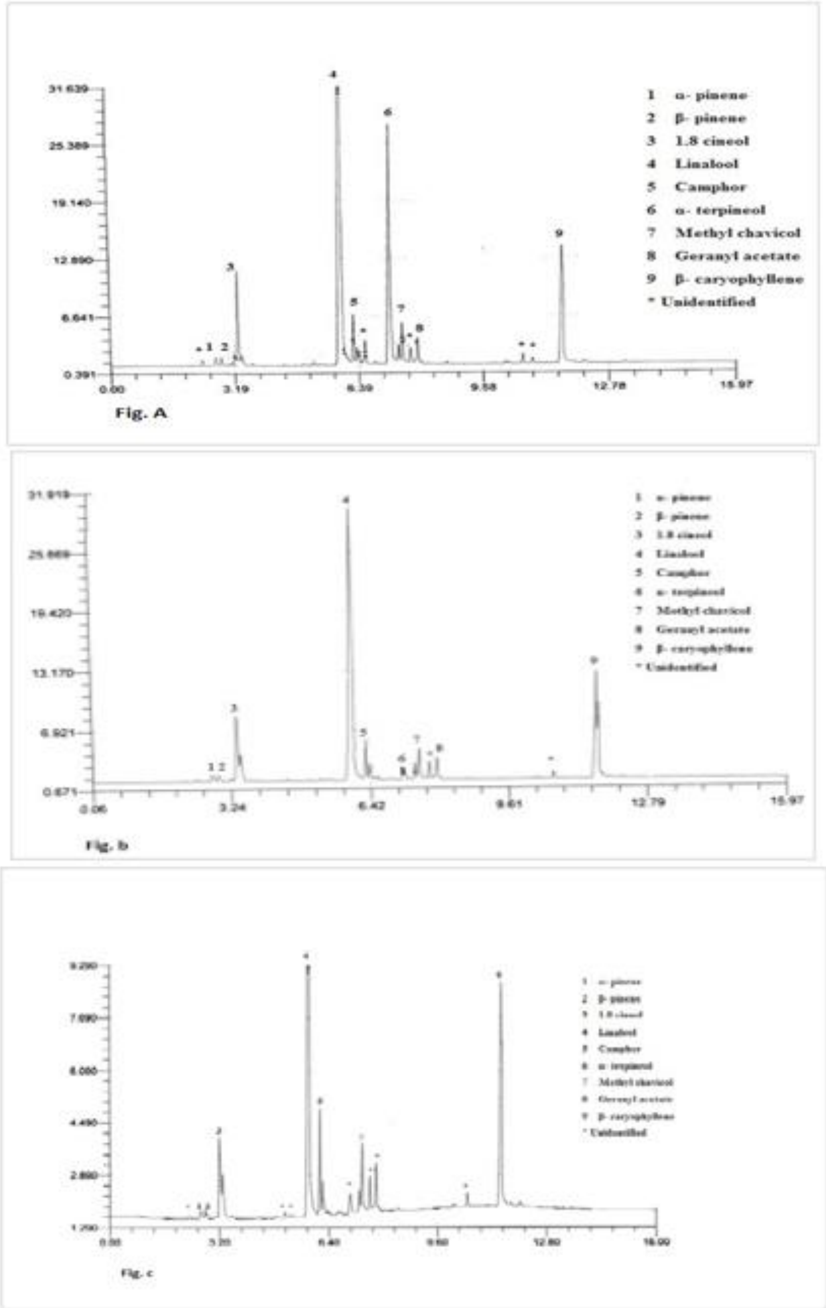

Fig. 2: Effect of chitosan nanoparticles(B) and NPK (recommended (A) and nanoparticles (C) on the chemical composition of Basil (*Ocimum basilicum* L.) plants during 2018/2019 seasons.

Result of Characterization of Nano-fertilizer:

a- Characterization of Chitosan Nanoparticles:

Figure 3 A shows the Physicochemical characterization of the synthesized CS nanoparticles to evaluate their properties using different techniques. nanoparticle morphology and size determination, HR-TEM electrograph shows a nearly spherical shape, smooth surface and size range of about 43.6 nm, Fig. 3B. Fig. 3C, represents the particle size distribution curve obtained from DLS measurements. The measurement of the CS nanoparticles surface charge, zeta potential, was (+47 mV) as measured by DLS technique.

X-Ray powder diffraction patterns of chitosan nanoparticles is shown in Figure 1D. No peak is found in the diffractograms. Chitosan nanoparticles are comprised of a dense network structure of interpenetrating polymer chains cross-linked to each other by TPP counter ions Tang *et al.* (2003). The XRD implicated greater disarray in chain alignment in the nanoparticles after crosslinks.

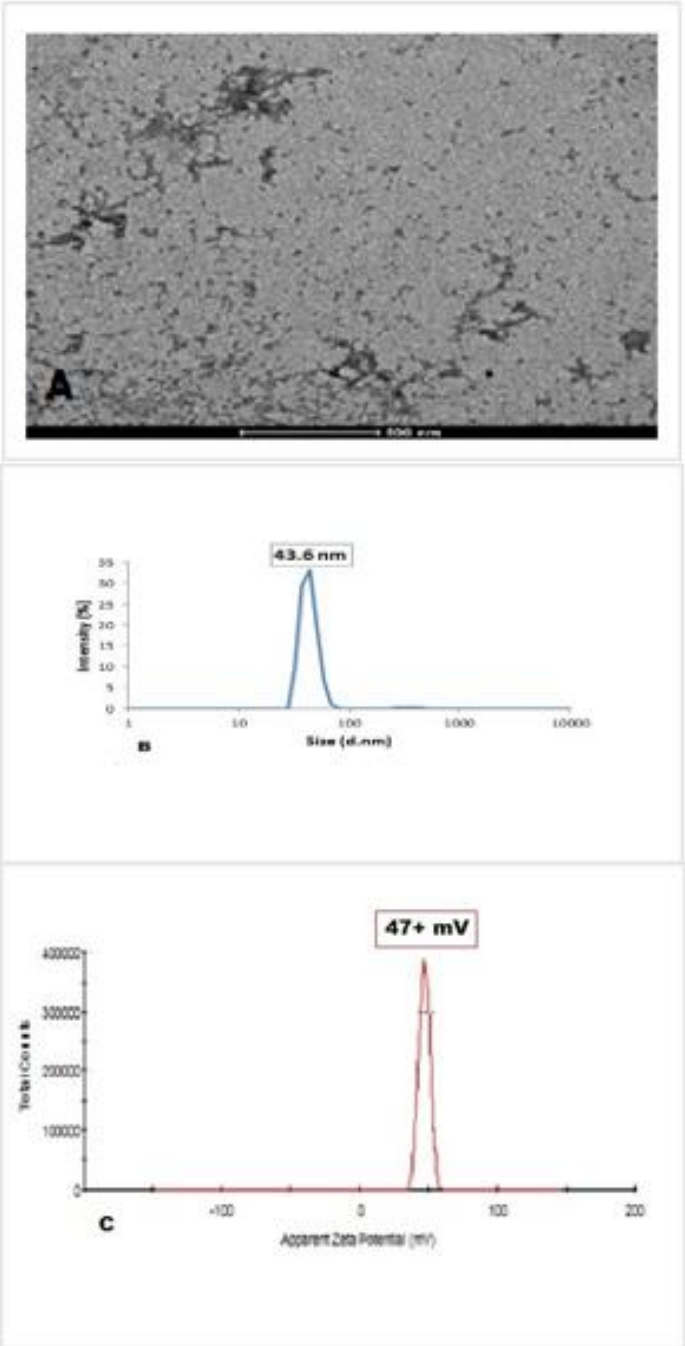

Fig. 3. (A) characterization of the synthesized CS nanoparticles morphology and size determination, (B) represents the particle size, (C) measurement of the CS nanoparticles zeta potential.

b- Characterization of chitosan-NPK nanoparticles Dynamic Light Scattering (DLS) Analysis:

DLS was used to measure hydrodynamic diameter in the nanometer range. The size of CS-NPK NF was 33.10 nm and the zeta potential was 39.7 mV (Fig. 4.B).

TEM Analysis Result:

The transmission electron microscope (TEM) gave us information on the particle shape and the determination of particle size. A typical TEM micrograph of the CS-NPK NF was shown in figure 4.A. CS-NPK NF has nearly spherical shape, smooth surface and average size of about 33.10 nm.

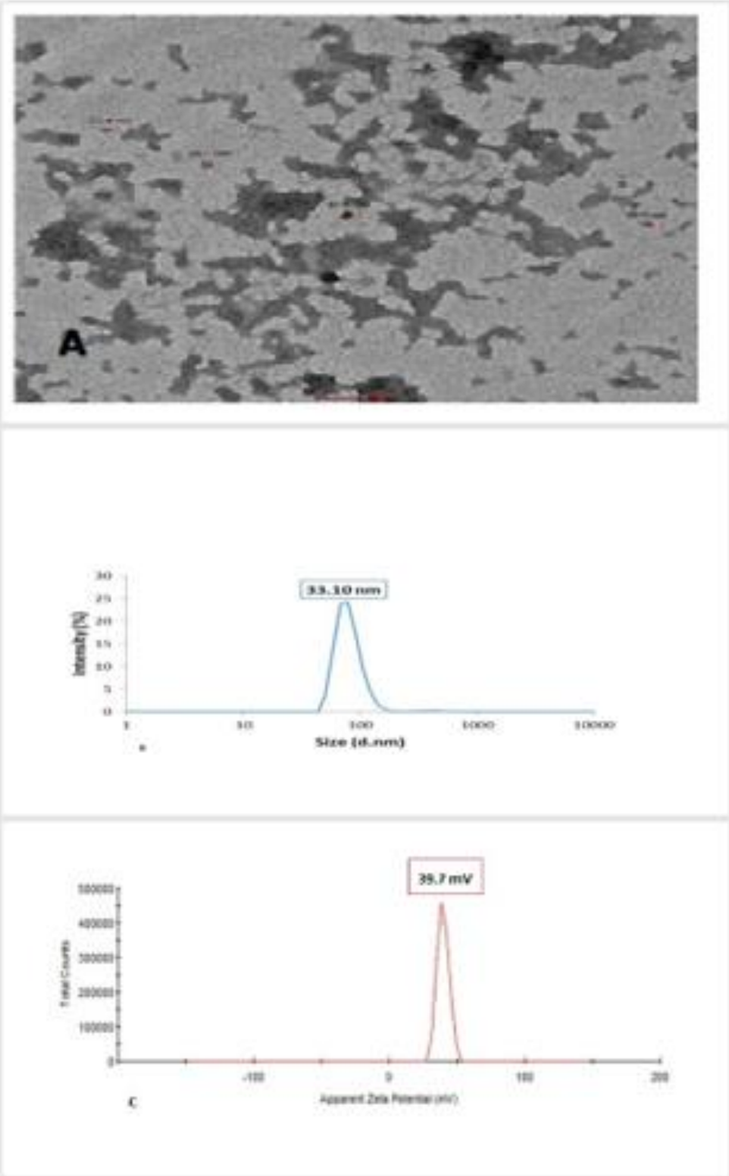

Fig. 4. (A) TEM image of CS-NPK NF1000 ppm, **(B)** DLS analysis of CS-NPK NF 500:60:40 ppm Particle size, **(C)** Zeta potential.

Toxicity Result:

As shown in Table (4) cytotoxic activity of both types of aqueous extract of plants treated with chitosan individually (T1) and chitosan loaded on NPK (T3) at the end of cutting compared with chitosan individually solution (T2) and chitosan loaded on NPK solution (T4) were varied among cells tested. The plants exposed to chitosan 4000 ppm during growth season at first cut gave the lowest toxicity of 0.24 % at a concentration of 12.5 ppm/ml with IC50 170.74 ppm/ml. On the other contrary, increasing concentrations of chitosan to 200 ppm/ml passed the toxicity from the common level (0.34 % ppm/ml for 100 ppm/ml) to a dangerous level (65.03%). The direct effect of chitosan on the was observed in Table (4-cut1) which scored a toxic effect on the cells (0.0986 %) after being treated at

25 ppm/ml, this level increased to 24.11 % for cells treated with chitosan directly at 200 ppm/ml. Moreover, applying chitosan nanoparticles loaded on NPK as a fertilizer at the end of cut 1 the aqueous extract of plants that were treated with this fertilizer scored the lowest toxicity level (0.0986 %) when diluted to 12.5 and 100 ppm/ml, respectively for each at IC50 191.53 ppm/ml. As compared to the direct effect of NPK fertilizer binding to chitosan NPs on Wi-38 normal fibroblast cells lowest toxic effect was 0.147 % for 25.0 ppm/ml.

At the second cut, The aqueous extract of plants treated with chitosan alone diluted to 12.5 ppm/ml had a positive effect to decrease the toxic effect to the lowest level of 0.363 % with IC50 190.28 ppm/ml. On the other hand, Wi-38 cells exposed to ChNPs solution directly at diluted concentrations of 6.25 and 25 ppm/ml decreased toxicity to 0.817 % for each, respectively with IC50 170.65 ppm/ml. The plants at the end of the second cut exposed to fertilizer contained NPK loading on chitosan NPs and diluted to 25 ppm/ml gave viability 100 % with toxicity 0.0 % at IC50 88.47 % this value increasing to 0.181 % with cells exposed to a solution containing fertilizer directly.

According to the data in cut (3), the cell line exposed to aqueous extraction of plants after being treated with chitosan NPs gave the lowest toxicity value (0.098 %) at 100 ppm/ml.

Moreover, at high dilutions of chitosan solution 187.5 ppm/ml gave 0.0 % toxicity with IC50 2014.25 ppm/ml.

When using chitosan loaded on NPK as a fertilizer, the aqueous extract of plants after the third cut decreased toxicity to 0.0 % when treated cells with 187.5 ppm/ml.

Table 4: Effect of aqueous solutions chitosan nanoparticles individually and loaded on NPK fertilizer on Wi-38 cell line cytotoxicity of basil during cut 1,2 and 3 season 2019/2020.

Cut 1 season 2019/2020				
ppm/ml	**ChNPs aqueous extract (T1)**	**ChNPs fertilizer (T2)**	**ChNPs +NPK extract (T3)**	**ChNPs + NPK fertilizer (T4)**
cont	0.00000	0.00000	0.00000	0.00000
200.0	65.03945	24.1124	53.5996	1.38067
100.0	0.34517	0.8383	0.0986	0.24655
50.00	0.73964	0.3945	0.1972	0.49310
25.00	0.29586	0.0986	0.4438	0.14793
12.50	0.24655	1.0355	0.0986	0.19724
6.250	0.39448	0.1479	0.2465	0.39448
IC50	170.74	----	191.53	----
Cut 2 season 2019/2020				
ppm/ml	**ChNPs aqueous extract**	**ChNPs fertilizer**	**ChNPs +NPK extract**	**ChNPs + NPK fertilizer**
cont	0.00000	0.00000	0.00000	0.00000
200.0	55.3134	68.3924	75.9310	3.17893
100.0	0.5450	5.7221	58.2198	0.36331
50.00	0.8174	1.4532	22.3433	1.08992
25.00	2.4523	0.8174	0.0000	0.99909
12.50	0.3633	1.3624	0.7266	0.99909
6.250	0.5450	0.8174	0.1817	0.18165
IC50	**190.28**	**170.65**	**88.47**	----

Cut 3 season 2019/2020					
ppm/ml	**ChNPs extract**	**ppm/ml**	**ChNPs fertilizer**	**ChNPs +NPK extract**	**ChNPs + NPK fertilizer**
cont.	0.00000	cont	0.00000	0.00000	0.0000
200	3.43137	3000	66.4706	87.3529	------
100	0.09804	1500	47.9412	71.6667	------
50	0.39216	750	24.9020	35.6863	------
25	1.07843	375	8.6275	5.3922	------
12.5	0.19608	187.5	0.0000	0.0000	------
IC50	------	IC50	2014.25	1089.03	------

Conclusion

Based on the experimental results, it is suggested to apply a foliar spray of chitosan solo or loaded on NPK NPs fertilizer getting high growth, yield, quality, the chemical composition of basil and returns thereby maintaining the soil health and effective treatments were CS-NPK NF 400:50:300; CS-NPK NF 300:60:400 and Chitosan Nano 250 under the study conditions.

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