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Effect Chitosan and Chitosan Nanoparticles on Marigold (*Calendula officinalis* L.) Plant Under Newly Reclaimed Soils Conditions

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Abstract: A field experiment was conducted during the 2019/2020 and 2020/2021 growing seasons at Qalabsho, Gamasa region, Dakahlia Governorate, Egypt, to evaluate the impact of foliar applications of chitosan (100, 200, 300 mg L^{-1}) and chitosan nanoparticles (20, 40, 60 mg L^{-1}) on *Calendula officinalis* L. cultivated under nutrient-deficient conditions in newly reclaimed soils. The results demonstrated that both chitosan and its nanoparticles significantly enhanced plant growth, floral yield, essential oil content, photosynthetic pigments, total flavonoids, and mineral uptake compared with untreated plants. Notably, chitosan nanoparticles at 40 mg L^{-1} followed by chitosan at 300 mg L^{-1}. recorded the highest improvements across most traits compared to the lowest concentrations of chitosan or its nanoparticles under study. These treatments improved plant height, branch number, flower yield, essential oil percentage, chlorophyll, carotenoids, flavonoids, and macro- and micronutrient contents, thereby alleviating the negative effects of soil nutrient deficiency. Overall, the study highlights that foliar application of chitosan nanoparticles, particularly at 40 mg L^{-1}, is a promising eco-friendly strategy to improve growth, productivity, and biochemical attributes of *Calendula officinalis* L. under challenging soil conditions.

Key words: *Calendula officinalis*, chitosan, nanoparticles, growth, flowers, flavonoids.

1. Introduction

Calendula (*Calendula officinalis* L.) belongs to Asteraceae (Compositae) family. marigold flowers were once as common on the medicine shelf as they were in the soup pot. It recommended to heal duodenal ulcers and liver ailments. *Calendula* is a popular salve and cream ingredient because it decreases the inflammation of sprains, soothes burns, stings and varicose veins, skin irritations, rashes and sunburn. *calendula* petals provided an inexpensive saffron substitute to color soups, stews and sauces. Petal give flavor and color to salad **Keville (1999)**. *Calendula* (*Calendula officinalis* L.) cultivation in newly reclaimed land has been studied by some authors such as **Khalid and da Silva (2010); Hashem (2016) and Sakr (2017)**. They recorded that it is possible to marigold plant under newly reclaimed land conditions but it negatively affected on productivity, quality, chemical constituents of and flavonoids and carotenoids. The results revealed that used some substances to help reduce stress on growth and productivity of the planted *Calendula* under newly reclaimed land conditions.

Chitosan is natural biopolymers and it's an effective natural marine bio-stimulant polysaccharide agent found in the shells of crustaceans, such as crab, shrimp, squid pen, crawfish mollusks, fishes, fungi and algae **No *et al*. (2002); Lárez-Velásquez (2023)**. Chitosan is effective against biotic and abiotic stresses in plants, to increase plant growth regulators such as indole acetic acid, gibberellin, and abscisic acid that protect plants from oxidative stress and increases crop yield. Hence, Chitosan has an effective bio-stimulative and metabolic profile of and its role in abiotic stress tolerance. it is a biopolymer, the involvement of it in agricultural applications is gaining momentum owing to its biodegradability, bio compatibility, plant growth regulation, nontoxicity and stress inhibitory activity in plants **Malerba and Cerana (2018)**. The role of chitosan to soil conditioning and bioremediation: Chitin is a recognized amendment that, providing nitrogen, can help in soil conditioning by freeing it from nematodes, either by promoting the growth of chitinolytic microorganisms **Sharp (2013)**. Additionally, the protective role of chitosan it is clears on plant growth that metal chelation **Chakraborty *et al*. (2020).**

The positive effects of chitosan on *Calendula officinalis* L. were noticed by many authors such as reported by **Abdel-Mola and Ayyat (2020)** revealed that foliar application of chitosan at concentrations 200 and 400 ppm improved the vegetative, flowering characters and chemical constituents of *Calendula officinalis* L. plant under saline water irrigation condition and helped to reduce salinity stress effect on all studied parameters compared to the control. **Akhtar *et al*. (2022)** evaluated the role of chitosan foliar application at as regulators to improve tolerance of *Calendula officinalis* L to drought stress. They reported that chitosan enhancing and significantly increased in physiological, morphological, anatomical parameters and biochemical of calendula under water stress. **Alsoufi *et al*. (2019)** and **Rogowska *et al*. (2023)** exposed to elicitation by chitosan in the context to stimulation of biosynthesis, accumulation and secretion of triterpenoids in vitro culture to increase root hair of *Calendula officinalis*. it was found to be effective elicitor, increasing both the accumulation of oleanolic acid saponins in the hairy root tissue. Chitosan slightly enlarged the accumulation and secretion of oleanolic acid saponins. Which results in clearly to root hair stimulation of *Calendula officinalis* by chitosan. As for the antimicrobial effect of chitosan **Abdel-Wahed and Shaker (2020)** they studied the effect of chitosan to control powdery mildew disease of marigold (*Calendula officinalis*). Results have shown that chitosan showed the best effect in decreasing disease, negative effects of the disease on the plant and positively expressed by an increase in crop parameters, chlorophyll and carotenoids. The positive effects of chitosan were more noticeable under abiotic stress conditions **Rojas-Pirela *et al*. (2024)**. The study analyzes the combined effects of plant growth-promoting bacteria (PGPB) and chitosan on plant growth and protection, with a focus on their crucial role in enhancing plant resistance to both biotic and abiotic stressors. Key bioactivities include the induction of phytohormone production, the mobilization of insoluble soil nutrients biological nitrogen fixation, ethylene level regulation and the control of soil phytopathogens.

Additionally, a previous studies and researches discussed about the role of chitosan to enhance growth parameters, plant height, fruit yield, pigments, carotenoid contents and antioxidant and non-antioxidative enzymes on different plants such as **Abd-El-Hady (2020)** on tuberose, showed that treated tuberose with chitosan at 60 ppm concentration resulted in significant increase in flowering, bulb production and plant growth compared to unsprayed plants under study. **Ibrahim and Arafa (2020)** they elucidated the effect of chitosan on germination, growth, yield and certain salinity stress-related metabolites in two barley cultivars. Chitosan treatment at 200 mgL-1 increased yield and its components in plants growing and alleviated the negative effects of salinity on all characters that were negatively-affected in salinity. On the other hand, Chitosan induced the content of TSS, proline and carotenoids. **El Amerany *et al*. (2022)** reported that the most effective chitosan increased leaf number, plant biomass, stomatal conductance, chlorophyll fluorescence and the levels of abscisic acid and jasmonoyl–isoleucine in wounded roots, stomata aperture, cell division and expansion, fruit maturation, mineral assimilation, and defense responses of tomato, whereas application at the fruit maturation stage reduced the Fv/Fm values. This decline was positively correlated with fruit shape and negatively correlated with the pH and the content of total flavonoids, soluble sugars, lycopene and nitrogen in fruits. **Ningsih and Sari (2023)** on the *Brassica juncea*, reported that the highest chlorophyll content is obtained from chitosan with a concentration of 3.5% and 0.5%. **Jacob *et al*. (2023)** on *Withania somnifera* the foliar spray treatments with chitosan were given better in most of the growth parameters (the number of branches and flowering branches) and had the positive effect on plant growth promotion than other treatments.

The feasibility of coupling nanotechnology with any material are very beneficial, which corresponding to their high surface area and very small size, which reduces wastage and motivates the efficacy. Hence the utilization of nano-chemicals prepared using chitosan is foreseen as an alternative to maintain the sustainability of the agroecosystems and environment. Since chitosan possess properties, these can be used as plant growth promoters and as inhibitors of micro-pests like bacteria and fungi **Maluin and Hussein (2020)**. These properties of chitosan-founded nanomaterials have put forward their use in different forms such as nanoparticles, microfibers, microbeads for several applications in agriculture **Yu *et al*. (2021)**.

The various studies have shown that chitosan nanoparticles applications and importance. **Behboud *et al*. (2019)** studied the role chitosan nanoparticles to mitigate adverse effects of drought in the wheat under drought stress. Added chitosan nanoparticles through soil and foliar application. Results indicated that application of the chitosan nanoparticles increased leaf area, yield, relative water content, photosynthesis rate, chlorophyll content and superoxide dismutase activities compared to the control. under drought stress under drought stress. **Mazrou *et al*. (2021)** investigated the impact of Chitosan Nanoparticles foliar application at 100, 200, 300 and 400 mg L^{-1} on the growth, antioxidant capacity and essential oil productivity of chamomile. Experience has shown Chitosan Nanoparticles foliar application enhanced the growth, photosynthetic pigment, productivity, N, P and K percentages and total soluble sugars of chamomile plants. **Ali *et al*. (2022)** recommended that chitosan nanoparticles as eco-friendly applications on maintaining the quality of damask rose flowers during storage at 4 or 20 ◦C. whereas the results demonstrated that chitosan nanoparticles application of 1% were help preserve the quality and extend the shelf life. **Mawale and Giridhar (2024)** recommend that the use of chitosan nanoparticles as an environmentally friendly approach to enhance growth, disease resistance and secondary metabolite production of *Capsicum annuum* plants. Whereas the application of chitosan nanoparticles with different concentrations on the leaves of Capsicum spp. plants resulted in improved physiological traits, content of chlorophyll, carotenoids, total phenolics, total flavonoids and protection against thrips.

There are some studies and researches discussed about the comparison between of the effects of chitosan and chitosan nanoparticles on plants. **Spricigo *et al*. (2020)** applied Chitosan and Chitosan nanoparticles as antimicrobial during postharvest storage of gerbera. The results showed chitosan nanoparticles solution prevented stem bending, enabled higher, established a suitable and controlled microbial growth more efficiently and inhibited yeasts and molds than chitosan solution the other solutions. **Bakhoum *et al*. (2022)** they investigated the effect of chitosan and chitosan nanoparticles on alleviating the adverse effect of drought stress on *Lupinustermis* L. Plant under drought conditions. The results recorded that seeds treated with chitosan or chitosan nanoparticles significantly increase in growth parameters, yield, pigments, proline, free amino acid contents, IAA and total Soluble Solids comparing with controls. However, chitosan nanoparticles were more effective than Chitosan on all parameters.

2. Materials and Methods

2.1. Study site

Two field experiments were carried out at one of the newly reclaimed agricultural lands, Qalabsho, Gamasa area, Dakahlia Governorate, Egypt, during two successive seasons of 2019/2020 and 2020/2021.

2.2. Soil analysis

A composite soil sample was taken from the surface soil with a different depth (0–50 cm). was collected from the soil used in the study to examine the Physic-chemical properties of the soil. The methods described by **Black (1965)** was followed to examine soil texture. CaCO3, soil pH, EC, N, P, K and cations exchange capacity (CEC), anions were determined **Page *et al*. (1982)** and presented in Table (1).

Table (1). The physical and chemical properties of the experimental soil

Mechanical analysis (%)				Chemical analysis		
soil Texture	Sand %	Silt %	Clay %	$CaCO_3$%	pH	EC ds m^{-1} (1:5)
sandy	58.2	9.2	32.5	0.001	7.90	3.31

Available (mg/100 g soil)			Cations (meq/100 g soil)				Anions (meq/100 g soil)			
N	P	K	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	Cl^{-}	SO_4^{--}
50.97	78.5	119	0.45	0.13	1.98	0.08	0.00	0.20	1.39	0.92

2.3. Plant material

Seeds of marigold were obtained from the Medicinal and Aromatic Plants Department. of Horticulture Research, Institute. Agriculture, Research Center.

Calendula seeds were sown at first week of September in seeding tray filled with standard peat moss, after 45 days from sowing date the seedlings were transferred to the experimental field area then sowing in hills at 40 cm apart in rows of 5 m^2 length and 50 cm apart between rows in both seasons.

The irrigation of the experiment was executed out was drip irrigation with the drippers of five liters/h for one hour twice every week. The spaces between plants were 40 cm and between pipes were 50 cm on the row. The experiment was fertilized according to the recommendation of the Egyptian Ministry of Agriculture. The rate of ammonium nitrate was 375 kg ha^{-1}, calcium superphosphate at the rate of 500 kg ha^{-1} and the rate of potassium sulfate was 250 kg ha^{-1} incorporated into the soil. All fertilization was applied in separate applications.

2.4. Foliar application materials

Chitosan Preparation

Stock solution (2% w/v) of chitosan (shrimp waste) was prepared by dissolving purified in 0.5% (v/v) acetic acid, under continues stirring and by using 1.0 M NaOH have been adjusting the pH of solution to 5. The stock solution was sterilized at 120°C for 20 minutes, and then by appropriate dilution with distillated water were made lower concentrations (100, 200 and 300 mg/L^{-1}) as described by **Du *et al*. (1997)**

Chitosan nanoparticles Preparation

A chitosan sample (1 g) was dissolved in acetic acid (100 mL^{-1}) to prepare a 10 g L^{-1} solution. The solution was stirred for 30 min at 60 C° and after that the stirring was continued at lab temperature. by using 1.0 M NaOH have been adjusting the pH of solution to 5. The Tween-20 surfactant (400 mL) was added to the chitosan solution and then centrifuged for 10 min at 10,000× g. The aqueous solution of Na-TPP was gradually added into the solution through magnetic stirring at room temperature, the mixture was re-centrifuged at 10,000× g for 35 min for CSNPs collection. Finally, the supernatant was preserved at 4 °C until application. before the foliar spray, to prepared three concentrations of nano chitosan particles (20m,40 and 60 mg/L^{-1}) as described by **Fan *et al*. (2012)**.

2.5. Experimental Design

The experiment was conducted in a completely randomized block design. the experiment included 7 treatments in three replicates. The treatments which were arranged as follow:

- Control.
- Chitosan at three concentrations (100,200 and 300 mg/L^{-1}).
- Chitosan Nanoparticles at three concentrations (20m,40 and 60 mg/L^{-1}).

All treatments were sprayed on the foliage of calendula plants till cover whole plant foliage completely after 15 days from transplanting date with 15 days intervals. Individual area (plot) containing 3 rows spaced at 50 cm with size were 8.75 m^2 (5×1.75 m^2).

2.6. Morphological growth Characteristics

A random sample of nine plants from each replicate was taken on 1st January after 100 days from transferred in the both seasons for determining the following characters as follows: number of branches /plant, plant height (cm) and fresh and dry weights of plant (g / plant).

Floral characters

Harvesting of fresh flower heads was collected from each treatment from beginning of flowering until end of flowering in both seasons and the data were recorded as follows: Number of flowers and flowers fresh and dry weights (g /plant).

Essential oil determination

Fresh flower heads (500 g) from each of treatments were collected in both seasons to extract the essential oil by subjecting flowers to hydro distillation for 3 h in Clevenger apparatus **Clevenger (1928)** according to the method described by **British Pharmacopoeia (2000)** to determination the oil percentage. Also, oil yield was calculated by multiplying the oil % by average fresh weight of flower heads yield per plant and expressed as volume in ml.

Estimation of Chlorophyll Pigments, carotenoids and total flavonoids

The photosynthetic pigments of total chlorophyll in the leaves and carotenoids contents in flowers were estimated according to **Sumanta *et al*. (2014)** as for determination of total flavonoids was according to **Pourmorad *et al*. (2006)**.

Mineral contents

The contents of N, P, K, Fe, Cu, Mn and Zn were measured **AOAC (1990)**. The samples were dried at 70 ℃ then crushed and digested using H_2SO_4 and HClO4 acids and Nitrogen, Phosphorus were determined according to **Cottenie *et al*. (1982)** and Potassium, iron, copper, manganese and zinc were determined as described by **Jackson (1967)**.

2.7. Statistical analysis

In this experiment, all physicochemical parameters were performed by using the complete randomized design. The data of the present study was analyzed with the Analysis of variance (ANOVA) procedure of the COSTAT program. When significant differences were detected, means were compared by LSD range test at the 5% level **Snedecor and Cochran (1980)**.

3. Results

3.1. Morphological growth Characteristics

According to results, nutrient deficiency at the newly reclaimed soil was significant negatively affected on plant growth characteristics of *Calendula officinalis* L. (Table 2). While, the foliar applications of chitosan and chitosan nanoparticles were significant positively affected on pant height (cm), number of branches / plants, plant fresh and dry weight (g). The results showed in (Table 2) indicated that with increasing concentration of the chitosan led to an increase in growth characteristics values. While, chitosan nanoparticle foliar application was superior chitosan in their effect on plant height (cm), number of branches / plants, plant fresh and dry weight(g) in both seasons. Meanwhile, sprayed with chitosan nanoparticle (40 mg/L-1) was the most effective in increasing growth characteristics compared to other treatments and control.

Table (2). Growth characteristics plant height (cm), number of branches / plants, plant fresh and dry weight (g) of calendula (*Calendula officinalis* L.) affected by foliar application of chitosan and chitosan nanoparticles in two seasons

Treatments	Plant height (cm)		Number of branches / plants		Plant fresh weight (g)		Plant dry weight (g)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	41.93	45.90	4.33	5	469.66	586.06	87.06	105.21
Chitosan 100 mg/L-1^{-1}	48.06	49.33	6.33	6.33	648.93	692.53	109.74	126.29
Chitosan 200 mg/L^{-1}	50.66	51.56	7.33	7.66	738.33	791.53	145.61	152.17
Chitosan 300 mg/L^{-1}	53.96	55.43	9	9.33	971.66	1004.03	191.61	195.193
Nan Chitosan 20mg/L^{-1}	51.3	51.73	8	8	836.90	919.56	174.09	179.27
Nan Chitosan 40 mg/L^{-1}	66.46	67.53	11.66	13.33	1356.86	1830	267.27	354.61
Nan Chitosan 60 mg/L^{-1}	60.23	60.20	9.66	10.33	1031.73	1156.80	206.94	216.41
L.S.D. at 5 %	**2.82**	**3.18**	**1.24**	**1.18**	**97.96**	**202.12**	**23.00**	**43.45**

3.2. Floral characters

Data mentioned in (Table 3) reveal that all treatments of foliar application (chitosan, chitosan nanoparticles) treated the negatively effect of nutritional deficiencies on flower yield in *Calendula officinalis* L.in both seasons whereas, the plants sprayed with chitosan at 100 mg/L^{-1} recorded the lowest effect to improving of number of flowers/ plant(30 and 31.33),flower fresh (159.70 and 229.97 g) and dry weight/ plant (25.46 and 29.73 g) while, chitosan at 200 mg/L^{-1} recorded number of flowers/ plant(39 and 42.33),flower fresh (264.12 and 276.63 g) and dry weight/ plant (39.24 and 41.07 g) however the best concentration of chitosan at 300 mg/L^{-1} number of flowers/ plant(43 and 46.33),flower fresh (294.25 and 303.01 g) and dry weight/ plant (40.69 and 43.84 g) .Meanwhile, the most effective to improving of number of flowers/ plant (60.67 and 66.33),flower fresh (430.56 and 452.73 g) and dry weight/ plant (49.83 and 51.43 g) were recorded from the plants were spray with chitosan nanoparticles at 40 mg/L^{-1} concentration followed by chitosan nanoparticles at 60 mg/L-1 compared to other treatments and the control treatments number of flowers/ plant(22 and 26),flower fresh (109.56 and132.96 g) and dry weight/ plant (20.35 and24.37 g).

3.3. Essential oil determination

Chitosan and chitosan nanoparticles were significantly affected on the essential oil percentage and yield of *Calendula officinalis* L. in (Table 4). Chitosan significantly increased gradually the essential oil percentage and oil yield per plant from plants sprayed with chitosan at 100 mg/L^{-1} (0.11and 0.13 %) and (0.013 and 0.018 ml/ plant) to from plants sprayed with chitosan at 200 mg/L^{-1} (0.13 and 0.15 %) and (0.020 and 0.035 ml/ plant) and plants sprayed with chitosan at 300 mg/L^{-1} (0.13 and 0.18 %) and (0.036 and 0.050 ml/ plant) respectively.

Application of chitosan nanoparticles at all concentrations caused more significant increases in the essential oil percentage and oil yield per plant from plants more than foliar application of chitosan and the control treatment in both seasons as presented in (Table 4). A maximum essential oil percentage (0.25 and 0.26 %) and oil yield per plant (0.10 and 0.11 ml/ plant), respectively were recorded from plants sprayed with chitosan nanoparticles at 40 mg/L^{-1}. Followed by plants sprayed with chitosan nanoparticles at 60 mg/L^{-1} was recorded (0.23and 0.24 %) and oil yield per plant (0.084 and 0.097 ml/ plant) compared with plants sprayed with chitosan nanoparticles at 20 mg/L^{-1} gave (0.20 and 0.24 %) and oil yield per plant (0.059 and 0.075 ml/ plant) respectively in both seasons.

Table (3). Floral characters (umber of flowers/ plant, flower fresh and dry weight (g) of calendula (*Calendula officinalis* L.) affected by foliar application of chitosan and chitosan nanoparticles in two seasons

Treatments	Number of flowers/ plant		Flower fresh weight/ plant (g)		Flower dry weight /plant (g)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	22	26	109.56	132.96	20.35	24.37
Chitosan 100 mg/L^{-1}	30	31.33	159.70	229.97	25.46	29.73
Chitosan 200 mg/L^{-1}	39	42.33	264.12	276.63	39.24	41.07
Chitosan 300 mg/L^{-1}	46.33	51	334.56	350.40	42.79	45.03
Nan Chitosan 20mg/L^{-1}	43	46.33	294.25	303.01	40.69	43.84
Nan Chitosan 40 mg/L^{-1}	60.67	66.33	430.56	452.73	49.83	51.43
Nan Chitosan 60 mg/L^{-1}	54	57.66	363.44	401.20	45.50	48.51
L.S.D. at 5 %	**2.14**	**3.88**	**24.63**	**36.03**	**3.05**	**3.08**

Table (4). Essential oil determination (essential oil % and essential oil yield ml / plant) of Calendula (*Calendula officinalis* L.) affected by foliar application of chitosan and chitosan nanoparticles in two seasons

Treatments	Essential oil %		Essential oil yield (ml / plant)	
	1st season	2nd season	1st season	2nd season
Control	0.11	0.13	0.013	0.018
Chitosan 100 mg/L^{-1}	0.13	0.15	0.020	0.035
Chitosan 200 mg/L^{-1}	0.13	0.18	0.036	0.050
Chitosan 300 mg/L^{-1}	0.16	0.21	0.053	0.073
Nan Chitosan 20mg/L^{-1}	0.20	0.24	0.059	0.075
Nan Chitosan 40 mg/L^{-1}	0.25	0.26	0.10	0.11
Nan Chitosan 60 mg/L^{-1}	0.23	0.24	0.084	0.097
L.S.D. at 5 %	**0.013**	**0.012**	**0.0066**	**0.0059**

3.4. Chlorophyll pigments, carotenoids and total flavonoids

A decreasing in the accumulation of pigments content in flowers as results to nutrient deficiency in soil was clearly in (Table 5).

Whereas chlorophyll pigments, carotenoids and total flavonoids content increased with foliar application of chitosan and chitosan nanoparticles. The increase in pigments content was significant increase gradually with increase of concentrations of chitosan from 100 to 200 to 300 mg/L^{-1} and while, chitosan nanoparticle concentrations were vary in their effect on chlorophyll pigments, carotenoids and total flavonoids content whereas, plants were spray with chitosan nanoparticles at 40 mg/L^{-1} concentration recorded the highest values followed by chitosan nanoparticles at 60 mg/L-1 concentration as for the chitosan nanoparticles at with 20 mg/L^{-1} recorded the lowest values of pigments content.

Table (5). Estimation Pigments (total chlorophyll, carotenoids and total flavonoids mg/ gm F.W) of calendula (*Calendula officinalis* L.) affected by foliar application of chitosan and chitosan nanoparticles in two seasons

Treatments	Total chlorophyll (mg/ gm F.W)		Carotenoids (mg/ gm F.W)		Total flavonoids (mg/ gm F.W)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	1.25	0.233	22.21	20.38	20.54	19.29
Chitosan 100 mg/L^{-1}	1.35	0.549	22.79	21.04	21.21	20.46
Chitosan 200 mg/L^{-1}	1.46	0.555	23.29	21.49	21.39	21.63
Chitosan 300 mg/L^{-1}	1.60	0.622	24.03	22.01	23.63	22.45
Nan Chitosan 20mg/L^{-1}	1.54	0.596	24.16	22.39	23.22	22.14
Nan Chitosan 40 mg/L^{-1}	1.72	0733	24.90	23.83	24.95	25.31
Nan Chitosan 60 mg/L^{-1}	1.67	0.647	24.61	23.51	24.15	24.38
L.S.D. at 5 %	**0.042**	**0.069**	**0.29**	**0.42**	**0.54**	**0.61**

3.5. Mineral contents

The data in (Table 6) noticed that the nutrient deficiency in soil led to decrease in content of macronutrients of N, P and K % and also the micronutrients of Fe, Cu, Mn and Zn % in head flowers of *Calendula officinalis* L. represented in the control treatment. However, foliar application of chitosan and chitosan nanoparticles were a significant increase in the content of macro- nutrients of N, P and K % in head flowers and also micronutrients of Fe, Cu, Mn and Zn % of *Calendula officinalis* L**.** The high content % of macronutrients and micronutrients observed from sprayed with chitosan at 300 mg/L^{-1} compared with chitosan at 100 mg/L^{-1} or 200 mg/L^{-1}. Plants sprayed by chitosan nanoparticles at different concentrations were superior to chitosan foliar application or the control. According to results, chitosan nanoparticles at 40 mg/L^{-1} concentrations was the most effective compared to other treatments in both seasons.

Table (6). Mineral contents (Macronutrients of N, P and K % and Micronutrients of Fe, Zn, Cu and Mn %) of calendula (*Calendula officinalis* L.) affected by foliar application of chitosan and chitosan nanoparticles in two seasons

Treatments	N%		P%		K%		Fe%		Zn%		Cu%		Mn%	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	1.06	1.16	0.0021	0.0026	0.466	0.520	0.56	0.66	0.13	0.16	0.20	0.20	0.13	0.17
Chitosan 100 mg/L^{-1}	1.31	1.40	0.0030	0.0034	0.586	0.633	0.66	0.73	0.20	0.20	0.23	0.24	0.20	0.20
Chitosan 200 mg/L^{-1}	1.45	1.57	0.0044	0.0048	0.667	0.666	0.67	0.73	0.25	0.26	0.26	0.26	0.27	0.26
Chitosan 300 mg/L^{-1}	1.73	1.73	0.0052	0.0057	0.746	0.836	0.80	0.80	0.30	0.30	0.33	0.40	0.40	0.47
Nan Chitosan 20mg/L^{-1}	1.68	1.67	0.0046	0.0050	0.733	0.750	0.73	0.76	0.23	0.27	0.30	0.33	0.36	0.37
Nan Chitosan 40 mg/L^{-1}	1.83	1.86	0.0063	0.0066	0.853	0.853	0.90	0.90	0.36	0.40	0.40	0.50	0.60	0.63
Nan Chitosan 60 mg/L^{-1}	1.81	1.82	0.0053	0.0065	0.826	0.840	0.80	0.83	0.32	0.33	0.40	0.40	0.53	0.56
L.S.D. at 5 %	**0.112**	**0.078**	**0.036**	**0.083**	**0.0200**	**0.052**	**0.077**	**0.088**	**0.071**	**0.072**	**0.059**	**0.11**	**0.107**	**0.080**

4. Discussion

Newly reclaimed soils often suffer from nutrient deficiencies due to a combination of factors. The primary issues include low organic matter content, which is essential for soil fertility, and poor water-holding capacity, which leads to nutrient leaching. Furthermore, these soils often have a high pH level, making critical nutrients like phosphorus and iron unavailable to plants. Another common problem is a nutrient imbalance, where high levels of certain elements, such as calcium and magnesium, can interfere with the uptake of other vital nutrients like potassium. All of these factors combined create an environment that is not conducive to healthy plant growth.

This study, is evident from the above results that the different concentrations of chitosan applied by foliar spraying encouraged growth parameters number of flowers / plant, flowers fresh and dry weights (g / plant and kg/ fed.), essential oil, total chlorophyll, carotenoids, total flavonoids and mineral contents of *Calendula officinalis* L. the antiperspirant effect of chitosan is important for C3 plants to benefit photosynthesis because it causes stomatal closure, which in turn reduces water loss through perspiration. This reduction in water loss helps regulate water availability for the plant's various processes. For C3 plants, which are often susceptible to water stress, this regulation is critical **Hidangmayum *et al*. (2019)**. When water is conserved, the plant can maintain its essential functions, including photosynthesis, more efficiently, even in conditions where water might be limited. Essentially, by helping the plant hold onto water, chitosan ensures that the plant has the resources it needs to keep its photosynthetic machinery running optimally, leading to improved growth and productivity **Iriti *et al*. (2009)**. These results are in accordance with those obtained by **Ibraheim and Mohsen (2015)** on summer squash plants, **Abd-El-Hady (2020)** on tuberose **Abdel-Wahed and Shaker (2020), Rogowska *et al*. (2023)** and **Rojas-Pirela *et al*. (2024)** on *Calendula officinalis* L.

In addition, chitosan nanoparticles were as significantly improving the marigold parameters number of flowers / plant, flowers fresh and dry weights (g / plant and kg/ fed.), essential oil, total chlorophyll, carotenoids, total flavonoids and mineral contents at three different concentrations. The study reveals that using chitosan nanoparticles as a bio stimulant significantly enhances the growth and productivity of plants. The improvement is achieved through several key mechanisms: Enhanced Photosynthesis: chitosan nanoparticles boost the plant's relative water content (RWC) and photosynthetic pigment levels, which are directly linked to improved growth **Zhang *et al*. (2017)**. Hormonal and Metabolic Regulation **Guan *et al*. (2009)** The nanoparticles activate signaling pathways for essential plant hormones like auxins and gibberellins, and they regulate carbon and nitrogen metabolisms. better water and nutrients uptake **Safikhan *et al*. (2018)**: chitosan nanoparticles increase root growth and adjust cell osmotic pressure, leading to more efficient water and nutrient absorption. Superior to Bulk Chitosan: The small size and large surface area of chitosan nanoparticles make them more effective than bulk chitosan. This positive effect on growth has also been observed in other medicinal plants, such as thyme **Pirbalouti *et al*. (2017)** and sweet basil **Bistgani *et al*. (2017)**. chitosan nanoparticles at 40 mg/L^{-1} concentration followed by chitosan at 300 mg/L^{-1} was the most effective compared to other treatments in both seasons.

5. Conclusions

The present study concluded that, foliar application of chitosan and chitosan nanoparticles had significant effect to reducing of the negative effect of the nutrient deficiency in soil at the newly reclaimed soil on growth parameters, floral character, essential oil, total chlorophyll, carotenoids, total flavonoids and mineral contents of *Calendula officinalis* L. The study cleared that, foliar application of chitosan was a significant increase in all studied parameters of *Calendula officinalis* L. while, plants sprayed by chitosan nanoparticles was superior to chitosan foliar application.

Finally, it could be concluded that treating *Calendula officinalis* L. plant by applying by foliar spraying with 40 mg/L^{-1} chitosan nanoparticles followed by chitosan at 300 mg/L^{-1} to give the best results regarding growth parameters, flower characters, essential oil, Chlorophyll pigments, carotenoids and total flavonoids and mineral of *Calendula officinalis* L. plant under newly reclaimed soil.

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