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Investigating the Physiological Roles of Bulk Chitosan and Nano-Chitosan in Growth, Flowering, Yield Attributes of *Brassica napus* L. and in Nutrient Delivery in Sandy Soils

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ABSTRACT

A field experiment was conducted during Winter season 2019/2020 at the Research and Production Station, National Research Centre, El-Nubaria Province, El-Behira Governorate, Egypt to investigate the different roles played by either bulk chitosan (CHT) (3000,5000, 7000,9000 ppm), or its nano-form (25,50,75,100,200 ppm) on growth, flowering, quality and quantity of the yielded seeds of *Brassica napus* L plant (cv. Serw 4) grown under sandy soil conditions. The results obtained in the present work indicate a significant positive effect of the relatively low concentrations of bulk chitosan (3000, 5000 ppm) and nano-chitosan (25, 50 ppm) on different growth, flowering, and fruiting attributes of *Brassica napus.* plants. Maximum response was attained upon using 5000 ppm of bulk CHT and 50 ppm of nano CHT. Upon applying the relatively highest concentration (9000 ppm of bulk CHT and 200 ppm of nano CHT), it was noted that growth, flowering, and fruiting parameters were negatively affected. CHT treatment proved its efficiency of increasing *Brassica napus* yield by 85.93% and 71.1%, in response to 5000 ppm of bulk CHT and 50 ppm of nano CHT, respectively beside improvement of the better consumer properties of *Brassica napus* yielded seeds with regard to increase content of oil, total carbohydrate, protein-N, nitrogen, phosphorous, potassium and increase of oleic acid content. Regarding the change in erucic acid content, the data obtained indicate appreciable increase and decrease in response to bulk CHT and nano CHT, respectively. The resultant defatted meal (seed cake) contained a relatively low content of glucosinolates. It could be concluded that 5000 ppm of bulk CHT and 50 ppm of nano CHT may be used to increase the quality and quantity of the yielded *Brassica napus* seeds grown under sandy soil conditions.

Keywords: *Brassica napus*, canola, chitosan, nano-chitosan, seed soaking, erucic acid.

1. Introduction

Plant oils represent a major agricultural commodity worldwide, with several nutritional and industrial uses. They constitute a significant portion of the daily human caloric intake and the raw materials for a variety of industries as surfactants, plastics, lubricants, biofuels, soaps, detergents, and paint industries. Egypt imports 2.5 million tons of oil annually as it produces less than 10 per cent of its consumption of vegetable oil. Only, about 48.5 thousand tons of this quantity is produced by oilseed extraction in local extraction plants (El-Hamidi *et al.,* 2020). In view of the rapid increase in the population in Egypt whereby Egypt's population grows by approximately 1.5 million people each year and in view of the fact that the consumption rate per capita of edible oils has been progressively increased in recent years, the consumption rate of edible oil has been greatly increased (El-Hamidi and Zaher, 2018). This issue became terrible because of the reliance of the edible oil industry in Egypt on imported crude materials. Thus, there is the need for both expansion of land cultivated with the oily plants through utilizing the newly reclaimed lands and the increase in plant productivity via its treatment with growth regulating substances.

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Cultivation of canola (*Brassica napus* L.) may provide an opportunity to cover some of the local deficit of vegetable edible oil production. Since, *Brassica napus* (oil seed rape) represents one of the important oily edible crops that are well adapted to grow under severe winter conditions (Sharaan *et al.,* 2002). Moreover, they are less exhaustive of soil nutrients, compared with cereals. Canola has become one of the most important oilseed crops worldwide (Kandil and Gad, 2012). Canola oil is the third largest vegetable oil after palm and soybean (Wittenberger, 2012). The oil content of canola seeds varies between 38 and 44%; having a relatively high level of oleic acid (60%), omega-3 (9-11%) (Dawood, 2005; Johnson *et al.,* 2007). Thus, canola oil is the most favorable of vegetable oils that has beneficial role as a part of a nutritious diet (Hodson *et al.,* 2001; Iggman *et al.,* 2011). Major advantage of growing canola is that it has an already established market for vegetable oil and other non-food uses such as industrial lubricants, cosmetics, candles etc., in addition to also being extensively developed as a biofuel feedstock (Chaganti *et al.,* 2021). Canola meal (cake after the oil is extracted) is a good source of protein, containing 38 to 42% protein. It can be used as a protein source in animal feeds or as an organic fertilizer. The meal protein has a reasonably balanced amino acid composition and rich in sulfur-containing amino acids (Campbell *et al.,* 2016). The increase in plant productivity to cope with the increase in both population and uncontrolled consumption of farmlands and the interest in the high quality and safety of foods have increased the research that can meet this demand. The searches were focused on using natural resources which are considered a novel eco-friendly farming practice that led to crop intensification and sustainability (Paradikovic *et al.,* 2019). The use of natural biopolymer-based materials is among the eco-friendly farming compounds which have been shown to increase productivity of agricultural plants.

Chitosan (polymer of glucosamine and N-acetyl glucoseamine) is a natural safe and cheap biopolymer established as a non-toxic, biodegradable, and biocompatible compound (Katiyar *et al.,* 2015; Shahrajabian *et al.,* 2021). It is now recorded to effectively act as a plant growth regulator and as effective signal biomolecules (Hadwiger *et al.,* 2002; Uthairatanakij *et al.,* 2007; Gornik *et al.,* 2008). Thus, it can be used for improving growth, productivity, and quality of different crops (Khan *et al.,* 2002; Chibu and Shibayama, 2003 and Zhang *et al.,* 2017). Besides, CHT may be enhanced enzyme activities of nitrogen metabolism, and improved the translocation of nitrogen thus increased plant growth and development (Sultana *et al.,* 2017). Further, chitosan treatment induced over-expression of genes involved in photosynthesis, changes in programming of protein metabolism with an enhancement of various storage proteins and hormone metabolism (Landi *et al.,* 2017). Moreover, CHT was recorded to effectively act as soil stabilizer, and improve major soil mechanical properties (Shariatmadari *et al.,* 2020).

Modernistic, nano-technology had proved its position in agriculture and related industries. Chitosan nano-particles (nano CHT) are environmentally friendly, bioactive substance (Agnihotri *et al.,* 2004) and an attractive and promising alternative to solve poor insolubility of bulk CHT (Harshil *et al.,* 2016), and proved its potentiality at a relatively low concentrations compared with bulk form. Moreover, nano CHT effectively acts as a delivery system of nutrients, pesticides, and hormones (Silva *et al.,* 2011; Vanti *et al.,* 2020). Moreover, nano-formulation of CHT proved its potential as a sustainable alternative in crop protection against pests, diseases, and its efficiency as a carrier of agrichemicals (Campos *et al.,* 2015; Kashyap *et al.,* 2015; Hernández-Téllez *et al.,* 2016).

This investigation aimed to study the different roles played by bulk CHT and nano CHT on growth; flowering, different fruiting attributes, quality, and quantity of the yielded seeds of *Brassica napus* L. cv. Serw- 4 grown under sandy soil conditions.

2. Materials and methods

2.1. Experimental site

The field experiment was conducted at Research and Production Station, National Research Centre, Al-Nubaria District, Al-Behaira Governorate, Egypt. The experiment was carried out in winter season (2019/2020) for the purpose of evaluating the different roles played by bulk CHT and nano CHT on growth, flowering, and different fruiting attributes of *Brassica napus L.* cv. Serw-4 plants.

2.2. Experimental design

The reclaimed sandy soil, used in the experiment was ploughed twice, ridged, and divided into plots (5.0 m long and 1.6 m width: with area $8m^2$). The experiment was a Complete Randomized Block

Design with three replications in lines 5-meter-long, 0.60 meter apart. Hill spacing was 15 cm within the lines. Calcium super-phosphate (15.50% P_2O_5) was added pre-sowing at 150 Kg/feddan to the soil. Similarly, potassium sulphate (48% K_2O) was added pre-sowing at the rate of 50 kg/feddan to the soil, while nitrogen fertilizer was added at the rate of 45 kg N/feddan as ammonium nitrate (33.5%N) in two equal doses at 21 and 35 days after planting, respectively.

2.3. Plant material preparation

Canola seeds (*Brassica napus* L. cv. Serw 4) were obtained from Oilseed Department, Agricultural Research Centre, Ministry of Agriculture and Land Reclamation, Egypt. A homogenous lot of *Brassica napus* L. seeds were selected for uniformity by choosing those of equal size and with the same color, washed, sterilized by soaking with sodium hypochlorite (1%) for two minutes and then washed thoroughly with distilled water. This experiment was divided into two parts. The first part included soaking seeds for 6 hours with bulk CHT at (3000,5000,7000 or 9000 ppm) before sowing and the developed plants were subjected to spraying with above concentrations of CHT twice at 50 and 90 days after sowing (DAS). The second part included spraying of canola plants developed from water-soaked seeds with nano- CHT at the concentrations 25, 50, 75, 100, or 200 ppm at 60 and 75 DAS. The soaked seeds were sown at 3-5 seeds on each hill in mid of November. Sprinkler irrigation took place immediately after sowing, then every week intervals according to agronomic practices in the district. Thinning was carried out 30 days after sowing to secure two plants per hill on one side of the ridge.

2.4. Data recorded

At vegetative stages at 75 DAS, a random sample of ten plants from each plot were taken to determine the following parameters: shoot length, root length, number of leaves per plant, leaf area/plant, shoot and root fresh and dry weights, as well as photosynthetic pigments content in leaf tissues.

At flowering stage at 105 DAS, a random sample of ten plants from each plot were taken to determine the number of flowering branches, number of fluorescences per plant, date of onset and ending of flowering.

At harvest time, random samples of ten plants from each plot were taken to determine yield attributes including: number of siliqua/plant, siliqua length, and number of seeds/ siliqua. Plants of two square meter from the middle rows of each plot were harvested, dried under sunshine for one week and seeds were cleaned after separation from the siliqua, then the seed yield (kg/faddan) was determined. Oil, protein, total carbohydrate, and mineral (NPK) contents were determined in the yielded seeds. Seed cake obtained after oil extraction of seeds subjected to analysis included its content of glucosinolates.

2.5. Biochemical analysis

The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were extracted and determined spectrophotometrically following the method recommended by Moran and Porath (1980). The oil content of the seeds was determined according to the procedure reported in the A.O.A.C. (1990). Preparation, determination, and identification of fatty acids were carried out according to the method of Fedak and Dela Roche (1977). The total nitrogen was determined by Micro-Kjeldahl method according to the procedure reported in the A.O.A.C. (1990). The nitrogen content was multiplied by the factor 6.25 to obtain the protein content. Total carbohydrate content was extracted and determined according to Dubois *et al.,* (1956). For estimating nitrogen (N), phosphorous (P), and potassium (K) content in seeds; seed samples were dried at 70°C until constant weight and digested according to Cottenie *et al.* (1982). Phosphorus content was estimated by spectrophotometric method as described by Cottenie *et al.* (1982). Potassium content was estimated by a flame photometer method according to Okalebo *et al.* (2002). Glucosinolates were extracted according to Rauchberger *et al.* (1979) and determined calorimetrically according to Nasirullah and Krishnamurthy (1996). Regarding determination of soil properties after harvesting, the soil porosity and water-holding capacity of the soil samples was determined as described by Piper (1947). The percolation and retention of water in soil was determined using rudimentary method.

2.6. Statistical analysis

The average data was statistically analyzed by using SPSS Version 26. The differences among means were determined by least significant differences (LSD) at 5% level according to Snedecor and Cochran (1982) and compared using Duncan's multiple range tests and standard error (± SE).

3. Results and Discussion

3.1. Effect of bulk chitosan on major growth attributes and photosynthetic pigments of *Brassica napus* L. plants

In response to application of bulk chitosan (seed soaking and foliar spray) at the relatively low concentrations, there was a significant increase at different stages of growth in shoots and roots length, number of leaves per plant, leaf area, fresh and dry weights of shoots and roots of *B.napus* plants (Table 1) as well as the content of photosynthetic pigments (Fig.1) with a maximum of response attained upon applying bulk chitosan at 5000 ppm relative to control. On the other hand, the relatively highest concentration of bulk chitosan (9000 ppm) induced a significant decrease in the above-mentioned growth parameters and non significant effect on photosynthetic pigments relative to control. These results obtained in the present work (Table 1) are confirmed by the work of Mohamed *et al.,* (2019) where they recorded appreciable increase in major growth parameters of orange as a result of using different concentrations CHT treatment. Similarly, Mohamed *et al.* (2018) reported that shoot height and diameter, leaf area, leaf fresh and dry weights of sour orange seedlings were affected positively by CHT spraying. Enhancement of growth induced by CHT may be attributed to its role in increasing efficiency of nutrient uptake, photosynthetic rate, stomatal conductance via regulating rate of ABA synthesis (Hidangmayun *et al.,* 2019, Chakraborty *et al.,* 2020, Monfared *et al.,* 2020). In addition, as chitosan is structurally related to oligosaccharides, it behaves as a signaling molecule and acts in a manner similar to plant growth regulators in regulation of morphogenesis, growth and development as reported by Cote and Hahn (1994); Hadwiger *et al.* (2002); Uthairatanakij *et al.* (2007); Gornik *et al.* (2008) and El-Bassiouny *et al.* (2023). Chitosan stimulated different plant responses associated with primary and secondary metabolism including: carbon and nitrogen metabolism, Calvin cycle, sucrose metabolism, glycolysis, tricarboxylic acid cycle, photosynthesis, as well as biosynthesis of phenolic compounds which enhanced plant growth and development (Zhang *et al.,* 2017; Ahmed *et al.,* 2020). In addition, the positive effects of chitosan on plant growth might be due to enhance stomatal conductance, and stimulate growth of xylem (Hidangmayum *et al.,* 2019) or may be due to increase the key enzyme activities of nitrogen metabolism (nitrate reductase, glutamine synthetase and protease) and improved the transportation of nitrogen in the functional leaves as well as increased photosynthesis which enhanced plant growth and development (Chibu and Shibayama, 2003 ; Gornik *et al.,* 2008; Mondal *et al.*,2012). Chitosan facilitates plant growth by increasing the uptake and availability of water and important nutrients by adjusting osmotic pressure in the cells (Guan *et al.,* 2009), and can promote the division of root cells by activating plant hormones including auxin and cytokinin that further lead to increased nutrient uptake (John *el al.,* 1997; Dzung *el al.,* 2011).

The significant increase in chlorophyll content of *B. napus* leaves obtained in the present work (Fig. 1) may be due to high rate of chlorophyll preservation induced by carotenoids which subjected to significant increase encountered in response to CHT application. In this regard, Pirbalouti *et al.,* (2017) and Rahman *et al.,* (2018) recorded appreciable increase in carotenoids in basil and strawberry tissues respectively following chitosan-elicitation. Likewise, Naderi *et al.* (2015) registered that chitosan increased chlorophyll and carotenoid of *Lepidium sativum* L plant by activating the genes involved in the biosynthesis of photosynthetic pigments. Stahl and Sies (2003) reported that carotenoids are pigments that play a very important role in the protection of plants against photooxidative processes in plants because of its antioxidants effect that play an extremely active role in eliminating the harmful effects of free oxygen radicals. In this connection, El-Bassiouny *et al.* (2023) explained the increase in photosynthetic pigments in wheat plant subjected to CHT treatment to be due to increased endogenous levels of cytokinins induced by CHT which play essential role in chlorophyll biosynthesis and decreasing the sensitivity of the pigments protein complex to light as reported by Chibu and Shibayama (2001).

Table 1: Effect of soaking and spraying with different concentrations of bulk-chitosan on growth parameters of *Brassica napus L.* plants at 75 DAS.

Treatments		Vegetative growth parameters							
		Shoot length (cm)	Root length (cm)	Number of leaves/ plant	Leaves area(cm^2)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot Dry Weight (g)	Root dry weight (g)
Bulk CHO (ppm)	Control	36.9 ± 0.33c	12.6 ± 0.24c	7.4 ± 0.24d	335.80 ± 4.92c	25.01± 0.40c	1.72± 0.03d	2.71 ± 0.05d	0.34 ± 0.007d
	3000	39.4 ± 0.24b	14.6 ± 0.18b	8.4 ± 0.24c	384.72 ± 2.38b	50.69± 0.46b	3.52± 0.09c	4.66 ± 0.09c	0.78 ± 0.006 c
	5000	46 ± 0.31a	15.4 ± 0.24a	10.4 ±0.24a	632.88 ± 4.82a	68.04± 0.62a	5.51± 0.13a	6.45 ± 0.08a	1.07 ± 0.016a
	7000	38.6 ± 0.48b	15.1 ± 0.10ab	9.4 ± 0.24b	378.91 ± 6.72b	51.56± 0.62b	4.73± 0.06b	5.22 ± 0.07b	0.97 ± 0.025b
	9000	27.9 ± 0.40d	9.5 ± 0.22d	6.4 ± 0.24e	225.29 ± 3.32d	17.56± 0.33d	1.27± 0.02e	1.71 ± 0.02e	0.23 ± 0.012e
L.S.D at 5%		0.51	0.29	0.34	6.61	0.71	0.11	0.09	0.02

Data are presented as mean of ten variables ± SE. Mean values in each column followed by different letter are significantly different by least significant difference test at 5%.

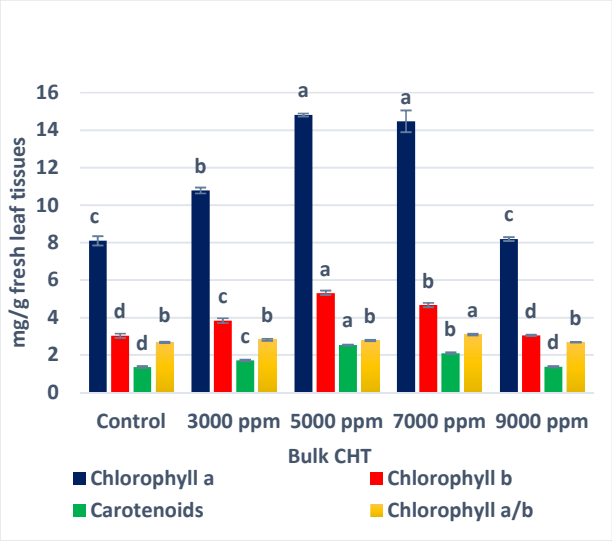

Fig. 1: Effect of soaking and spraying with different concentrations of bulk-chitosan on photosynthetic pigments content of *Brassica napus L.* plants at 75 DAS. Data are presented as mean of five replicates ± SE. Mean values in each parameter followed by different letter are significantly different by least significant difference test (LSD) at 5%.

3.2. Effect of nano-chitosan on major growth attributes and photosynthetic pigments of *Brassica napus* L. plants

In a comparable manner, *Brassica napus* L growth and development responded to application of nano CHT, where there was a significant increase in growth attributes (length of roots and shoots, leaf area per plant, fresh and dry weights of roots and shoots) (Table 2) and the content of photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) (Fig. 2) with an optimum increase obtained at 50 ppm followed by a significant decrease on applying 200 ppm of nano CHT relative to control. It was declared that chitosan nanoparticles are easily absorbed by the epidermis of leaves and translocated to stem which facilitated the uptake of active molecules and enhanced growth and productivity of plants (Malerba and Cerana, 2016). When chitosan nanoparticles entered through stomata, osmosis pressure of the stomata increased that lead to increase the opening of stomatal cells, stomatal conductance and CO_2 concentration (Van *et al.,* 2013). Moreover, chitosan and chitosan nanoformulations significantly increased seedling growth, seed germination, biomass accumulation and used as growth promoters by

enhancing the nutrient uptake (nitrogen, potassium, phosphorus, calcium and magnesium), chlorophyll content and photosynthesis rate (Van *et al.,* 2013; Anusuya and Banu, 2016; Saharan *et al.,* 2016; Sathiyabama and Manikandan, 2021).

Likewise, in green house condition, chitosan nanoparticles had significant impacts on biophysical characteristics of coffee seedlings such as increasing content of the pigments, nutrient uptake, photosynthesis net rate, CO_2 concentration integrated and switching on the genes related to the chlorophyll synthesis (Van *et al.,* 2013). They added that spraying coffee seedlings with three times of chitosan nanoparticles increased chlorophyll a, b and carotenoid content from 38.8 to 72.2% higher than the control.

Table 2: Effect of spraying with different concentrations of nano-chitosan on growth parameters of *Brassica napus L.* plants at 75 DAS.

Treatments	Shoot length (cm)	Root length (cm)	Number of leaves/ plant	Leaves area (cm^2)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot Dry Weight (g)	Root dry weight (g)
Control	39.02 ± 0.458 c	9.4 ± 0.244e	6.6 ± 0.244c	135.752 ± 1.852d	25.98 ± 0.515d	1.272 ± 0.022 e	2.472 ± 0.056 d	0.206 ± 0.005 e
25 ppm	47.6 ± 0.400a	15.5 ± 0.223 b	9.5 ± 0.223 b	482.216 ± 3.695 b	65.05 ± 0.723 b	4.596 ± 0.073 b	6.732 ± 0.060 b	0.912 ± 0.012 b
50 ppm	48.6 ± 0.509a	16.3 ± 0.200 a	10.4 ± 0.244 a	683.788 ± 6.627 a	74.296 ± 0.666 a	5.714 ± 0.055 a	7.566 ± 0.108a	1.146 ± 0.049 a
75 ppm	41.7 ± 0.538b	12.3 ± 0.200c	10 ± 0.316a/b	437.154 ± 3.886c	37.53 ± 0.432 c	2.476 ± 0.063c	3.636 ± 0.072c	0.492 ± 0.008 c
100 ppm	40.6 ± 0.244b	11.2 ± 0.122d	6.4 ± 0.244c	436.96 ± 5.173c	19.69 ± 0.085e	1.544 ± 0.015d	2.452 ± 0.036d	0.376 ± 0.006d
200 ppm	32.3 ± 0.374d	9.9 ± 0.400e	6.6 ± 0.244c	111.052 ±1.97e	17.934 ± 0.367f	1.068 ± 0.021f	1.59 ± 0.028e	0.22 ± 0.003e
L.S.D at 5%	0.61112	0.34881	0.36056	5.96583	0.72179	0.06756	0.09353	0.0302

Data are presented as mean of ten variables ± SE. Mean values in each column followed by different letter are significantly different by least significant difference test at 5%.

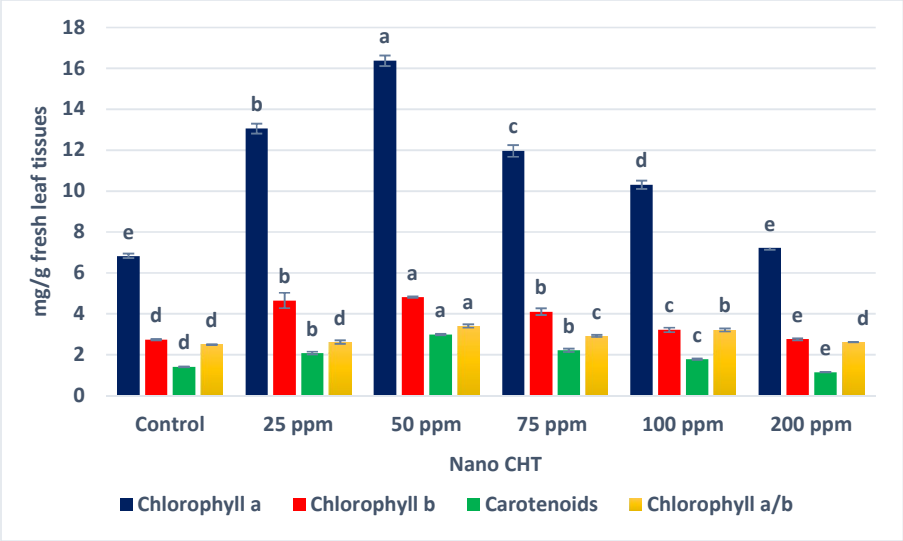

Fig. 2: Effect of spraying with different concentrations of nano-chitosan on photosynthetic pigments content of *Brassica napus L.* plants at 75 DAS. Data are presented as mean of five replicates ± SE. Mean values in each parameter followed by different letter are significantly different by least significant difference test (LSD) at 5%.

A comparison between the results obtained on application of bulk CHT and nano-CHT, it is obvious that maximum response in growth enhancement was attained at a relatively low concentration of nano CHT (50 ppm) compared with (5000 ppm) in case of using bulk CHT. Increased efficiency of

nano CHT may be due to that nano form of CHT characterized by a highly positive surface coating that can passively penetrate across the membrane of organelles as plastids, where these nano-particles exhibit both confined diffusion and convection before reaching an irreversible tapped state (Wong *et al.,* 2016). Thus, nano CHT may act as molecular transporters into organelles. Moreover, nano-particles were reported to be easily absorbed to epidermis of leaves and stems, thus prolonging the contact time facilitating their uptake (Yanat and Schroënk, 2021). Also, it was reported that both bulk CHT and nano CHT bind to the cells extracellularly with nano CHT being showing more intense localization (Navarro *et al.,* 2008), hence the dose demand of nano CHT is substantially lower and its efficiency is higher compared to bulk CHT. Nano formulation of agrochemicals, in general, are characterized by unique properties as having large specific surface area, high surface energy and quantum confinement (Nel *et al.,* 2006). These characteristics may acquire nano-particles additive functions, behaviors and environmental fate compared with their bulk counterparts (Taylor and Walton, 1993). Moreover, promotion of plant growth induced by nano-materials, in general, may be at least due to their potentialities of absorption of nutrients and water (Dubey and Mailapalli**,** 2016, Shojaci *et al.,* 2019), the slow or controlled release or loss of nano-fertilizers which permit the long-term availability for plant throughout its life cycle (Lateef *et al.,* 2016).

3.3. Effects of bulk chitosan and nano chitosan on flowering, and fruiting of *Brassica napus* L. plants

It is obvious from the data represented in Tables 3 and 4 that in a comparable manner to the response of vegetative growth of *B. napus* to chitosan treatments (bulk or nano CHO), the flowering and fruiting processes were affected. Thus, seed priming with bulk CHT followed by foliar spraying of bulk and nano CHT at the early vegetative and flowering stages had increased the number of inflorescences per plant with a maximum increase attained on applying 5000 ppm of bulk CHT and 50 ppm of nano CHT (Tables 3 and 4). This increase in number of inflorescences of CHT-treated *B. napus* may be attributed mainly to the obvious increase encountered in number of lateral flowering branches recorded in concentrations 5000 ppm (bulk CHT) and 50 ppm of nano CHT. Pre-harvest foliar application of chitosan on Washington navel orange tree increased number of inflorescences per plant, and number of flowers per tree (Mohamed *et al.,* 2019). Moreover, a positive effect of CHT in stimulating flowering and increasing percentage of flower number was reported by Wanichpongpan *et al.,* (2001) on gerbera. Similarly, Ramos-Garcia *et al*. (2009) recorded enhancement of major flowering criteria of gladiolus in response to CHT treatment.

The role of CHT in transition of plants to flowering stage is uncertain. However, it is known that CHT is one of the first signaling molecules in the jasmonic acid signaling pathway that led to high rate of jasmonic acid (JA) biosynthesis (Doares *et al.,* 1995). The presence of high level of JA in the reproductive structures of plants (flowers, fruits, and seeds), indicates that it has a crucial role in the flowering and fruiting stages. So**,** it may conclude that CHT, via induction of JA biosynthesis may enhance flowering of CHT-treated plants. Moreover, CHT was reported to enhance the biosynthetic pathway of gibberellins (El-Bassiony *et al.,* 2014**)** which efficiently has a crucial role in encouragement of flowering of plants (Taiz and Zeiger, 2006).

Bulk CHT and nano CHT treatments obviously interfered with the date of onset of flowering of *B.napus* plants (Tables 3 and 4). The transfer from vegetative to flowering stage was suggested to be merely a consequence of growth regulator - caused rapid growth and not really a direct effect of them, which lead to earliness of flower initiation (McDaniel, 1996). In the present work, bulk-CHT treatment was observed to enhance vegetative growth and accelerated development of treated plants which was recorded in earliness of flower initiation by 11 days before control (5000 ppm of bulk CHT) and from 5-12 days, (in response to applied concentrations of nano CHT). In this regard, it may be suggested that earliness of flowering of *B. napus* induced by CHT may be due to its effect on the change over from the vegetative buds to flowering state as a result of developmental enhancement and substantial change in bud organization as supposed by Blazquez and Weigel (2000). There was also an earliness of the ending of flowering which coincides with the start of siliqua formation in response to treatment with the concentrations 3000, 5000 and 7000 ppm achieving a maximum of 15 days in using the concentration 5000 ppm of bulk CHT. Upon applying nano CHT, there was an earliness of flowering of treated plants in a reverse concentration-dependent manner, where earliness of flowering reached 16 days (in response to the relatively low concentrations) and 7 days in response to the higher

concentrations (l00 and 200 ppm). In a comparable manner, the start of siliqua formation is affected by the concentration applied of nano CHT where it begins earlier 6 days on applying 100 and 200 ppm of nano CHT, compared with 16 days upon using the relatively low concentrations (25, 50,75 ppm). In a similar manner, CHT was reported to induce earliness of flowering of *Gompey fressia* as mentioned by Salachna and Zawadzińska (2014). Moreover, CHT treatment was recorded to cause shortening of the life cycle of nano-fertilized - treated plants where there was a reduction of about 25% in number of days required for yield production (Abdel-Aziz *et al.,* 2016). Such an acceleration of flowering and of plant production demonstrate the potentiality of nano-materials as effective tools in agricultural practices, particularly in sudden change in environment, in sudden flash flood-porne areas where the early maturity of crops protects them from exposure to this type of environmental hazards.

Table 3: Effect of seed soaking and foliar spraying with different concentrations of bulk chitosan on major flowering parameters of *Brassica napus* L. plants (105 DAS)

Treatments		Flowering parameters			
		Number of flowering branches plant^{-1}	Number of inflorescences plant^{-1}	Time of initiation of flowering (DAS)	Time of the beginning of siliqua formation (DAS)
Bulk – CHT (ppm)	Control	2.2 ± 0.20d	17.4 ± 0.24b	91	144
	3000	3.6 ± 0.24c	18 ± 0.45b	87	138
	5000	5.4 ± 0.24a	20.2 ± 0.37a	80	129
	7000	4.6 ± 0.24b	18.2 ± 0.37b	87	138
	9000	1.6 ± 0.2d	9.6 ± 0.24c	91	143

Data are presented as mean of ten variables ± SE. Mean values in each column followed by different letter are significantly different by least significant difference test at 5%.

Table 4: Effect of foliar spraying with different concentrations of nano chitosan on major flowering parameters of *Brassica napus* L. plants (105 DAS)

Treatments		Flowering parameters			
		Number of flowering branches plant^{-1}	Number of inflorescences plant^{-1}	Time of initiation of flowering (DAS)	Time of the beginning of siliqua formation (DAS)
Nano-CHT	Control	2.2±0.20c	6.8 ± 0.20d	92	145
	25	4.6±0.40a	17 ± 0.45c	80	129
	50	4.6±0.24a	23.2 ± 0.58a	80	129
	75	3.4±0.24b	19.6 ± 0.25b	80	129
	100	3.6±0.24b	16.8 ± 0.49c	87	138
	200	2.4±0.24c	17 ± 0.45c	87	138

Data are presented as mean of ten variables ± SE. Mean values in each column followed by different letter are significantly different by least significant difference test at 5%.

3.4. Effects of bulk chitosan and nano chitosan on components of yielded seeds of *Brassica napus* L plants

As indicated in Tables 5 and 6, a highly significant increase in major yield attributes was observed. Thus, treatment with CHT (bulk and nano-form) at different concentrations lead to a significant increase in length of siliqua, number of siliqua per plant, seeds number per siliqua, increase in percentage of yield per feddan with a maximum increase obtained on applying 5000 ppm and 50 ppm of bulk CHT and nano CHT, where it reached 85.93% and 71.1%, respectively. The strategies employed by chitosan in enhancing crop yield are diverse. The significant increase in yield of *Brassica napus* obtained in the present work could be explained on the bases of the obvious increase encountered in the number of inflorescences per plant, number of flowering branches (Tables 3 and 4), siliqua length, number of siliqua per plant and number of seeds per siliqua (Tables 5 and 6). Further, chitosan

stimulated flowering and increased translocation of sufficient assimilate to the passion fruit (sink), thus enhanced yield (Utsunomiya and Kinai, 1994).

Table 5: Effect of seed soaking and foliar spraying with different concentrations of bulk chitosan on major yield attributes of *Brassica napus* L. plants.

Treatments		Yield attributes				
		Length of silique (cm)	Number of Silique plant^{-1}	Number of seeds siliqua^{-1}	Seed yield (kg feddan^{-1})	% of change in yield feddan^{-1} relative to control
Bulk CHT (ppm)	Control	5.19±0.06c	173.8±3.72e	20.66±0.38d	1564	--------
	3000	6.66±0.09b	347.2±9.63c	25.26±0.49c	1948	24.0%
	5000	7.54±0.11a	1042.4±10.92a	35.46±1.16a	2908	85.93%
	7000	6.71±0.04b	581.0±3.39b	29.33±0.34b	2311	47.76%
	9000	4.73±0.03d	197.4±4.38d	19.20± 0.24d	1671	6.84%

Data are presented as mean of ten variables ± SE. Mean values in each column followed by different letter are significantly different by least significant difference test at 5%.

Table 6: Effect of foliar spraying with different concentrations of nano chitosan on major yield attributes of *Brassica napus* L. plants.

Treatments		Yield attributes				
		Length of silique (cm)	Number of silique plant^{-1}	Number of seeds siliqua^{-1}	Seed yield (kg feddan^{-1})	% of change in yield feddan^{-1} relative to control
Nano-CHT (ppm)	Control	5.65±0.07d	203.6±1.69e	21.46±0.66e	1406	-------
	25	7.13±0.07b	614.2±7.50b	31.39±0.60b	2270	61.45%
	50	7.33±0.03a	810.8±6.08a	37.13±0.62a	2405	71.05%
	75	6.16±0.03c	345.6±11.36c	23.79±0.13c	1766	25.6%
	100	6.05±0.03c	343.5±5.82c	23.16±0.16cd	1699	18.71%
	200	5.74±0.06d	286.2±1.85d	22.19±0.22de	1532	8.96%

Data are presented as mean of ten variables ± SE. Mean values in each column followed by different letter are significantly different by least significant difference test at 5%.

The action mechanism of CHT in increasing plant yield was suggested by Hidangmayum *et al.* (2019); Monfared *et al.* (2020) to be via its effect on enhancing transport of photoassimilates, mobilization of metabolites to the reproductive sinks, increasing nutrient uptake, photosynthetic rate, antioxidant enzyme activities which can eventually lead to increase in crop yield. Similarly, Masjedi *et al.* (2017) concluded that foliar application of chitosan at vegetative stage enhanced the plant growth and development and increased grain yield of wheat by 76% than control under normal irrigation. Previously, Dzung and Thang (2004) stated that plant growth parameters are significantly enhanced by the application of oligoglucosamine. It stimulates plant growth and immunity through regulating various physiological processes such as cell elongation, cell division, protein synthesis, enzymatic activation and uptake of nutrients by roots, which ultimately contribute to an increase in crop yield.

Moreover, CHT and nano CHT has been recommended as an efficient biofertilizers, they proved its high efficiency in controlling the release of inorganic fertilizers (Ghormade *et al.,* 2011), hence limit need of using excessive toxic amount of inorganic fertilizers. Nano CHT, on the other hand, besides acting as plant growth promoter (Kong *et al.,* 2010; Maluin and Hussein, 2020) and as biofertilizer proved its potential as a carrier of agrochemicals which via their encapsulation led to slow release into the soil, hence minimize losses of fertilizers and hence a decrease in resource losses, and simultaneously increase their delivery and availability for plants and an increase in its product. Moreover, CHT via increasing JA content plays an important role in production and maturation of fruits and seeds. In this regard, JA was reported to participate in fruit ripening via induction of expression of genes involved in the biosynthesis of storage proteins, activation of the enzymes involved in biosynthesis of ethylene which participate in completion of fruit set (Czapski and Saniewski, 1992).

Above important feature of CHT recommended, it is not only to increase the yield but also the quality of the yielded seeds. In the present work, application of CHT to *B. napus* plants gives along with an increase of yield, a better quality of seeds was obtained with regard to increased content of carbohydrate, oil, protein-N, and the macroelements N, P and K as shown in Figure (3a and b) for bulck CHT and Figure (4a and b) for nano CHT, thus having a better consumer properties. Chitosan application may affect biosynthesis and translocation of carbohydrates during stimulation of cell division and the synthesis of DNA and RNA (Phothi and Theerakarunwong, 2017). As reported by Geng *et al.* (2020) and based on metabolic pathways, the chitosan could be converted directly to other sugars and pyruvate that participates in the tricarboxylic acid cycle. On the other hand, the enhancing effect of chitosan on protein content may be due to its enhancing effect on amino acids (Li *et al.,* 2017). Rabêlo *et al.* (2019) stated that application of chitosan derivatives increased amounts of amino acids in the leaves, which implied greater nitrogen uptake and/or mobilization or greater efficiency in the use of this nutrient in the plants. Chitosan treatment induced changes in programming of protein metabolism with an enhancement of various storage proteins and hormone metabolism (Landi *et al.,* 2017), modulated redox homeostasis, increased the expression of the genes of enzymes involved in glycolysis, carbohydrate metabolism and photosynthesis, (Chamnanmanoontham *et al.,* 2015; Zhang *et al.,* 2017). Recently, Chakraborty *et al.* (2020) suggested that chitosan working as a consequence of other metabolic processes rather than merely enhancing nitrogen nutritional quality or as a source of energy for the production of carbohydrates.

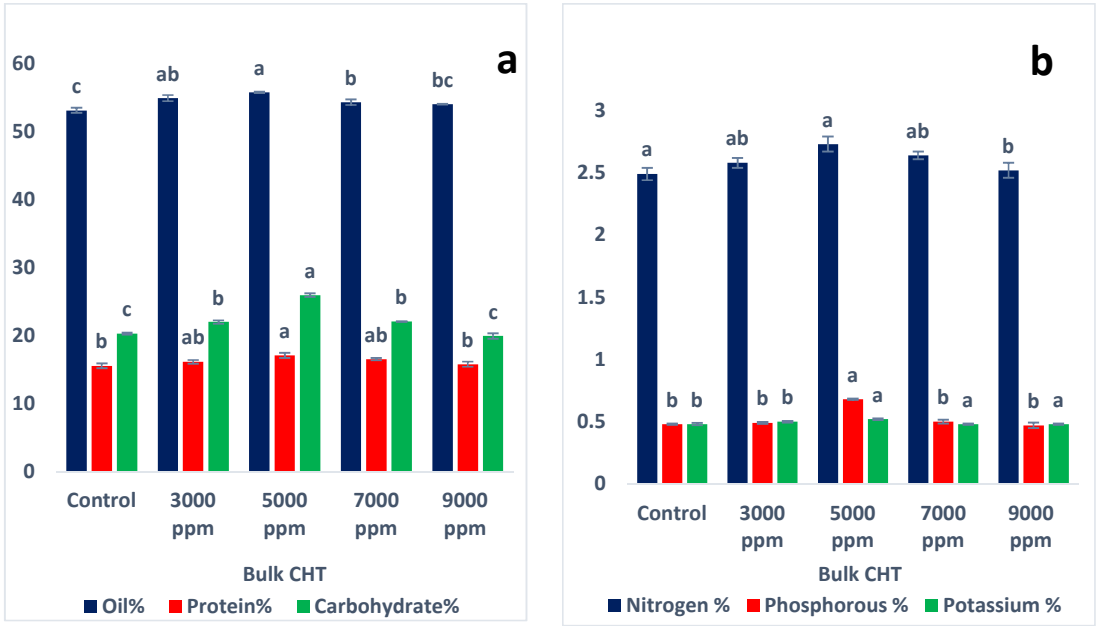

Fig. 3 (a, b): Effect of seed soaking and foliar spraying with different concentrations of bulk chitosan on some biochemical composition of the yielded seeds of *Brassica napus L.* plants. Data are presented as mean of ten variables ± SE. Mean values in each column followed by different letter are significantly different by least significant difference test at 5%.

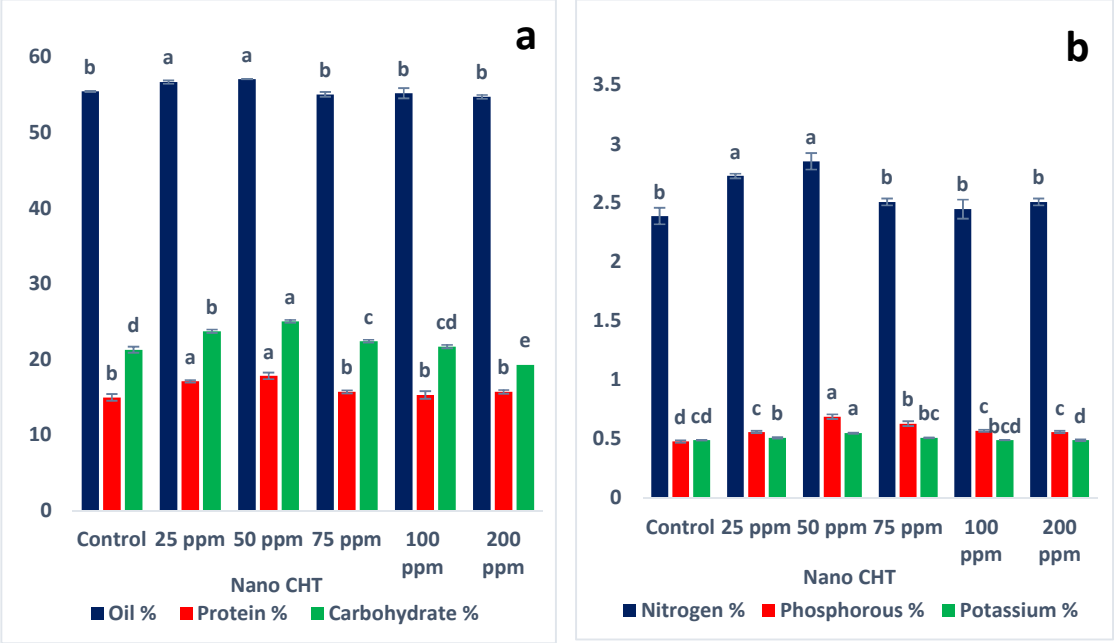

Fig. 4 (a, b): Effect of foliar spraying with different concentrations of nano chitosan on some biochemical composition of the yielded seeds of *Brassica napus L.* plants. Data are presented as mean of five replicates ± SE. Mean values in each column followed by different letter are significantly different by least significant difference test at 5%.

Regarding the change in the fatty acids composition of yielded seeds of *B. napus* seeds induced by chitosan, the data represented in Table 7 refer to that treatments with bulk CHT at the concentrations 5000 and 7000 ppm led to a significant increase in the content of oleic and linoleic acids associated with obvious decrease in linolenic and erucic acids content compared with the control. On the other hand, on applying the relatively highest concentration (9000 ppm) decreased oleic acid content together with inducing non-significant and significant increase in linoleic and erucic acids content, respectively.

Table 7: Effect of seed soaking and foliar spray with different concentrations of bulk chitosan on fatty acids profile of yielded seeds of *Brassica napus L.* plants.

Treatments		Fatty acid composition (%)				
		Palmitic acid	Oleic acid	Linoleic acid	Linolenic acid	Erucic acid
Bulk-CHT (ppm)	Control	3.47 ± 0.00	42.3 ±0.04c	14.69 ± 0.10b	8.01 ± 0.005	12.09 ± 0.010 c
	3000	3.5 ±0.080	39.96 ±0.15 d	15.34 ± 0.11a	7.79 ± 0.045	14.23 ± 0.035 b
	5000	3.46 ± 0.01	43.80 ±0.025b	15.31 ± 0.01a	7.77 ± 0.155	11.87 ± 0.025 c
	7000	3.38 ± 0.065	46.16 ±0.045a	15.47 ± 0.01a	7.96 ± 0.00	10.27 ± 0.050 d
	9000	3.42 ± 0.01	37.83 ±0.08 e	15.51 ± 0.18a	8.02 ± 0.14	16.29 ± 0.175 a

Data are presented as mean of ten variables ± SE. Mean values in each column followed by different letter are significantly different by least significant difference test at 5%.

In this connection, Monfared *et al.* (2019) found that application of CHT at a relatively low concentrations caused an increase of palmitic, oleic, and linoleic acids content and a decrease of linolenic and erucic acids content. Accumulation of erucic acid in seeds was reported to dominate in plants exposed to drought stress (Razaeizadeh *et al.,* 2019). In the present work, *B. napus* treatment with the relatively highest concentration (9000 ppm) lead to growth retardation (decrease in root and shoot length, fresh and dry weights, leaf area, (Table 1) which may be due to interference of CHT with ABA signaling pathway, hence decreased stomatal conductance and retardation of water translocation as reported by Bittelli *et al.* (2001) exposing plants to water stress. Water stress was observed to alter

profile of fatty acids towards increase in harmful fatty acids including erucic acid and a decrease in useful fatty acids as oleic acid (Rezaeizadeh *et al.,* 2019).

In response to nano CHT treatments, although there is no obvious change in the content of palmitic and linolenic acids at the different applied concentrations, yet appreciable increase in oleic and linoleic acids content was obtained (Table 8). On the other hand, erucic acid content of the yielded seeds exposed to significant decrease with a maximum response (47.55 and 55.08% reduction) was attained upon applying 25 and 50 ppm of nano CH respectively. The association of the increase encountered in oleic acid content with a decrease in erucic content may refer to a role of nano CHT either on elongase enzyme converting oleic acid (18:1, 9c) into erucic acid (22:1, 13c) (Kunst *et al*.,1992 and James *et al.,* 1995) and, or in regulation of the chain shortening of erucic acid to oleic acid by peroxisomal pathway, which was one of the fundamental breakthrough in regulating erucic acid content in canola oil (Black and Bewley, 2000).

Table 8: Effect of foliar spray with different concentrations of nano chitosan on fatty acids profile of yielded seeds of *Brassica napus L.* plants.

Treatments		Fatty acid composition (%)				
		Palmitic acid	Oleic acid	Linoleic acid	Linolenic acid	Erucic acid
Nano-CHT (ppm)	Control	3.34 ± 0.02 c	43.415 ± 0.005	15.90 ± 0.075b	7.835 ±0.025	12.09 ± 0.01 a
	25	3.69 ± 0.09 b	52.43 ± 0.00 b	16.68 ± 0.1 a	7.805 ±0.065	6.34 ± 0.02d
	50	4.35 ± 0.01 a	53.35 ± 0.005 a	16.02 ± 0.03 b	7.78 ± 0.08	5.43 ± 0.005e
	75	3.59 ± 0.08 b	47.51 ± 0.00 d	16.05 ± 0.01 b	7.85 ± 0.05	9.25 ±0.085c
	100	3.54 ±0.02bc	47.00 ± 0.01 e	16.05 ± 0.065b	7.615 ±0.035	9.18 ±0.005c
	200	3.54 ±0.11bc	48.72 ±0.015 c	15.15 ± 0.01 c	7.67 ±0.06	9.93 ±0.05 b

Data are presented as mean of ten variables ± SE. Mean values in each column followed by different letter are significantly different by least significant difference test at 5%.

Analysis of the seed cake (residue left after oil extraction) refer to, in general, a significant decrease in its content of glucosinolates in response to the different concentrations applied of bulk CHT or nano CHT, except in applying the highest concentration of nano CHT (200 ppm) which induced a significant increase in this content (Tables 9 and 10).

Table 9: Effect of seed soaking and foliar spray with different concentrations of bulk chitosan on glucosinolates content of seed cake of *Brassica napus* L. yielded seeds

Treatment		Glucosinolates content (µ mole/g defatted dried meal)
Bulk CHT (ppm)	Control	7.30 ± 0.013 a
	3000	6.62± 0.011d
	5000	6.24 ± 0.009 e
	7000	6.94 ± 0.010c
	9000	7.20 ± 0.003b

Data are presented as mean of ten variables ± SE. Mean values in each column followed by different letter are significantly different by least significant difference test at 5%.

Table 10: Effect of foliar spraying with different concentrations of nano chitosan on glucosinolates content of seed cake of *Brassica napus* L. yielded seeds

Treatments		Glucosinolates content (µ mole/g defatted dried meal)
Nano CHT (ppm)	Control	6.46 ± 0.004 b
	25	6.18 ± 0.011d
	50	6.05 ± 0.018e
	75	6.27 ± 0.008c
	100	6.51 ± 0.026b
	200	6.92 ± 0.054a

Data are presented as mean of ten variables ± SE. Mean values in each column followed by different letter are significantly different by least significant difference test at 5%.

3.5. Effect of bulk chitosan and nano-chitosan on major soil properties

Enhancement of canola growth induced by bulk CHT or nano CHT may be due to their efficiency in amelioration of the major mechanical properties of sandy soils. The data obtained in the present work concerned with the effect of bulk and nano CHT on major mechanical properties of the soil indicate that in spite of the obvious increase recorded in soil porosity in response to the different applied concentrations of bulk CHT, a significant increase was obtained in percentage of water holding capacity may be via decreasing the percolation rate (Table 11). Nano CHT, on the other hand, particularly at the most promotive concentration via decreasing soil porosity and percolation rate lead to marked increase in percentage of water holding capacity (Table 12). In this regard CHT when was investigated for its effect on soil properties was recorded to effectively acts as soil stabilizer, where it was found to have the potential of enhancing inter-particle interaction which lead to improvement of soil mechanical properties (Shariatmadari *et al.,* 2020), and via gathering sandy soil particles, it enhances its porosity increasing its holding capacity of water and its availability to plant growth (Hataf *et al.,* 2018).

Table 11: Effect of seed soaking and foliar spraying with different concentrations of bulk chitosan on major soil properties.

Treatments		Soil properties		
		Soil porosity	Water holding capacity	Percolation rate
Bulk-CHT (ppm)	Control	26.33±0.33d	38.33±0.33d	2.07±0.05a
	3000	25.55±0.33d	43.67±0.33b	1.68±0.02b
	5000	30.33±0.33a	45.67±0.33a	1.50±0.01c
	7000	29.00±0.58b	44.67±0.33ab	1.55±0.02c
	9000	27.67±0.33c	41.33±0.67c	1.68±0.02b

Data are presented as mean of ten variables ± SE. Mean values in each column followed by different letter are significantly different by least significant difference test at 5%.

Table 12: Effect of foliar spraying with different concentrations of nano chitosan on major soil properties.

Treatments		Soil properties		
		Soil porosity	Water holding capacity	Percolation rate
Nano-CHO (ppm)	Control	25.50±0.50a	39.53±0.29bc	1.33±0.015a
	25	23.87±0.47bc	40.83±0.6 a	1.18±0.013c
	50	23.43±0.43c	41.87±0.47ab	0.87±0.012d
	75	24.90±0.15ab	38.00±1.00cd	1.25±0.026b
	100	24.93±0.58ab	36.00±1.00d	1.32±0.012a
	200	24.67±0.33abc	36.33±0.33d	1.35±0.003a

Data are presented as mean of ten variables ± SE. Mean values in each column followed by different letter are significantly different by least significant difference test at 5%.

References

A.O.A.C. 1990. Official methods of analysis. 20th edition. Association of official analytical chemists, Arlington, Virginia, U.S.A.

Abdel-Aziz, H.M.M., M.N.A. Hassaneen, and A.M. Omer, 2016. Nano chitosan-NPK fertilizer enhances the growth and productivity of wheat plants grown in sandy soil. Spanish Journal of Agricultural Research, 14: 1-9.

Acemi, A., G.P. Ece, Ç. Merve, D. Elif, Y. Bahar, and Ö. Fazıl, 2021. Molecular weight and concentration of chitosan affect plant development and phenolic substance pattern in arugula.Not Bot Horti Agrobo (NotulaeBotanicae Horti Agrobotanici Cluj-Napoca) 49(2):12296.

Agnihotri, S.A., N.N. Mallikarjuna, and T.M. Aminabhavi, 2004. Recent advances in chitosan-based micro-and nanoparticles in drug delivery. J. Control Rel., 100, 5-28.

Ali, M., C.M. Ayyub, Z. Hussain, R. Hussain, and S. Rashid, 2020. Optimization of chitosan level to alleviate the drastic effects of heat stress in cucumber (*Cucumis sativus* L.). Journal of Pure and Applied Agriculture, 5(1): 30-38.

Al-Tawaha, Abdel R., A.T. Munir, M.A. Abdel Razzaq, H.A. Muhammad, W. Mohammad, M.A. Ezz Al-Dein, and H. AnhTuan, 2018. Using chitosan to improve growth of maize cultivars under salinity conditions. Bulgarian Journal of Agricultural Science, 24 (3):437–442.

Anusuya, S., and K.N. Banu, 2016. Silver-chitosan nanoparticles induced biochemical variations of chickpea (*Cicer arietinum* L.). Biocatalysis and Agricultural Biotechnology, 8: 39-44. https://doi.org/10.1016/j.bcab.2016.08.005

Bakhoum, G., M. Sadak, and M. Tawfic, 2022. Chitosan and chitosan nanoparticle effect on growth, productivity and some biochemical aspects of *Lupinus termis* L plant under drought conditions. Egyptian Journal of Chemistry, 65(5): 537-549.

Bittelli, M., *et al.,* 2001. "Reduction of transpiration through foliar application of chitosan." Agricultural and Forest Meteorology 107.3: 167-175.

Black, M., and J. Derek Bewley, eds. 2000. *Seed technology and its biological basis*. Crc Press.

Blázquez, M.A., and W. Detlef, 2000. "Integration of floral inductive signals in Arabidopsis." Nature 404.6780 : 889-892.

Campbell, L., B.R. Curtis, and P.D.W. Janitha, 2016. Canola/Rapeseed Protein: Future Opportunities and Directions—Workshop Proceedings of IRC 2015. Plants (Basel). 2016 Jun; 5(2): 17. Published online 2016 Apr 13. doi: 10.3390/plants5020017

Campos E.V.R., J.L. de Oliveira, L.F. Fraceto, and B. Singh, 2015. Polysaccharides as safer release systems for agrochemicals. *Agron. Sustain. Dev.,* 35:47–66. doi: 10.1007/s13593-014-0263-0.

Chaganti V.N., G. Girisha, N. Genhua, U. April, M.E. Juan, F. Robert, M. Norman, and R.K. James, 2021. Yield response of canola as a biofuel feedstock and soil quality changes under treated urban waste water irrigation and soil amendment application. Industrial Crops and Products. 170, 15 October 2021, 113659. https://doi.org/10.1016/j.indcrop.2021.113659

Chakraborty, M., M. Hasanuzzaman, M. Rahman, M.A.R. Khan, P. Bhowmik, N.U. Mahmud, M. Tanveer, and T. Islam, 2020. Mechanism of plant growth promotion and disease suppression by chitosan biopolymer. Agriculture, 10, 624.

Chamnanmanoontham, N., W. Pongprayoon, R. Pichayangkura, S. Roytrakul, and S. Chadchawan, 2015. Chitosan enhances rice seedling growth via gene expression network between nucleus and chloroplast. Plant Growth Regulation, 75:101-114. https://doi.org/10.1007/s10725-014-9935-7.

Chapman, H.D., and P.F. Pratt, 1961. Method for Analysis of Soils, Plants and Waters. Univ. of California, USA.

Chibu H., and H. Shibayama, 2001. Effects of chitosan applications on the growth of several crops. P. 235-239. In: Uragami T., K. Kurita, T. Fukamizo (eds) Chitin and chitosan in life science. Yamaguchi, Japan.

Chibu, H. and H. Shibayama, 2003. Effects of chitosan application on the growth of several crops. In: Chitin and Chitosan in Life Science, Uragami, T., Kurita, K. and Fukamizo, T. (eds.). Yamaguchi, Japan. 235-39.

Cote, F., and M.G. Hahn, 1994. Oligosaccharin: Structures and signal transduction. Plant Molecular Biolology, 26: 1379–1411.

Cottenie, A., M. Verloo, L. Kiekens, G. Velghe, and R. Camrbynek, 1982. Chemical analysis of plant and soil. Lab. Anal.Agrochem. State Univ. Ghent, Belgium, 63 .

Czapski, J., and S. Marian, 1992. "Stimulation of ethylene production and ethylene-forming enzyme activity in fruits of the non-ripening nor and rin tomato mutants by methyl jasmonate." Journal of plant physiology, 139.3: 265-268.

Dawood, M.G., 2005. Study of some changes in chemical composition during germination of some canola varieties. Ph. D. Thesis in Biochemistry, Faculty of Science, Cairo University. Egypt.

Doares, S.H., *et al.,* 1995. "Oligogalacturonides and chitosan activate plant defensive genes through the octadecanoid pathway." *Proceedings of the National Academy of Sciences* 92.10: 4095-4098.

Dubey, A., and R.M. Damodhara, 2016. "Nanofertilisers, nanopesticides, nanosensors of pest and nanotoxicity in agriculture." Sustainable Agriculture Reviews, 19: 307-330.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem., 28: 350-356.

Dzung N.A., V.T.P. Khanh, and T.T. Dzung, 2011. Research on impact of chitosan oligomers on biophysical characteristics, growth, development, and drought resistance of coffee. Carbohydr. Polym., 84: 751-755.

Dzung, N.A., and N.T. Thang, 2004. Effect of chitooligosaccharides on the growth and development of peanut (*Arachis hypogea* L.). In Proceedings of the 6th Asia-Pacific on Chitin, Chitosan Symposium. Ed. E. Khor, D. Hutmacher and L. L. Yong. Singapore, ISBN: 981-05-0904-9.

El-Bassiony, A.M., Z.F. Fawzy, M.A. El-Nemr and Y. Li, 2014. Improvement of growth, yield, and quality of two varieties of kohlrabi plants as affected by application of some bio stimulants. Middle East Journal of Agriculture Research, 3(3): 491-498.

El-Hamidi, M., A.Z. Ferial, and S. Atteia, 2020. Edible Oil Production in Egypt: An Overview. Current Science International. DOI: 10.36632/csi/2020.9.4.58. 09(04): 649-655.

El–Hamidi, M., and F.A. Zaher, 2018. Production of Vegetable Oils in the World and in Egypt: an Overview. Bulletin of the National Research Centre, 42:19. https://doi.org/10.1186/s42269-018-0019-0

El-Tantawy, E.M., 2009. Behaviour of tomato plants as affected by spraying with chitosan and amino fort as natural stimulator substances under application of soil organic amendments. Pak. J. Biol. Sci.,12:1164-1173.

Farouk, S., K.M. Ghoneem, and Abeer A. Ali, 2008. Induction and expression of systematic resistance to downy mildew disease in cucumber plant by elicitors. Egyptian Journal Phytopathology, 1-2: 95-111.

Fedak, G. and I. De La Roche, 1977. Lipid and fatty acid composition of barley kernels. Can. J. Plant Sci., 57(1): 257-260.

Geng, W., L. Zhou, J.H. Muhammad and P. Yan, 2020. Chitosan regulates metabolic balance, polyamine accumulation, and Na+ transport contributing to salt tolerance in creeping bentgrass Geng *et al.,* BMC Plant Biology, 20:506

Ghoname, A.A., M.A. EL-Nemr, A.M.R. Abdel-Mawgoud, and W.A. El-Tohamy, 2010. Enhancement of sweet pepper crop growth and production by application of biological, organic and nutritional solutions. Research Journal of Agriculture and Biological Science, 6(7): 349-355.

Ghormade, V., V.D. Mukund, and M.P. Kishore, 2011. "Perspectives for nano-biotechnology enabled protection and nutrition of plants." Biotechnology advances 29.6: 792-803.

Gornik, K., M. Grzesik and B.R. Duda. 2008. The effect of chitosan on rooting of grapevine cuttings and on subsequent plant growth under drought and temperature stress. J. Fruit Ornamental Plant Res. 16:333-343.

Guan, Y.J., J. Hu, X.J. Wang and C.X. Shao, 2009. Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. J. Zhejiang Univ. Sci. 10:427-433.

Hadwiger, L.A., S.J. Klosterman and J.J. Choi, 2002. The mode of action of chitosan and its oligomers in inducing plant promoters and developing disease resistance in plants, 452-457. In: K. Suchiva, S. Chandrkrachang, P. Methacanon and M.G. Peter (eds.), Advances in Chitin Science, 5; Bangkok, 452-457, ISBN 974-229-412-7.

Harshil, B.B.P., M. Patel1, and N.S. Chainesh, 2016. Nanosuspension: a novel approach to enhance solubility of poorly water soluble drugs - a review, Int. J. Adv. Pharm. 5 (2): 2320–4923

Hataf, N., Gh. Pooria, and R. Navid, 2018. "Investigation of soil stabilization using chitosan biopolymer." Journal of cleaner production, 170 : 1493-1500.

Hernández-Téllez, C.N., M. Plascencia-Jatomea, and M.O. Cortez-Rocha, 2016. Chitosan-based bionanocomposites: development and perspectives in food and agricultural applications. Chitosan in the preservation of agricultural commodities, 315-338.

Hidangmayum, A., P. Dwivedi, D. Katiyar, and A. Hemantaranjan, 2019. Application of chitosan on plant responses with special reference to abiotic stress. Physiol. Mol. Biol. Plants., 25: 313–326.

Hodson, L., C.M. Skeaff, and W.A. Chisholm, 2001. The effect of replacing dietary saturated fat with polyunsaturated or monounsaturated fat on plasma lipids in free-living young adults. Eur. J. Clin. Nutr., 55:908–915.

Iggman, D., I.-B. Gustafsson, L. Berglund, et al., 2011. Replacing dairy fat with rapeseed oil causes rapid improvement of hyperlipidaemia: a randomized controlled study. J. Intern. Med., 270:356–364.

James, Jr., W. Douglas, *et al.,* 1995. "Directed tagging of the Arabidopsis Fatty Acid Elongation1 (FAE1) gene with the maize transposon activator." The Plant Cell 7.3: 309-319.

John, M., H. Röhrig, J. Schmidt, R. Walden, and J. Schell, 1997. Cell signalling by oligosaccharides. Trends Plant Sci., 2: 111–115.

Johnson, G.H., D.R. Keast, and P.M. Kris-Etherton, 2007. Dietary modeling shows that the substitution of canola oil for fats commonly used in the United States would increase compliance with dietary recommendations for fatty acids. J. Am. Diet. Assoc., 107:1726–1734.

Jones, Jr.J.B., B.J. Wolf, and H.A. Mills, 1990. Plant analysis handbook: A practical sampling, preparation, analysis, and interpretative guide. MicroMacro Publishing Inc., Athens, Georgia, 127–189.

Kandil, H., and N. Gad, 2012. Growth and oil production of canola as affected by different sulphur sources. J. Basic Appl. Sci. Res., 2(5): 5196-5202.

Kashyap, P.L., X. Xu, and H. Patricia, 2015. "Chitosan nanoparticle-based delivery systems for sustainable agriculture." International journal of biological macromolecules, 77: 36-51.

Katiyar, D., A. Hemantaranjan and B. Singh, 2015. Chitosan as a promising natural compound to enhance potential physiological responses in plant: a review. Indian Journal of Plant Physiology, 20: 1-9.

Khan, W.M., B. Prithiviraj and D. Smiyh, 2002. Effect of foliar application of chitin oligosaccharides on photosynthesis of maize and soybean. Photosynthetica, 40(4): 621-626. https://doi.org/10.1023/A:1024320606812

Kong, M., *et al.,* 2010. "Antimicrobial properties of chitosan and mode of action: a state of the art review." International journal of food microbiology 144.1 : 51-63.

Kunst, L., C.T. David, and W. Edward, 1992. Underhill. "Fatty acid elongation in developing seeds of *Arabidopsis thaliana*." Plant Physiol Biochem., 30.4: 425-434.

Landi, L., R.M. De MiccolisAngelini, E. Pollastro, S. Feliziani, F. Faretra, and G. Romanazzi, 2017. Global transcriptome analysis and identification of differentially expressed genes in strawberry after preharvest application of benzothiadiazole and chitosan. Frontiers in Plant Science, 8:235. https://doi.org/10.3389/fpls.2017.002 35

Lateef, A., R. Nazir, N. Jamil, S. Alam , A. Shah, M.N. Khan , and M. Saleem, 2016. Synthesis and characterization of zeolite based nano–composite: An environment friendly slow release fertilizer. Microporous and Mesoporous Materials, 232: 174-183

Lee, Y.S., Y.H. Kim and S.B. Kim, 2005. Changes in the respiration, growth, and vitamin C content of soybean sprouts in response to chitosan of different molecular weights. HortScience, 40:1333-1335.

Li, K.C., X.Q. Zhang, Y. Yu, R.E. Xing, S. Liu and P.C. Li 2020. Effect of chitin and chitosan hexamers on growth and photosynthetic characteristics of wheat seedlings Photosynthetica, 58 (3): 819-826. DOI: 10.32615/ps.2020.021

Malerba, M. and C. Raffaella, 2018. Recent Advances of Chitosan Applications in Plants. Polymers (Basel). 10 (2): 118. Published online 2018 Jan 26. doi: 10.3390/polym10020118

Maluin, F.N., and Z.H. Mohd, 2020. "Chitosan-based agronanochemicals as a sustainable alternative in crop protection." *Molecules 25.7: 1611.*

Masjedi, M.H., R. Arash and B. Mahdi, 2017. Assessment effect of chitosan foliar application on total chlorophyll and seed yield of wheat (*Triticum aestivum* L.) under water stress conditions. Journal of Crop Nutrition Science, 3(4):14-26.

McDaniel, C.N., and K.H. Laura, 1996. "Flowering as metamorphosis: two sequential signals regulate floral initiation in *Lolium temulentum*." Development, 122.11: 3661-3668.

Meara, M.L., and C.B. Patel, 1950. "The component acids and glycerides of dika fat." Journal of the Science of Food and Agriculture, 1.2: 48-51.

Mohamed, S. A., and H.S. Ahmed, 2019. "Study effect of chitosan and gibberellic acid on growth, flowering, fruit set, yield and fruit quality of Washington navel orange trees." Mid. East J., 8: 255-67.

Mohamed, S. A., H.S. Ahmed and A. A. El-Baowab, 2018. Effect of chitosan, putrescine, and irrigation levels on the drought tolerance of sour orange seedlings. Egypt. J. Hort. 45(2): 257-273.

Mondal, M.M.A., A.B. Puteh, and N.C. Dafader, 2016. Foliar application of chitosan improved morpho-physiological attributes and yield in summer tomato (*Solanum lycopersicum). Pak. J. Agric. Sci., 53: 339–344.*

Monfared B.B., N. Ghorban, H.S.R. Amir, and M.H. Eslam, 2020. Effects of sowing date and chitosan on some characters of canola (*Brassica napus* L.) genotypes. J. Crop Sci. Biotech., 23 (1): 65-71.

Monfared B.B., N. Ghorban, H.S.R. Amir, and M.H. Eslam, 2019. effects of sowing date, cultivar and chitosan on quality and quantity of rapeseed *(Brassica napus* L.) oil. Journal of Agricultural Sciences, 25: 508-517.

Moran, R., and P. Dan, 1980. "Chlorophyll determination in intact tissues using N, N-dimethylformamide." Plant physiology 65.3: 478-479.

Naderi, S., H. Khajeh, and H. Ahmadi, 2015. The Effect of Chitosan on Some Morphological Indices in *Lepidium sativum* L. The First Conference of Medicinal Herbs and Herbal Medicines, Tehran, Center for the Sustainable Development of Science and Technology Farzin.

Nasirullah and M.N. Krishnamurthy, 1996. A method for estimating glucosinolates in mustard/rapeseeds and cake. J. Food Sci. Technol., 33(6): 498-500

Navarro, E., A. Baun, R. Behra, N.B. Hartmann, J. Filser, A. Miao, A. Quigg, P.H. Santschi, and L. Sigg, 2008. Environmental behaviour and ecotoxicity of engineered nanoparticles to algae, plants and fungi. Ecotoxicology, 17: 372-386. http:// dx.doi.org/10.1007/s10646-008-0214-0

Nel, A., *et al.,* 2006. "Toxic potential of materials at the nanolevel." Science, 311.5761: 622-627.

Okalebo, J.R., K.W. Gathua, and P.L. Woomer, 2002. Laboratory methods of soil and plant analysis: a workingmanual second edition. Sacred Africa, Nairobi, 21.

Paradikovic, N., T. Teklic, S. Zeljkovic, M. Lisjak, and M. Spoljarevic, 2019. Biostimulants research in some horticultural plant species-a review. Food Energy Secur., 8 (2): 1–17.

Phothi, R., and C.D. Theerakarunwong, 2017. Effect of chitosan on physiology, photosynthesis and biomass of rice (*Oryza sativa* L.) under elevated ozone. Aust. J. Crop Sci., 11(5): 624–630.

Piper, C.S., 1947. Soil and plant analysis. Interscience Publisher inc., New York.

Pirbalouti, G.A., F. Malekpoor, A. Salimi, and A. Golparvar, 2017. Exogenous application of chitosan on biochemical and physiological characteristics, phenolic content, and antioxidant activity of two species of basil (*Ocimum ciliatum* and *Ocimum basilicum*) under reduced irrigation. Sci. Hortic., 217: 114–122.

Rabêlo, M., C.M. Paulo, A.B. Letícia, T.C. Diogo, O.dosR. Caroline, K. Decio, C.D. Antônio, H.dos S. Marcelo, dos S.F. Plínio Rodrigues and C.deS. Thiago, 2019. the foliar application of a mixture of semisynthetic chitosan derivatives induces tolerance to water defcit in maize, improving the antioxidant system and increasing photosynthesis and grain yield Valquíria . Scientific Reports, 9:8164 | https://doi.org/10.1038/s41598-019-44649-7

Rahman, M., J.A. Mukta, A.A. Sabir, D.R. Gupta, M. Mohi-Ud-Din, M. Hasanuzzaman, and T. Islam, 2018. Chitosan biopolymer promotes yield and stimulates accumulation of antioxidants in strawberry fruit. PLoS ONE 13(9):e0203769. https://doi.org/10.1371/journal.pone.0203769.

Ramos-García, M., *et al.,* 2009. "Response of gladiolus (Gladiolus spp) plants after exposure corms to chitosan and hot water treatments." Scientia Horticulturae, 121.4: 480-484.

Rauchberger, Y., M. Shoshana, and C. Uri, 1979."The effect of aqueous leaching of glucosinolates on the nutritive quality of rapeseed meal." Journal of the Science of Food and Agriculture, 30.1: 31-39.

Rezaeizadeh, M., *et al.,* 2019. "Influence of drought stress and chitosan on fatty acid compounds of rapeseed varieties." Iranian Journal of Plant Physiology, 9.3: 2819-2825.

Saharan, V., and A. Pal, 2016. *Current and Future Prospects of Chitosan-Based Nanomaterials in Plant Protection and Growth*; Springer: Berlin/Heidelberg, Germany, 43–48.

Salachna, P and A. Zawadzińska, 2014. Effect of chitosan on plant growth, flowering and corms yield of potted. J. Ecol. Eng. 15(3):97–102. https://doi.org/10.12911/22998993.1110223

Sathiyabama, M., and A. Manikandan, 2021. Foliar application of chitosan nanoparticle improves yield, mineral content and boost innate immunity in finger millet plants. Carbohydr. Polym., 258: 117691.

Shahrajabian, M.H., *et al.,* 2021. "Sustainable agriculture systems in vegetable production using chitin and chitosan as plant biostimulants." Biomolecules 11.6: 819.

Sharaan, A.N., K.H. Ghallab and K.M. Yousif, 2002. Performance and water relations of some rapeseed genotypes grown in sandy loam soils under irrigation regimes. Annals of Agriculture Science, Moshtohor, 40: 751-767.

Shariatmadari, N., M. Reza, A. Tasuji, P. Ghadir, and A. Akbar Javadi, 2020. Experimental study on the effect of chitosan biopolymer on sandy soil stabilization. E3S Web of Conferences 195: 06007. https://doi.org/10.1051/e3sconf/202019506007

Shojaei, T.R., *et al.,* 2019. "Applications of nanotechnology and carbon nanoparticles in agriculture." *Synthesis, technology, and applications of carbon nanomaterials*. Elsevier, 247-277.

Silva dos Santos, M., D.S. Cocenza, R. Grillo, N.F.S. de Melo, P.S. Tonello, L.C. de Oliveira, ... and L.F. Fraceto, 2011. Paraquat-loaded alginate/chitosan nanoparticles: preparation, characterization and soil sorption studies. Journal of hazardous materials, 190(1-3): 366-374.

Smith, F., M.A. Gilles, J.K. Hamilton, and P.A. Godees, 1956. Colorimetric method for determination of sugar related substances. Anal. Chem., 28: 350.

Snedecor G.W., and W.G. Cochran, 1982. Statistical Methods. The Iowa State University Press.7th Ed., 2ndPrinting, 507

Stahl, W. and S. Helmut, 2003. "Antioxidant activity of carotenoids." Molecular aspects of medicine , 24,6: 345-51. doi:10.1016/s0098-2997(03)00030-x

Sultana, S., M. Islam, M.St. Khatun, M. Hassain, and R. Huque, 2017. Effect of foliar application of oligo-chitosan on growth, yield and quality of tomato and eggplant. Asian J. of Agricultural Res., 11 (2): 36-42 (). DOI: 10.3923/ajar.2017.36.42.

Taiz, L., and Z. Eduardo, 2006. "Secondary metabolites and plant defense." Plant physiology, 4: 315-344.

Taylor, R., and R.M.W. David, 1993. "The chemistry offullerenes." Nature, 363.6431: 685-693.

Uthairatanakij, A., J.A. Teixeira da Silva, and K. Obsuwan, 2007. Chitosan for improving orchid production andquality. Orchid Science and Biotechnology, 1(1): 1-5.

Utsunomiya, N. and H. Kinai, 1994. Effect of chitosan-oligosaccharides soil conditioner on the growth of passion fruit (in Japanese). J. Jpn. Soc. Hort. Sci. 64 (Suppl.1):176–177.

Van S., M.H. Dinh, and A.D. Nguyen, 2013. Study on chitosan nanoparticles on biophysical characteristics and growth of Robusta coffee in green house. Biocatal. Agric. Biotechnol., 2(4): 289-294.

Vanti, G.L., *et al.,* 2020. "Synthesis and application of chitosan-copper nanoparticles on damping off causing plant pathogenic fungi." International Journal of Biological Macromolecules, 156: 1387-1395.

Waewthongrak, W. and S. Nurahayatee, 2020. Effect of Chitosan on Growth of Seed Tomato in Vegetative Stage. Thaksin University Journal, 23(3):

Walker-Simmons, M., L.A. Hadwiger, and C.A. Ryan, 1983. Chitosan and peptic polysaccharides both induce the accumulation of the antifungal phytoalexin pisatin in pea pods and antinutrient proteinase inhibitors in tomato leaves. Biochem. Biophys. Res. Commun. 110 : 194- 99.

Wanichpongpan, P., K. Suriyachan, and S. Chandrkrachang, 2001. Effects of chitosan on the growth of gerbera flower plant (*Gerbera jamesonii*). In: T. Uragami, K. Kurita, T. Fukamizo (Eds.), Chitin and Chitosan in Life Science, Yamaguchi, 198–201.

Watanabe, F.S., and S.R. Olsen, 1965. "Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil." Soil Science Society of America Journal 29.6: 677-678.

Wittenberger, K., 2012. USDA Economic Research Service. ERS/USDA Briefing Room – Soybeans and Oil Crops: Canola Seed, Oil, and Meal.

Wong, M.H., R.P. Misra, J.P. Giraldo, S.Y. Kwak, Y. Son, M.P. Landry, and M.S. Strano, 2016. Lipid exchange envelope penetration (LEEP) of nanoparticles for plant engineering: a universal localization mechanism. Nano letters, 16(2): 1161-1172.

Yanat, M., and S. Karin, 2021. "Preparation methods and applications of chitosan nanoparticles; with an outlook toward reinforcement of biodegradable packaging." Reactive and Functional Polymers, 161: 104849.

Zhang, X.Q., K.C. Li, R. Xing, S. Liu, and P. Li, 2017. Metabolite profiling of wheat seedlings induced by chitosan: revelation of the enhanced carbon and nitrogen metabolism. Front Plant Sci., 8:2017–29.

Zong, H., S. Liu, R. Xing, X. Chen, and P. Li, 2017. Protective effect of chitosan on photosynthesis and antioxidative defense system in edible rape (*Brassica rapa* L.) in the presence of cadmium. Ecotoxicol. Environ. Saf., 138: 271–278.