Archives of Agriculture Sciences Journal
Print ISSN: 2535-1680
Online ISSN: 2535-1699

ARCHIVES OF AGRICULTURE SCIENCES JOURNAL

Volume 3, Issue 2, 2020, Pages 205–223

Available online at www.agricuta.edu.eg

DOI: https://dx.doi.org/10.21608/aasj.2020.178054

The role of vermicompost and chitosan nanoparticles as foliar application to enhancing growth, yield and oil of black cumin (*Nigella sativa* L.) plants

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Abstract

A field experiment was carried out in Faculty of Agriculture Farm, Al-Azhar University, Assiut, Egypt during two successive seasons 2018/2019 and 2019/2020 aiming to study the effect of four rates of vermicompost (zero, 2, 4 and 6 tons /feddan) (feddan = 4200 m² = 0.420 hectares = 1.037 acres) and three concentrations of chitosan nanoparticles (ChNP) at zero, 20 and 40ppm, as well as, their interaction on growth, yield and chemical constituents of black cumin (*Nigella sativa* L.) plants. The obtained results showed that all vermicompost levels increased plant height, branch number, herb fresh and dry weights, capsule number and weight/plant, seed weight/plant, 1000 seed weight, fixed and volatile oil percentages and yields and NPK percentages in herb. However, the high rate of vermicompost (6 tons /feddan) gave the best results for these characteristics. Also, treat plants with all concentrations of chitosan nanoparticles (ChNP) showed significant increases in all parameters, except 20 ppm in the second season, regarding branches number comparing to control. The best results were obtained by 40 ppm ChNP in comparison with control. The combined treatment of vermicompost (6 tons /feddan) with 40 ppm ChNP recorded the best results of most parameters.

Keywords: *Nigella sativa*, vermicompost, chitosan nanoparticles, oil production.

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1. Introduction

Black cumin (*Nigella sativa*, L.) is an annual plant belongs to family Ranunculaceae, grows at 20-90 cm tall, leaves are finely divided, the flowers are white or pale purple color, with 5-10 petals. The fruit is a bloated capsule consists of 3-7 united follicles, each one contains several seeds small in size, odor slightly aromatic and taste bitter (Goreja, 2003). It is a herbaceous plant, one of the most promising medicinal and aromatic plants. This plant is vastly cultivated in the middle and Upper Egypt regions, for seed and oil production. The seeds contain 30-35% of fixed oil, which has several uses in pharmaceutical and food industries (Ustun *et al.,* 1990). Also, the seeds containing volatile oils (0.8 – 1.1%, which mainly contain on nigellon compound), glycosides, saponines, albuminous proteins, tannins, glucose and mucilage resins (Burits and Bucar, 2000). *Nigella sativa* seeds are used in treatment a lot of diseases, including diabetes, inflammatory diseases, rheumatism, anti-hypertensive, as well as, improved kidney function and liver in cases of old age and increases the activity of immune cells (Bashandy, 1996; Houghton *et al*., 1995; Salomi and Rajgopalar, 1989). In the last years, consumers are increasingly concerned about issues such as food quality, environmental safety and soil conservation have led to a substantial increase in the use of sustainable agricultural practices (Tilman *et al*., 2002). Pollution is increasing day by day, as a result of an accumulation of organic waste and on the other side there is a huge shortage of organic manure. The organic manure could increase the fertility and productivity of the land and produce nutritive and safe food (Ramesh *et al.,* 2005). Recycling organic waste of different resources in the form of compost can be an alternative to meet the increasing demands for organic manures, this will also help to reduce environmental pollution arising out of accumulated organic wastes (Kumar, 2005). Vermicompost is one of the most important fertilizers, which depended on recycling organic waste, where he is a low- tech operation, environmentally friendly process, so vermicompost proved to have many positive effects on plant growth and health (Cristina and Jorge, 2011). Vermicompost is bio-organic manure obtained by passing semi-decomposed organic matter through the digestive system by various species of earthworms like *Eisenia foetida*, *Eudrilus sp*. and its disposal from their body when the materials pass through the worm body, inseminated with intestinal mucosa, vitamins and enzymes. The result is an enriched organic fertilizer which has major applications in agricultural lands, nursery and greenhouses. Recently, there has been much interest in the potential of vermicompost, which are products of a mesophilic, aerobic biodegradation and stabilization of organic matters, produced through interactions between earthworms and microorganisms, as plant growth media and soil amendments (Arancon *et al.,* 2008). Nanotechnology is multi-specialty in research field. In this time, a lot of efforts have been made possibility to improve agricultural crops through exhaustive research in nanotechnology (Duhan *et al*., 2017). Nanotechnology

includes nanoparticles having one or more dimensions in the order of 100 nm or less (Auffan *et al*., 2009). Nanoparticles have found applications in plant protection, nutrition and management of farm practices due to small size and high surface to volume ratio. Chitosan is one of the most important natural polymers. It is polysaccharides called 2-Amino-2-deoxybeta-D-glucosasmine, consist of N-acetyl-D-glucosamine (acetylated unit) and *β*-(1-4)-linked D-glucosamine (Peniston and Johnson, 1980). Chitosan is derived from chitin, which is considered the external component of marine crustaceans like, shrimp, crabs, shellfish, as well as, cell wall of fungi (Wojdyla, 2001). Chitin can be easily converted to chitosan through extracting the acetyl group and turn it into amino (Sugiyama *et al.*, 2001). Chitosan and its derivatives are nontoxic, biodegradable and friendly to environment. It has been recognized as a product to improve crop production due to their bioactivities to plants, which included stimulating growth of plants and seed germination (Chandrkrachang, 2002). Chitosan nanoparticles (ChNP) has the same characteristics of chitosan and the properties of nanoparticles like, surface and interface effect, small size and quantum size effects (Ingle *et al*., 2008). The main aim of the present work is to determine the impact of vermicompost fertilizer and chitosan nanoparticles, as well as, their interaction on growth, yield and active ingredients of black cumin plant.

2. Materials and Methods

This investigation was carried out at the Experimental Farm, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt during 2018/2019 and 2019/2020 seasons to study the influence of the addition of vermicompost and chitosan nanoparticles, as well as, their interactions on vegetative growth, yield components, fixed and volatile oil productivity and some chemical constituents of black cumin (*Nigella sativa* L.) plants. The seeds of black cumin were sown in November 10th of both seasons. The experimental design was a split plot design with three replicates. Vermicompost fertilizer levels were considered as main plots (A), while chitosan nanoparticles concentrations were arranged in the sub plots (B). The experimental unit was 2.0 × 2.4 m with 60 cm distance between the rows, in hills 25 cm apart on one side of the ridge, each plot contained 3 rows and 21 hills. The plants were thinned after 30 days from planting to one plant/hill to be the number of plants in experimental plot 21 plant. Some physical and chemical properties of the experimental soil (average of the two seasons) are shown in Table (1). The main plots (A) included 4 levels of vermicompost fertilizer, while sub plots contained of foliar spray by three concentrations of chitosan nanoparticles (ChNP). Vermicompost levels were as follows:

- Control treatment.
- 2 tons vermicompost /feddan (feddan = 4200 m² = 0.420 hectares = 1.037 acres).
- 4 tons vermicompost/ feddan.

- 6 tons vermicompost/ feddan.

The concentrations of chitosan nanoparticles treatments were as follows:

- Control (Tap water).
- Chitosan nanoparticles (ChNP) at 20 ppm.
- Chitosan nanoparticles (ChNP) at 40 ppm.

Vermiompost fertilizer was obtained from Central Laboratory for Agricultural Climate (CLAC), Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. Vermicompost was added during the preparation of soil in both seasons. Chitosan nanoparticles was obtained from Naqaa Foundation for Scientific Research, technology and development.

Table (1): Physical and chemical properties of the used soil.

Texture	PH (1:2.5)	E.C. (m.mo hs/cm)	CaCo3 (%)	O.M. (%)	Total N (%)	Available		Water soluble ions (Meq/l) in the soil paste				
						P (ppm)	K (mg/ 100g soil)	Ca	Mg	Co3+ Hco3	Cl	So4
Loamy	7.5	2.2	2.53	0.50	0.12	0.14	3.5	3.4	1.9	2.9	2.2	6.6

[a] = SE: standard error. [b] = RR_{50}: resistance ratio = LC_{50} of the selected generation / LC_{50} of the Susceptible strain. [c] = P: Parent field strain.

2.1 Chitosan nanoparticles preparation method

One gram chitosan was dissolved in 250 ml distilled water contains 1% acetic acid for 20 mints, then 50 ml TPP Sodium Tri polyphosphate solution was dropped into the chitosan beaker at room temperature, after that chitosan solution was magnetically stirred for 45 mints in order to obtain chitosan nanoparticles. The prepared chitosan nanoparticles solution at a concentration of 1mg/ml (1000 ppm), finally, chitosan nanoparticles stored in distilled water at 2 -8 °C, where it will be stable at the prepared size 50 nm for two months. These concentrations of chitosan nanoparticles were applied as foliar spray twice at one-month intervals, the first was after 45 days from planting, the second was a month later in both seasons. All other agricultural practices were performed as usual. Chemical analysis of vermicompost manure used in this work are shown in Table (2).

Table (2): Chemical analysis of vermicompost manure used in this work.

Properties	Vermicompost
pH $_{(1:2.5)}$ suspension	8.1
$EC_{(1:5)}$ (dS m^{-1})	6.8
Organic matter %	43.3
Total N %	1.7
Total P %	1.15
Total K %	0.61
Fe ppm	1654
Zn ppm	102
Mn ppm	98

2.2 Measured parameters

2.2.1 Vegetative growth characteristics

The plants were randomly selected from each experimental plot at 150 days after planting date and transferred to

laboratory to record the following data: (plant height (cm), branch number/plant, herb fresh weight g/plant and herb dry weight g/plant).

2.2.2 Yield component characters

Capsule number /plant, capsule weight/plant, weight of seeds /plant and 1000 seed weight.

2.2.3 Oil production

2.2.3.1 Fixed oil determination

The fixed oil % of the seeds was evaluated by soxhlet apparatus according to A.O.A.C. (1970), then fixed oil yield (ml /plant) was calculated by multiplying fixed oil % in seed weight (g /plant).

2.2.3.2 Volatile oil determination

The volatile oil of seeds was isolated by hydro distillation for 3 hours by Clevenger apparatus according to Guenther (1961), then volatile oil yield (ml /plant) was calculated by multiplying volatile oil % in seed weight (g /plant).

2.2.4 Chemical constituents

2.2.4.1 Nitrogen, phosphorus and potassium percentages

The three studied elements of N, P and K % in the dry herb were estimated as follows:

- Nitrogen percentage was determined according to the modified micro-kjeldahel method as described by Wildy *et al.* (1985).
- Phosphorus % was estimated calorimetrically by the spectrophotometer according to Chapman and Pratt (1975).
- Potassium % was determined by using the Flame-photometer method according to Cottenie *et al.* (1982).

The Analysis of Variance (ANOVA) and L.S.D multiple range tests at 0.05 % level of probability were used to test the significance of differences between the treatments. Statistical data analyses were performed using Costat software (Steel and Torrie, 1986).

3. Results and Discussion

3.1 Vegetative growth parameters

The presented data in Table (3) indicated that plant height, branch number, herb fresh weight (g /plant) and herb dry weight (g /plant) of black cumin was significantly augmented, in the two seasons, due to utilizing all levels of vermicompost, except for the low rate of vermicopost 2 tons /feddan in the first season, concerning to plant height as compared to untreated plants. It is clear that the highest value of such traits was obtained due to adding the high level of vermicompost, which increased plant height, branch number, herb fresh weight (g /plant) and herb dry weight (g /plant)

by 9.4 and 10.5, 19.6 and 22.4, 67.0 and 58.1 and 66.8 and 60.5 % over control in the two experimental seasons, respectively.

Table (3): Influence of vermicompost and chitosan nanoparticles on vegetative growth parameters of black cumin (*Nigella sativa* L) plants during the seasons of 2018-2019 and 2019-2020.

Vermicompost Levels (A)	Chitosan nanoparticles concentrations (B)							
	Plant height							
	First season				Second season			
	Control	20 ppm	40 ppm	Mean (A)	Control	20 ppm	40 ppm	Mean (A)
Control	55.9	64.8	67.9	62.9	58.4	67.9	68.7	65.0
2 tons /feddan	58.8	67.0	69.6	65.1	62.4	68.2	71.6	67.4
4 tons /feddan	61.4	67.2	70.3	66.3	64.2	68.7	72.5	68.5
6 tons /feddan	63.7	69.7	72.9	68.8	65.9	73.4	76.0	71.8
Mean (B)	60.0	67.2	70.2		62.7	69.6	72.2	
LSD 0.5%	A: 2.2 B: 1.1 AB: 2.2				A: 1.2 B: 1.2 AB:2.4			
Vermicompost Levels (A)	Chitosan nanoparticles concentrations (B)							
	Branch number							
	First season				Second season			
	Control	20 ppm	40 ppm	Mean (A)	Control	20 ppm	40 ppm	Mean (A)
Control	8.5	9.2	10.0	9.2	8.9	9.8	10.7	9.8
2 tons /feddan	9.5	10.1	10.9	10.2	10.1	10.3	11.7	10.6
4 tons /feddan	9.8	10.4	11.3	10.4	10.4	11.3	12.2	11.3
6 tons /feddan	10.2	10.8	11.9	11.0	11.0	11.4	13.6	12.0
Mean (B)	9.5	10.1	11.0		10.1	10.7	12.1	
LSD 0.5%	A: 0.7 B: 0.5 AB: 1.1				A: 0.7 B: 0.6 AB: 1.3			
Vermicompost Levels (A)	Chitosan nanoparticles concentrations (B)							
	Herb fresh weight (g/plant)							
	First season				Second season			
	Control	20 ppm	40 ppm	Mean (A)	Control	20 ppm	40 ppm	Mean (A)
Control	42.3	45.4	49.8	45.8	46.9	50.1	52.7	49.9
2 tons /feddan	56.7	65.2	66.8	62.9	61.3	71.4	72.8	68.5
4 tons /feddan	69.6	73.1	75.2	72.6	68.3	76.9	77.8	74.3
6 tons /feddan	72.5	77.1	79.8	76.5	74.3	80.1	82.4	78.9
Mean (B)	60.3	65.2	67.9		62.7	69.6	71.4	
LSD 0.5%	A: 1.4 B: 1.5 AB: 2.9				A: 1.3 B: 1.6 AB: 3.2			
Vermicompost Levels (A)	Chitosan nanoparticles concentrations (B)							
	Herb dry weight (g/plant)							
	First season				Second season			
	Control	20 ppm	40 ppm	Mean (A)	Control	20 ppm	40 ppm	Mean (A)
Control	10.8	11.9	12.8	11.8	12.1	12.9	13.6	12.9
2 tons /feddan	14.7	16.8	17.7	16.4	15.7	18.1	18.9	17.6
4 tons /feddan	17.8	18.9	19.5	18.7	17.5	19.5	20.3	19.1
6 tons /feddan	18.6	19.8	21.1	19.8	19.2	20.7	22.2	20.7
Mean (B)	15.5	16.9	17.8		16.1	17.8	18.8	
LSD 0.5%	A: 1.4 B: 0.6 AB: 1.3				A: 1.5 B: 0.9 AB: 1.8			

The efficiency of vermicompost in improving plant height, branch number, herb fresh weight (g /plant) and herb dry weight (g /plant) were indicated by Vadiraj *et al*. (1993) on cardamom (*Elettaria cardamomum*), Karmegam *et al*. (1999) on green gram, Atiyeh *et al*. (2000) on pot marigold, Bongkyoon (2004) on *Solanum tuberosum* L., Pande *et al*. (2007) on *Bacopa monnieri* Ahmed *et al*. (2011) on *Hibiscus sabdariffa* L., Singh *et al*. (2012) on *Coleus forskohlii*, Aryafar *et al*. (2013) on *Nigella sativa* L and Al- Sayed *et al*. (2019) on roselle

(*Hibiscus sabdariffa* L.). As for ChNP treatments, the listed data in Table (3) revealed that plant height, branch number, herb fresh weight (g /plant) and herb dry weight (g /plant) were significantly increased, in both seasons as a result of supplying the plants with these materials, except for 20 ppm chitosan nanoparticles treatment in the second season, with respect to branch number comparing to control. Moreover, the most effective treatment was obtained by using 40 ppm chitosan nanoparticles which increased these traits by 17.0 and 15.2, 15.8 and 19.8, 12.6 and 13.9 and 14.8 and by 16.8% in comparison with those obtained by other treatments and control. The positive effect of ChNP treatments on enhancing these characteristics was emphasized by Choi *et al*. (2000) on soybean, Khan *et al*. (2002) on maize and soybean, El-Tantawy (2009) on *Solanum lycopersicum*, Farouk, *et al*. (2011) on radish (*Raphanus sativus*, L. var. sativus), Ma *et al.*(2011) on wheat, Mahdavi *et al.* (2011) on Safflower (*Carthamustinctorius* L.), Mahdavi and Rahimi (2013) on ajowan (*Carum copticum*), Massoud *et al*. (2016) on coriander and Dzung *et al*. (2017) on chili plant. The interaction between the two studied factors had significant effect on herb dry weight /plant in first and the second seasons. It was found that most combined treatments significantly affected plant height, branch number, herb fresh weight (g /plant) and herb dry weight (g /plant) in comparison with untreated plants. Furthermore, the addition of vermicompost at the high level (6 tons /feddan) plus 40 ppm ChNP proved to be more effective than other combinations, as clearly declared in (Table 3).

3.2 Yield parameters

The presented data in Table (4) indicated that capsule number /plant, capsule weight /plant, seed weight (g /plant) and weight of 1000 seed /plant were significantly augmented, in the two seasons, due to using all levels of vermicompost as compared to unfertilized plants. The maximum capsules number /plant**,** capsule weight/plant, seed weight (g /plant) and weight of 1000 seed/plant was noticed by utilizing the high level of vrmicompost as ranged 41.7 and 40.2, 42.1 and 39.9, 31.7 and 30.3 and 33.9 and 24.9 % over the check treatment during the two experimental seasons, respectively. The capability of vermicompost application on enhancing yield components was emphasized by Jadhav *et al.* (1997) on sunflower (*Helianthus annuus* L.), Vadiraj *et al*. (1998) on coriander (*Coriandrum sativum*), Reddy *et al.* (1998) on *Chrysanthemum chinensis*, Atiyeh *et al*., (2002) on some vegetables, Senthilkumar *et al.* (2004) on *Rose sp*, Nagavallemma *et al*. (2004) on mung bean (*Vigna radiate* L.), Alam *et al.* (2007) on red amaranth, Chamani *et al*. and (2008), Darzi *et al.* (2012) on anise (*Pimpinella anisum* L.), Bahrampour

and Ziveh (2013) on tomato, Maji *et al*. (2016) on pea (*Pisum sativum* L.).

Table (4): Influence of vermicompost and chitosan nanoparticles on yield parameters of black cumin (*Nigella sativa* L.) plants during the seasons of 2018-2019 and 2019-2020.

Vermicompost Levels (A)	Chitosan nanoparticles concentrations (B)							
	Capsule number/plant							
	First season				Second season			
	Control	20 ppm	40 ppm	Mean (A)	Control	20 ppm	40 ppm	Mean (A)
Control	45.7	53.2	54.4	51.1	50.9	56.3	60.7	56.0
2 tons /feddan	58.1	63.5	66.7	62.8	62.9	67.4	70.2	66.8
4 tons /feddan	63.7	68.1	71.5	67.8	66.4	73.9	77.7	72.7
6 tons /feddan	64.9	74.2	78.1	72.4	68.1	81.7	85.6	78.5
Mean (B)	58.1	64.8	67.7		62.1	69.8	73.6	
LSD 0.5%	A: 0.4 B: 0.7 AB: 1.4				A: 0.9 B: 1.0 AB: 1.9			
Vermicompost Levels (A)	Chitosan nanoparticles concentrations (B)							
	Capsule weight/plant							
	First season				Second season			
	Control	20 ppm	40 ppm	Mean (A)	Control	20 ppm	40 ppm	Mean (A)
Control	13.8	16.7	17.2	15.9	15.9	17.2	18.9	17.3
2 tons /feddan	18.1	19.8	20.8	19.6	19.1	20.9	21.8	20.6
4 tons /feddan	19.6	21.6	22.4	21.2	20.1	23.0	24.2	22.4
6 tons /feddan	19.9	23.5	24.4	22.6	20.6	25.5	26.4	24.2
Mean (B)	17.9	20.4	21.2		18.9	21.7	22.8	
LSD 0.5%	A: 1.0 B: 0.9 AB: 1.9				A: 1.2 B: 1.0 AB: 2.1			
Vermicompost Levels (A)	Chitosan nanoparticles concentrations (B)							
	Seed weight (g/plant)							
	First season				Second season			
	Control	20 ppm	40 ppm	Mean (A)	Control	20 ppm	40 ppm	Mean (A)
Control	18.1	20.7	21.0	19.9	19.6	21.4	22.4	21.1
2 tons /feddan	20.4	22.6	23.4	22.1	21.3	23.2	24.1	22.9
4 tons /feddan	22.1	23.3	25.1	23.5	22.5	24.9	26.2	24.5
6 tons /feddan	23.0	26.2	29.3	26.2	23.6	28.2	30.8	27.5
Mean (B)	20.9	23.2	24.7		21.8	24.4	25.9	
LSD 0.5%	A: 0.15 B: 0.11 AB: 0.23				A: 0.10 B: 0.11 AB: 0.24			
Vermicompost Levels (A)	Chitosan nanoparticles concentrations (B)							
	Thousand seeds weight/plant							
	First season				Second season			
	Control	20 ppm	40 ppm	Mean (A)	Control	20 ppm	40 ppm	Mean (A)
Control	1.55	1.57	2.02	1.71	1.65	1.71	2.08	1.81
2 tons /feddan	1.80	2.00	2.16	1.99	1.70	2.07	2.12	1.96
4 tons /feddan	2.00	2.32	2.37	2.23	1.82	2.24	2.29	2.12
6 tons /feddan	1.96	2.44	2.46	2.29	1.84	2.37	2.57	2.26
Mean (B)	1.83	2.08	2.25		1.75	2.10	2.27	
LSD 0.5%	A: 0.15 B: 0.11 AB: 0.23				A: 0.10 B: 0.11 AB: 0.24			

With regard to chitosan nanoparticles (ChNP) treatments, the obtained results in Table (4) emphasized that foliar spray with chitosan nanoparticles at all concentrations, during the two seasons, caused a significant augment in capsule number /plant**,** capsule weight/plant, seed weight (g /plant) and weight of 1000 seed /plant comparing to control. Obviously, the application of ChNP at the high concentration (40 ppm) gave the highest values of these traits as ranged 16.5 and 18.5, 18.4 and 20.6, 18.2 and 18.8 and 22.9 and 29.7% over no sprayed ones in the first and second seasons, respectively. The augment in capsule number /plant**,**

capsule weight /plant, seed weight (g /plant) and weight of 1000 seed/plant due to applying ChNP was also demonstrated by Dzunga *et al.* (2011) on coffee, Abdel-Aziz *et al*. (2016) on wheat, Zagzog *et al.* (2017) on mango (*Mangifera indica* L.). Regarding the combination, the obtained results in Table (4) cleared a significant effect on capsules number /plant**,** capsule weight /plant, seed weight (g /plant) and weight of 1000 seed /plant, during the two experimental seasons. However, most of combined treatments caused a significant increment in these traits, during the two seasons, comparing to untreated plants. Therefore, adding vermicompost at 6 tons/feddan + 40 ppm chitosan nanoparticles proved to be more effective in increasing capsule number /plant**,** capsule weight /plant, seed weight (g /plant) and weight of 1000 seed /plant than those of by other combinations.

3.3 Oil production

3.3.1 Volatile oil and fixed oil percentages and their yield (ml/plant)

The obtained results in Table (5) indicated that volatile oil and fixed oil % and volatile oil and fixed oil yield (ml /plant) of black cumin seeds was significantly increased as a result of vermicompost application at all levels compared to the check treatment, during the two consecutive seasons. Clearly, the use of vermicompost at the high level (6 tons /feddan) gave the highest values of volatile oil & fixed oil % and volatile oil and fixed oil yield (ml /plant) comparing to other treatments. This treatment augmented such traits by 49.3 and 49.4, 23.0 and 21.3% 98.1 and 99.4 and 70.6 and 65.6% over the check treatment in the two seasons, respectively. The role of vermicompost in enhancing volatile oil & fixed oil % and volatile oil and fixed oil yield (ml /plant) was reported by Hadi *et al.* (2011) on *Matricaria chamomila* L., Singh (2011) on coriander (*Coriandrum sativum* L.), Moslemi *et al*. (2012) on Coriander (*Coriandrum sativu* L.), Singh and Wasnik (2013) on rosemary (*Rosmarinus officinalis* L.) and Abbaszadeh *et al.* (2016) on *Lavandula officinalis*. It is evident from the revealed data in Table (5) that volatile oil & fixed oil% and volatile oil & fixed oil yield (ml/plant) of black cumin was statistically significant, in both seasons, due to applying ChNP treatments as compared to untreated ones during the two experimental seasons. Clearly spraying the plants by 40 ppm ChNP yielded the maximum values of volatile oil and fixed oil % and volatile oil and fixed oil yield (ml /plant) by range 24.2 and 29.8, 8.9 and10.3, 49.2 and 57.4 and 26.7 and 39.3% in the first and second seasons, respectively. Accordingly, the combination of the two studied factors on volatile oil and fixed oil% and volatile oil and fixed oil yield (ml/plant) of black cumin had significant effect during the two experimental seasons (Table 5). From the recorded data, it could be noticed that applying most combined treatments significantly increased these

traits, in the first and second seasons, as compared to control. Clearly, the application of vermicompost at the high level (6 tons /feddan) with 40 ppm chitosan nanoparticles proved to be more effective in increasing volatile oil & fixed oil% and volatile oil & fixed oil yield (ml /plant) than those obtained by other combination treatments in both seasons.

Table (5): Influence of vermicompost and chitosan nanoparticles on volatile & fixed oil% and volatile & fixed oil yield (ml /plant) of black cumin (*Nigella sativa* L.) plants during the seasons of 2018-2019 and 2019-2020.

Vermicompost Levels (A)	Chitosan nanoparticles concentrations (B)							
	Volatile oil (%)							
	First season				Second season			
	Control	20 ppm	40 ppm	Mean (A)	Control	20 ppm	40 ppm	Mean (A)
Control	0.74	0.79	0.85	0.79	0.77	0.82	0.91	0.83
2 tons /feddan	0.90	1.08	1.11	1.03	0.96	1.24	1.28	1.16
4 tons /feddan	0.96	1.17	1.23	1.12	1.00	1.24	1.29	1.18
6 tons /feddan	1.02	1.19	1.34	1.18	1.04	1.26	1.41	1.24
Mean (B)	0.91	1.06	1.13		0.94	1.14	1.22	
LSD 0.5%	A: 0.04 B: 0.05 AB: 0.16				A: 0.04 B: 0.06 AB: 0.19			
Vermicompost Levels (A)	Chitosan nanoparticles concentrations (B)							
	Fixed oil (%)							
	First season				Second season			
	Control	20 ppm	40 ppm	Mean (A)	Control	20 ppm	40 ppm	Mean (A)
Control	23.33	24.30	25.73	24.45	23.51	25.70	26.70	25.30
2 tons /feddan	24.73	25.67	26.77	25.72	25.63	26.17	27.73	26.51
4 tons /feddan	26.91	28.03	28.33	27.76	27.07	28.30	29.07	28.15
6 tons /feddan	28.37	30.10	31.51	29.99	28.83	30.61	32.63	30.69
Mean (B)	25.84	27.03	28.09		26.26	27.70	29.03	
LSD 0.5%	A: 0.35 B: 0.48 AB: 0.97				A: 0.32 B: 0.64 AB: 1.28			
Vermicompost Levels (A)	Chitosan nanoparticles concentrations (B)							
	Volatile oil yield (ml/plant)							
	First season				Second season			
	Control	20 ppm	40 ppm	Mean (A)	Control	20 ppm	40 ppm	Mean (A)
Control	0.134	0.162	0.178	0.158	0.140	0.175	0.203	0.173
2 tons /feddan	0.183	0.244	0.260	0.229	0.205	0.287	0.308	0.267
4 tons /feddan	0.212	0.272	0.309	0.264	0.226	0.309	0.338	0.291
6 tons /feddan	0.234	0.312	0.394	0.313	0.246	0.355	0.434	0.345
Mean (B)	0.191	0.248	0.285		0.204	0.282	0.321	
LSD 0.5%	A: 0.017 B: 0.023 AB: 0.049				A: 0.023 B: 0.024 AB: 0.052			
Vermicompost Levels (A)	Chitosan nanoparticles concentrations (B)							
	Fixed oil yield (ml/plant)							
	First season				Second season			
	Control	20 ppm	40 ppm	Mean (A)	Control	20 ppm	40 ppm	Mean (A)
Control	4.223	5.023	5.412	3.119	4.237	5.512	5.991	3.279
2 tons /feddan	5.053	5.807	6.254	3.705	5.458	6.068	6.672	4.168
4 tons /feddan	5.962	6.542	7.121	4.727	6.096	7.046	7.606	4.734
6 tons /feddan	6.524	7.886	9.244	5.321	6.796	8.627	10.042	5.430
Mean (B)	3.692	4.284	4.678		3.575	4.655	4.979	
LSD 0.5%	A: 0.319 B:0.384 AB: 0.769				A: 0.492 B: 0.414 AB: 0.828			

3.4 Chemical constituent parameters

3.4.1 Nitrogen, phosphorus and potassium (%)

The presented data in Table (6) showed that the nutrient elements of N, P and K % in black cumin herb were significantly augmented due to the use of vermicompost treatments as compared to unfertilized plants for the two

experimental seasons. It is noticed that supplying the plants with vermicompost at the high rate (6 tons /feddan) gave the maximum values of the three examined elements of N, P and K % in both seasons, which increased N % by 58.3 and by 55.9 %, augmented P % by 28.1 and by 26.9% and also increased K % by 16.4 and by 18.7% over the check treatment during the two seasons, respectively. The promoting effect of vermicompost treatments was reported by Sainz *et al*. (1998) on rice (*Oryza sativa* L.), Premuzic *et al*. (1998) on petunia (*Petunia hybrida* L.), Senthilkumar *et al.* (2004) on *Rose sp*., Kumari and Ushakumari (2002) on cowpea (*Vigna unguiculata* L. Walp), Llaven (2008) on bell pepper, Lazcano and Dominguez (2010) on ornamental plant species and Manh and Wang (2013) on *Cucumis melo* L.

Table (6): Influence of vermicompost and chitosan nanoparticles on NPK percentages in the dry herb of black cumin (Nigella sativa L) plants during the seasons of 2018-2019 and 2019-2020.

Vermicompost Levels (A)	Chitosan nanoparticles concentrations (B)							
	Nitrogen (%)							
	First season				Second season			
	Control	20 ppm	40 ppm	Mean (A)	Control	20 ppm	40 ppm	Mean (A)
Control	1.33	1.39	1.46	1.39	1.38	1.42	1.49	1.43
2 tons /feddan	1.48	1.53	1.57	1.53	1.54	1.58	1.61	1.58
4 tons /feddan	1.68	1.76	1.87	1.77	1.63	1.73	1.88	1.75
6 tons /feddan	2.02	2.18	2.40	2.20	2.03	2.22	2.43	2.23
Mean (B)	1.63	1.72	1.83		1.65	1.74	1.85	
LSD 0.5%	A: 0.06 B: 0.07 AB: 0.18				A: 0.06 B: 0.07 AB: 0.18			
Vermicompost Levels (A)	Chitosan nanoparticles concentrations (B)							
	Phosphorus (%)							
	First season				Second season			
	Control	20 ppm	40 ppm	Mean (A)	Control	20 ppm	40 ppm	Mean (A)
Control	0.266	0.332	0.342	0.313	0.272	0.337	0.347	0.319
2 tons /feddan	0.356	0.361	0.370	0.362	0.350	0.354	0.364	0.356
4 tons /feddan	0.363	0.378	0.387	0.376	0.369	0.381	0.403	0.384
6 tons /feddan	0.392	0.403	0.409	0.401	0.393	0.407	0.414	0.405
Mean (B)	0.344	0.369	0.377		0.346	0.370	0.382	
LSD 0.5%	A: 0.016 B: 0.016 AB: 0.032				A: 0.016 B: 0.017 AB: 0.035			
Vermicompost Levels (A)	Chitosan nanoparticles concentrations (B)							
	Potassium (%)							
	First season				Second season			
	Control	20 ppm	40 ppm	Mean (A)	Control	20 ppm	40 ppm	Mean (A)
Control	1.24	1.27	1.32	1.28	1.21	1.23	1.26	1.23
2 tons /feddan	1.29	1.40	1.43	1.37	1.26	1.35	1.40	1.34
4 tons /feddan	1.33	1.45	1.50	1.43	1.32	1.43	1.46	1.40
6 tons /feddan	1.37	1.52	1.57	1.49	1.35	1.49	1.55	1.46
Mean (B)	1.31	1.41	1.46		1.29	1.38	1.42	
LSD 0.5%	A: 0.02 B:0.03 AB: 0.09				A: 0.03 B: 0.03 AB:0.10			

Data in Table (6) indicated that the percentages of N, P and K in dry herb of black cumin were significantly augmented due to the use of ChNP treatments comparing to untreated treatment in the two concoctive seasons. In this regard, the maximum values of the three tested elements of N, P and K % were obtained by applying 40 ppm ChNP concentration without significant differences between the two superior treatments, in the first and the second

seasons, respectively. The positive effect of ChNP treatments on the elements of N, P and K % was insured by Dzunga *et al.* (2011) on coffee, Zagzog (2017) on mango (*Mangifera indica* L.). Regarding the combined between the vermicompost manure and chitosan nanoparticles (ChNP) treatments, the result in Tables (6-8) revealed that it was significant effect on the three studied elements N, P and K % for the two experimental seasons. However, the most effective treatments were vermicompost at the high level (6 tons /feddan) plus 40 ppm chitosan nanoparticles concerning N, P and K% in comparison with other combined treatments in both seasons. From the obtained data, it could be discussed as follows: the improvement of plant height, branch number, herb fresh weight, herb dry weight, number of capsule and weight, seed weight, 1000 seed weight, fixed and volatile oil percentages and yields of seed of black cumin in this study as a result of using organic manure (vermicompost) reflected the biological and physiological roles of organic fertilization which was explained that organic matter as a source of elements namely, N, P and K as well as, it is considered as a source of energy for the growth of *microorganisms*. Dominguez (2004) showed that vermicompost is a nutrient rich (macro and micronutrients.), microbiologically active. It is a stabilized, finely divided peat-like material with a low C:N ratio, high porosity, a slow release and high-water holding capacity, in which most nutrients are present in forms that are readily taken up by plants. Anwar *et al*. (2005) studied the effect of vemicompost on growth and productivity of some medicinal and aromatic plant and showed that positive effects due to the use of vermicompost. Darzi *et al.* (2012) showed that, the using of vermicompost at (0, 5 and 10 tons /ha) significantly affected on plant height, umbel number per plant, biological and seed yield of anise. The maximum values of these traits were obtained by vermicompost at 10 tons/ha. Also, the enhancement of the previous traits in the present work might be attributed to the biological and physiological roles of chitosan may stimulate a signal to synthesize plant hormones such as gibberellins. In addition to chitosan may enhance growth and development by some signalling pathway related to auxin biosynthesis via a tryptophan- independent pathway Uthairatanakij *et al.* (2007). Moreover, chitosan has been shown to stimulate plant growth (Kim, 2005; Mondal *et al*., 2012), to possess antioxidant activity (Chen *et al*., 2009; Xie *et al*., 2001). Van *et al*. 2013 showed that ChNP was impacted on the biophysical characteristics of coffee seedlings, through increasing pigment content, the rate of photosynthesis and nutrient uptake. It could be recommended to supply black cumin plants with vermicompost at 6 tons /feddan in combination with 40 ppm ChNP for improving plant height, branch number, herb fresh weight, herb dry weight, capsule number and weight, seed weight, 1000 seed weight, fixed and volatile oil percentages and yields of the seeds.

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