

Response of *Pimpinella anisum* L. Plant For Graphite and Silica Nanoparticles

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THERE has been a rising demand for nanotechnology-based products in recent years, particularly in agriculture application. In this effort, the phytotoxicity and stimulatory effects of graphite and silica nanoparticles (NPs) on seed germination, yield characteristics, chemical conformation and root anatomical characteristics of (*Pimpinella anisum* L.) cv. anise were studied. Seeds were soaked in six concentrations of the two NPs (0, 20, 40, 60, 80 and 100 ppm) for 24 hr to investigate germination under laboratory conditions. The same concentrations were used for soaking and foliar application in the open field. The results displayed that silica NPs improved seed germination traits comparing with graphite NPs. Silica NPs at 20 and 40 ppm, showed the highest germination parameters for anise seeds. The increase in germination % reached 21.88%. Meanwhile, 80ppm silica NPs enhanced the seeds yield fed.⁻¹ by 64.8%, Number of umbels by 50%, root diameter plant⁻¹, epidermis thickness and cortex thickness. Silica NPs (60ppm) increased biochemical characteristic. Otherwise, graphite NPs (80ppm) enhanced volatile oil% and root vascular cylinder thickness. In general, there was a considerable response by anise seeds to graphite and silica nano-sized presenting the possibility of a new methodology to overcome problems with seed germination and increasing the oil percent in some plant species, mostly medicinal plants.

Keywords: Graphite NPs, Silica NPs, Anise, Seeds germination, Yield, Essential oil, Root anatomical characteristics.

Introduction

Nano-agriculture involves the employment of nanoparticles in agriculture, with the particles imparting specific beneficial effects to the crops (Morla et al., 2011). Among the nanoparticles, nano-silicon has had a special consideration mostly in the last few years. Silicon is an abundant element in the soils which accounts for approximately 32% of the total weight of the soil and has been known as a beneficial nutrient for growth and development of most plants (Khodakovskaya et al., 2009, Siddiqui and Al-Whaibi 2014 and Abdel Latef & Tran, 2016), but silicon still failed to get assessment as a necessary nutrient for plant growth and development, while favorable belongings of this element have been determined in a wide variety of plant species as a physic mechanical barrier, and is deposited on the epidermal cell walls and leaf sheath, vascular tissues of the stem and hull in most plants particularly monocots (Saqib et al., 2008 and Pei

et al., 2010). Silicon has had a very important role in a lot of metabolic and physiological plant activities, for example keeping plant cells turgid and plant size by improving water use efficacy and leaf relative water content. Implementation of silicon fertilizers in silicon-deficient soil can promote plant growth, increase plant resistance to disease, cold and heavy metals such as copper, aluminum, manganese, and iron consequently encourage photosynthesis (Guo, 2000 and Hu & Schmidhalter, 2005).

In the recent years, carbon nanomaterials are used in agriculture to rise the crop production. As an important category of carbon nanomaterials such as fullerenes, graphene carbon nanotubes, known for their light weight, super strength and extreme conductivity occupy a unique place in applications of agriculture through their effects on germinations of the seeds, seedling growth, and plant development of various plant species. Similarly Khodakovskaya et al. (2009) found that

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carbon nanotubes (10–40 µg/ml) improve seed sprouting and root development by penetrating the thick coat of tomato seeds and support water uptake inside seeds. In addition, the expression of the tobacco aquaporin (NtPIP1) gene, as well as the production of the (NtPIP1) protein considerably increases in the cells which treated with carbon nanotubes (Khodakovskaya *et al.*, 2012). While, they are known to be beneficial in seed germination, root growth, and photosynthesis. Although, the quick development that occurred in the synthesis and use of carbon nanoparticles, but till now the mode of action of these materials of interaction with the plant is not completely understood because opposite reports from various quarters have been received (Canaset *al.*, 2008 and Lin *et al.*, 2009).

Seed germination is an important phenomenon in modern agriculture because it is a thread of plants lives that guarantee its survival and used as acute phytotoxicity test with numerous benefits like sensitivity, low cost, simplicity, and suitability for unstable chemicals or samples (Wang *et al.*, 2005). Recently, some chemical constituents were applied in a wide range for enhancing seed germination and overcoming seed dormancy problem in plants. Applications of nanomaterials can accelerate plant germination/ production, affect plant protection, above of that help in decrease environmental contaminations as compared with the traditional methods (Khotet *al.*, 2012). Nanoparticles improve the chemical and physical properties of the soil which reflected on plant growth (Amin *et al.*, 1999).

Pimpinella anisum L. (anise) Fam: Apiacea is one of the most important annual medicinal plants, white-flowered, about 44 cm high, a native of Iran, Turkey, Egypt, Greece, Crete and Asia Minor. Its main active components are used in pharmaceutical industries and cosmetics besides its use as flavoring materials in food stuffs (Nabizadeh *et al.*, 2012). The seeds contain volatile oil and active constituents specially anethole that has been used in pharmaceutical and food products (Klaus *et al.*, 2009). The influence of NPs on plants differs from plant to another and from species to others. However, poor seed germination is a common occurrence in medicinal and aromatic plants especially, seeds after dry storage often display slow and non-uniform germination due to compromised vigor. Moreover, germinating seeds and young seedlings are susceptible to dehydration stress due in part

to the progressive loss of desiccation tolerance upon seed hydration. As above mentioned, this study was done to examine the effect of SiO₂ and graphite NPs concentrations on anise seed germination, yield characteristics, chemical composition, oil productivity and root anatomical characteristics.

Materials and Methods

The experiments were performed during the two seasons of 2013-2014 and 2014-2015.

Laboratory experiment

This experiment was carried out in the lab of Sakha Horticulture Research station, Kafr El-Sheik Governorate, Egypt to study the germination characteristics. Local variety of healthy anise seeds from Medicinal and Aromatic plants Department, Agricultural Research Center, Egypt were surface sterilized by dipping them in sodium hypochlorite (5%) solution for 5 min, washed more than one time with tap water then rinsed with distilled water, and dried by blotting in filter paper. The sterilized seeds were divided into six portions. Equal portions of sterilized seeds were soaked in five concentrations (20, 40, 60, 80 and 100 ppm) of each nanoparticles (silicone and graphite) in addition to soaking in distilled water as the control. Seeds were soaked for 24 hr at ±2°C under dark conditions. Forty soaked seeds of each treatment were sown in 10 cm width Petri dishes top of two moistened layers of Whatman No. 1 filter paper and incubated at 25±2°C with 16 hr illumination in four replicates. Germination data were documented every day for 9 days; seed with 2 mm radicle protrusion was considered as germinated. Radicle and plumule lengths as well as fresh and dry weights were measured at seventh day.

Germination parameters

Germination percentage (G) was computed following the ISTA (2011).

$$G (\%) = (\text{germinated seeds no} / \text{total number of seeds}) \times 100$$

Mean germination time (MGT) was calculated based on Matthews and Khajeh-Hosseini (2007).

$$MGT = \Sigma F \times X / \Sigma F$$

Where F is the number of seeds newly germinated at the time of X, and X is the number of days from sowing. Seedling vigors were calculated based on Vashisth and Nagarajan (2010)

$$\text{Vigor index I VI (I)} = \text{Germination\%} \times \text{Seedling length (cm)}$$

Vigor index II VI (II) = Germination% × Seedling weight (g)

Evaluations of Mean Daily Germination (MDG), Pick Value (PV) and Germination Value (GV) were calculated by the following equations:

$$MDG = \text{Germination\%} / \text{Total experiment days.}$$

$$PV = \text{Maximum germinated seed number at one day} / \text{day number.}$$

$$GV = PV \times MDG$$

Mean Daily Germination (MDG), Pick Value (PV) and Germination Value (GV) were documented by Hartmann et al. (1990).

Field experiment

Two field experiments were performed through

the two different seasons of 2013-2014 and 2014-2015 at El-Khashaa area, El-Hamoul, Kafr El-Sheik Governorate to calculate the response of *Pimpinella anisum* L. for silica and graphite nanoparticles on yield characteristics, chemical composition, oil productivity and characteristics of root anatomy.

Plant material and procedure

The second part of primed seeds treated by nanoparticles were sown in the field on November 27th in 1st season and November 25th in 2nd one on rows at 60 cm apart in hills and 30cm in between. Plot area was 4m² for each replicate contained 18 plant. After the complete germination, the seedlings were thinned to 2 plants per hill 30 days later of emergence. The experimental soil was analyzed and illustrated in Table 1

TABLE (a). Some physical and chemical soil properties of the experimental site as mean values of the two growth seasons

Organic matter %	Total N ppm	Total p ppm	Total k ppm	Sand (%)	Silt (%)	Clay (%)	Texture class	pH.
0.94	32.70	22.50	780	10.78	19.85	69.37	Clayey	7.7
EC _e			Anions concentration meq/L			Cations concentration meq/L		
(dS m ⁻¹)	CO ₃ ⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻	Ca ⁺⁺	Mg ⁺⁺	Na ⁺	CaCO ₃ %
5.6	---	2.8	104	79	55	61	94	2.5

TABLE 1. Effect of graphite and silica nanoparticles on GP %, MGT (day), seedling length (cm), Seedling weight (g), VI I (cm), VI II (g), MDG, PV and GV of *Pimpinella anisum* L. seeds

Traits	GP %	MGT (day)	Seedling length	Seedling weight	VI I (cm)	VI II (g)	MDG	PV	GV
Control	80.0 d	4.87 g	5.461 abc	0.040 b	436.88 ab	2.88 d	8.00 d	2.16 b	17.280 c
GNps 20 ppm	72.5 g	6.31 b	5.419 abc	0.044 b	392.85 ab	3.19 c	7.25 g	1.30 i	9.425 i
GNps 40 ppm	67.5 h	6.11 c	4.530 bc	0.031 c	305.75 b	2.09 i	6.75 h	1.42 g	9.585 h
GNps 60 ppm	75.0 f	6.40 b	5.673 abc	0.043 b	425.45 ab	3.22 b	7.50 f	1.57 f	11.775 g
GNps 80 ppm	85.0 c	5.47 e	3.768 c	0.030 c	320.25 b	2.55 e	8.50 c	2.00 d	17.00 d
GNps 100 ppm	60.0 j	5.75 d	6.181 ab	0.042 b	370.84 b	2.52 f	6.00 j	2.20 a	13.200 e
Si Nps 20 ppm	97.5 a	5.77 d	5.652 abc	0.040 b	551.02 a	3.90 a	9.75 a	2.20 a	21.450 a
Si Nps 40 ppm	50.0 k	5.25 f	6.795 a	0.050 a	339.72 b	2.50 g	5.00 k	1.40 h	7.000 k
Si Nps 60 ppm	62.5 i	5.36 ef	5.420 abc	0.033 c	338.66 b	2.06 j	6.25 i	1.16 j	7.250 j
Si Nps 80 ppm	77.5 e	6.67 a	5.207 abc	0.030 c	403.54 ab	2.32 h	7.75 e	1.66 e	12.865
Si Nps 100 ppm	95.0 b	5.73 d	4.255 bc	0.022 d	404.17ab	2.09 i	9.50 b	2.14 c	20.330 b

Means, in each column, followed by similar letter are not significantly different at the 5% probability level – using Duncan’s Multiple Range Test.

The recommended chemical fertilizer doses were added according to the Ministry of Agriculture as follows (300, 150 and 50 kg fed⁻¹) for ammonium nitrate (33.5%N), calcium superphosphate (15.5 %P₂O₅) and potassium sulphate (48% K₂O), respectively. After thinning, plants were sprayed twice with the same nanoparticles levels used in germination test on January 23rd and 21th in the first and second season, respectively and before flowering stage on February 26th and 19th in both seasons, respectively. The following parameters were documented at the end of growth seasons when plants were harvested in the ripe dry fruits stage:

Yield characteristics

1. Number of umbels per plant.
2. Seed index (weight of 1000 seeds).
3. Seeds yield g/plant and/fed.

Biochemical characteristics

Essential oil productivity and components

Essential oil percentage was determined in the ripe dried fruits according to British Pharmacopoeia (1963). Gas chromatographic analysis was determined for essential oil in nine samples only for anise plant especially highly percentage oil samples which were obtained from treated plants with 20, 60, 80 and 100 ppm silica and graphite nanoparticles in addition to the control. GC/mass analysis of essential oil of each treatment was achieved with the specification of the apparatus used according to Robert (1995). Chlorophyll a, b and carotenoids were determined in fresh samples at the flowering stage according to the technique cleared by (Wettstein, 1957).

Characteristics of root anatomy

Roots samples of *Pimpinella anisum* L. were gathered from the mature plants during February 2015. The samples were taken from approximately the beginning of lowest half of the plant root and

then fixed in formalin-acetic acid alcohol (FAA) using 70% ethanol. The samples were gradually dehydrated in a tert-butyl alcohol (TBA) series (Johansen, 1940) and embedded in paraffin wax (m.p. 56 °C). Sections were cut on a rotary microtome at a thinness of 8-10 µm. Paraffin was removed with xylol, after that slides were stained with safranin FCF methanol and fast green, then mounted in Canada balsam (Johansen, 1940). The best sections were selected and examined then photographed by the light microscope.

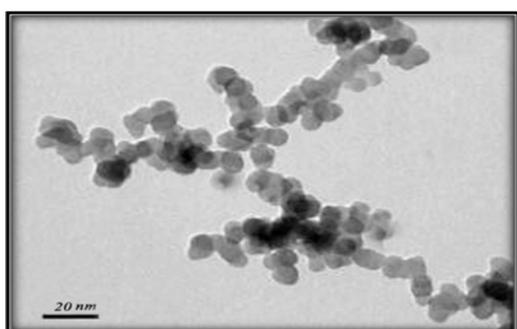
Nanomaterials type

Silica nanoparticles (SiNPs)

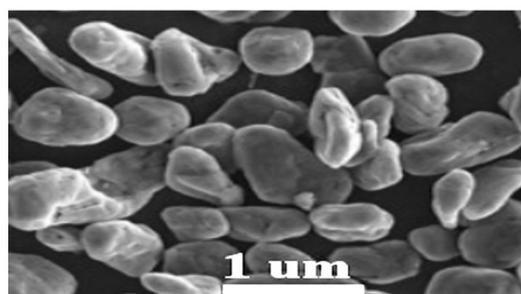
SiNPs; 18 nm in average diameters, were prepared from rice husk by **Dr. Magdy F. El-Samahy**, Plant Protection Research Institute, Agriculture Research Center, Egypt, in the spherical form (98% purity). The SiNPs size was examined by transmission electron microscopy (TEM). It was found that the obtained SiNPs size ranged from 10 to 12 nm (Fig. 1). Using the Brunauer–Emmett–Teller (BET) method (Brunauer, 1945), the result showed that the highest specific surface of the sample was about 320 m² g⁻¹. The obtained results in the mentioned method were proved that the rice husk from agricultural wastes can be used for the production of silica nanoparticles.

Graphite nanoparticles were purchased from NanoTech Egypt Co., Dreamland, Wahat Road, 6th October, Egypt. The properties of graphite nanoparticles were purity: 99.9% (metal base), range: 400 nm-1.2 µm, pH = 6-7, fixed Carbon: 99%, ash: <0.5%, impurities: (quartz + mica) < 0.1%, H₂O~0.2%, morphology: flaky, color: black and the surface area: 6-8 m²/g (Fig. 1).

Concerning statistical analysis, the experimental layout was a complete randomized blocks design with three replicates. The main values of treatments were compared by Duncan's Multiple Range Test according to Snedecor and Cochran (1980) using MSTATC computer program.



Silicon nanosized



graphite nanosized

Fig.1. Images of SiO₂ nanosized and graphite nanosized by Transmission Electron Microscope (TEM)

Results and Discussion

Germination parameters

Results in Table 2 showed significant positive or negative effects of graphite and silica nanoparticles on all germination parameters of anise seeds. The highest significant G%, vigor indices, MDG, PV and GV of seeds obtained from 20 ppm silica nanoparticles compared with the control. Moreover, the highest significant length and weight of seedling were recorded from 40 ppm silica nanoparticles. Exposing the seeds to 80 ppm silica nanoparticles led to increasing MGT. These findings could state that less and moderate concentration of silica nanoparticles could penetrate intact cell walls and transport different

loads into plant cell organelles. Thus, NPs are able to penetrate plant cell walls and it is probable that the same process may be in place during seed germination (Lin et al., 2009) and might help the water absorption by the seeds (Zheng et al., 2005). Moreover, silica nanoparticles enhancement of the performance of chitinase and phytoalexin enzymes which help plants to prevent the contagion of microbial diseases (Luan et al., 2006). Similarly (Zhang et al., 2015, Agrawal & Rathore, 2014 and Clement et al., 2013) showed the affirmative morphological effects of nanoparticles including better germination percentage and rate, length of root and shoot and their ratio, and vegetative biomass.

TABLE 2. Effect of graphite and silica nanoparticles on Number of umbels /plant, seeds yield/plant and /fed and 1000 seeds weight of Pimpinella anisum L plants in the two seasons 2014 and 2015

Traits	Umbels no/plant		Seeds yield/plant (g)		Seeds yield/fed (kg)		1000 seeds weight (g)	
	1 st seasons	2 nd seasons	1 st seasons	2 nd seasons	1 st seasons	2 nd seasons	1 st seasons	2 nd seasons
Control	60.00 c	52.33 bc	5.19 c	6.56 bc	232.74 c	293.81 b-e	4.15 bcd	3.90 abc
GNps 20 ppm	67.00 b	61.00 b	6.16 b	7.38 b	276.04 b	330.40 bc	3.99 d	3.60 cd
GNps 40 ppm	47.00 e	43.66 c	4.73 c	6.39 bc	211.83 cd	286.34 cde	3.87 d	3.79 bcd
GNps 60 ppm	35.33 h	28.33 d	3.53 d	3.53 e	158.07 e	158.92 g	4.08 cd	3.93 abc
GNps 80 ppm	38.66 gh	45.00 c	2.93 e	6.53 bc	131.18 f	292.32 b-e	3.37 e	3.43 d
GNps 100 ppm	53.33 d	44.66 c	4.53 c	5.78 cd	202.86 d	258.72 ef	4.64 a	4.02 abc
Si Nps 20 ppm	64.00 bc	52.66 bc	6.49 b	7.14 bc	290.97 b	319.94 bcd	4.13 bcd	4.35 a
Si Nps 40 ppm	41.00 fg	41.00 c	3.76 d	5.06 d	168.52 e	226.61 f	4.49 ab	4.32 a
Si Nps 60 ppm	45.33 ef	50.00 bc	4.59 c	6.34 bc	205.87 cd	284.10 de	4.39 abc	4.14 ab
Si Nps 80 ppm	90.00 a	80.00 a	8.56 a	9.34 a	383.56 a	418.50 a	4.10 cd	3.90 abc
Si Nps 100 ppm	59.00 c	61.00 b	4.86 c	7.43 b	217.80 cd	332.64 b	4.39 abc	3.96 abc

Means, in each column, followed by similar letter are not significantly different at the 5% probability level – using Duncan's Multiple Range Test.

Phytotoxicity and stimulatory effects of graphite and silica nanoparticles

All graphite and SiO₂ nanoparticles concentration reduced germination % up to 37.5% at 40 ppm SiO₂ NPs except for 80 ppm graphite NPs, 20 and 100 ppm SiO₂ NPs compared to the control (Fig. 2). In addition, all graphite and SiO₂ nanoparticles treatments had a negative effect on MGT, 80 ppm silica nanoparticles greatly decreased MGT up to 26.99% compared to the control seeds. It seems that seedling length and weight were not significantly affected by nanoparticles concentration. Application of 80 ppm graphite NPs and 100 ppm SiO₂ NPs extremely decreased

seedling elongation and seedling weight up to 31 and 22.08%, respectively compared with unexposed seeds but 40 ppm SiO₂ NPs did not demonstrate such reduction in seedling length and weight. Nonetheless, this concentration (40 ppm SiO₂ NPs) led to enhancing length and weight of seedling by 19.63 and 20%, respectively compared to the non-exposure treatment. The reason for this can be attributed to increasing the concentration of nanoparticles may induced aggregation of particles and resulted in clogging of root pores that interrupted water uptake by seeds (Feizi et al., 2013). In addition, application of nanoparticles decreased vigor indices, MDG,

PV, and GV except application of 20ppm SiO₂ NPs greatly increased up to 20.71, 26.15, 17.95, 1.82 and 19.44%, respectively compared to the control. The reason for this decrease may be that nanoparticle could stimulate the process of seed germination like water and oxygen uptake resulting in improving seed germination percentage but in later growth stages, seedling might respond as different (Feizi *et al.* 2013). Also, it is possible that the seeds enhanced by nanoparticle and then cultivated in soil in the field. In this condition it is possible physico-chemical properties of soil modify adverse effects on plant growth and weights. Moreover, the key reason for this growth increasing could have been the photo-sterilization and photo-generation of active oxygen like, superoxide and hydroxide anions by SiO₂ NPs that enhanced seed stress resistance and encouraged capsule penetration for intakes of water and oxygen needed for quick germination (Khot *et al.*, 2012).

Yield characteristics

Graphite and silica nanoparticles (Table 3) significantly diminished the number of umbels plant⁻¹

and seeds yield plant⁻¹ except for 20ppm GNPs and 20, 80 and 100 ppm SiO₂ nanoparticles. In this regard, high concentration 80ppm of SiO₂ nanoparticles recorded the highest number of umbels plant⁻¹ and seeds yield plant⁻¹ and fed. Otherwise, useful effects of most SiO₂ nanoparticles concentration on 1000 seeds weight. These results may be because of the increasing amounts of pigments which led to increasing yield (Yang and Hong, 2006). These results respectively agree with those obtained by Abdul Qados and Moftah (2015). The stimulatory impacts on umbels number and seeds yield plant⁻¹ and fed. SiO₂ nanoparticles 80ppm increased the number of umbels /plant by 50 and 52.88%, seeds yield/plant by 64.93 and 42.38% and seeds yield/fed by 64.8 and 42.44% in both seasons, respectively (Fig. 3). An increase in germination indices by using SiO₂ NPs could be effective for the growth and yield of crops. However, the interaction mechanism between nanosilica and plants which establishes that nano SiO₂ could be used as a fertilizer for the crop improvement (Siddiqui and Al-Whaibi, 2014).

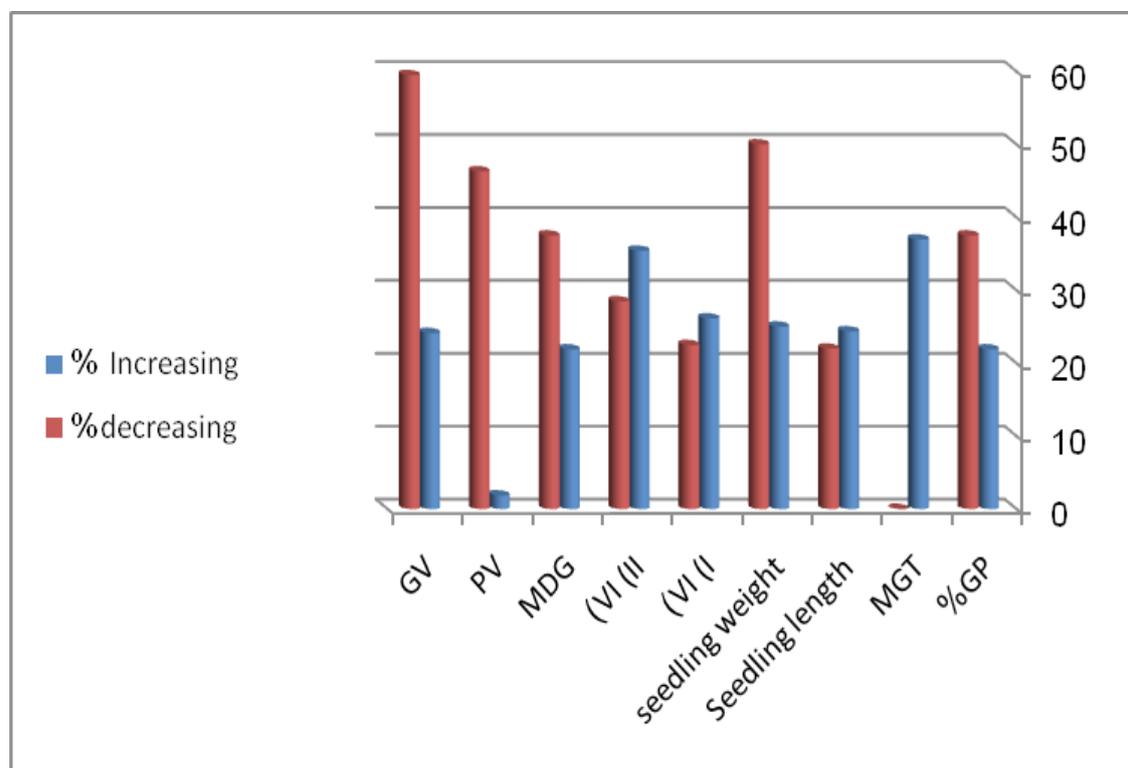


Fig.2. Impact of graphite and silicananoparticles on increasing and decreasing% of all germination anise traits

TABLE 3. Effect of graphite and silica nanoparticles on chlorophyll a, chlorophyll b, carotenoids and Volatile oil% of pimpinella anisum L plants in the two seasons 2014 and 2015

Traits	Chlorophyll a		Chlorophyll b		Carotenoids		Volatile oil%	
	1 st seasons	2 nd seasons						
Control	1.53 bc	1.42 c	1.38 b	1.72 cd	7.08 bc	5.64 c	1.39 ab	1.19 ab
GNps 20 ppm	1.71 b	1.55 bc	1.89 a	1.85 c	8.10 ab	6.71 b	1.20 c	1.09 b
GNps 40 ppm	1.33 d	1.40 c	0.86 c	1.57 d	6.18 c	5.54 c	0.95 d	0.79 cd
GNps 60 ppm	1.66 b	1.44 c	1.61 ab	1.69 cd	8.45 ab	5.88 c	0.64 e	0.46 e
GNps 80 ppm	1.23 d	1.36 c	0.63 c	1.31 e	4.16 d	4.96 d	1.45 a	1.32 a
GNps 100 ppm	1.39 cd	1.48 bc	1.44 ab	1.68 cd	8.24 ab	6.04 c	1.41 ab	1.22 ab
Si Nps 20 ppm	1.92 a	1.73 ab	1.64 ab	2.32 a	8.28 ab	7.00 b	1.35 ab	1.20 ab
Si Nps 40 ppm	1.65 b	1.65 abc	1.57 ab	1.81 c	8.05 ab	6.23 c	0.66 e	0.50 e
Si Nps 60 ppm	1.93 a	1.77 a	1.79 ab	2.34 a	8.96 a	7.52 a	0.89 d	0.69 d
Si Nps 80 ppm	1.56 bc	1.62 abc	1.43 ab	2.00 b	7.52 abc	6.04 c	1.28 bc	1.18 ab
Si Nps 100 ppm	1.60 bc	1.64 abc	1.48 ab	1.75 cd	7.08 bc	6.02 c	1.02 d	0.88 c

Means, in each column, followed by similar letter are not significantly different at the 5% probability level – using Duncan’s Multiple Range Test.

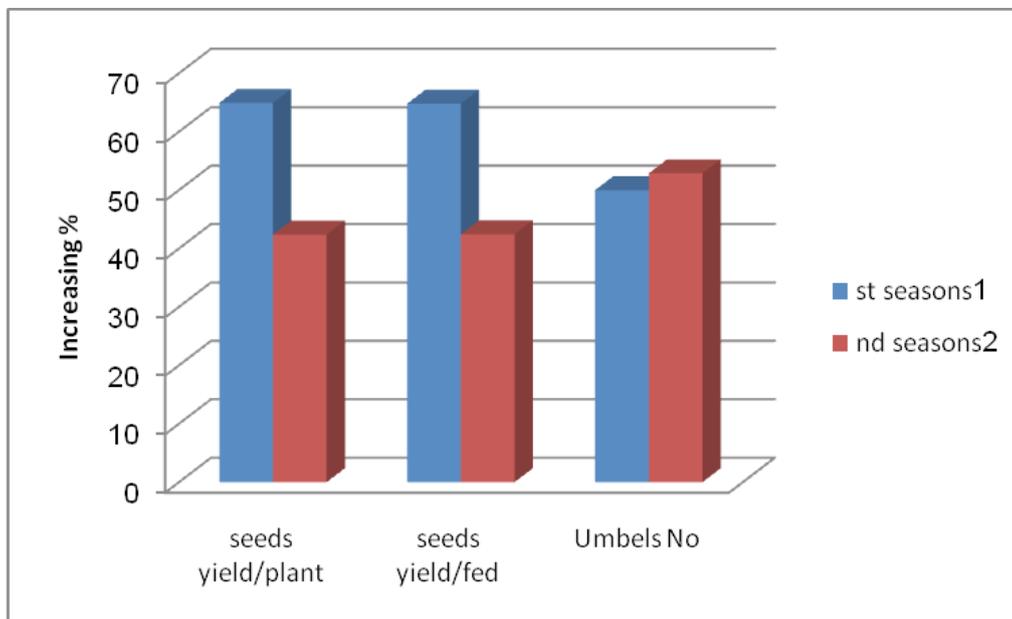


Fig. 3. Stimulatory impacts of 80 ppm SiO₂ nanoparticles on umbels number and seeds yield /plant and/fed as comparing with untreated

Concerning the correlation between umbels number/plant and seed yield/plant, the relation can be shown in Fig. 4. There is a complete correlation $R^2=1$ in the two seasons. Furthermore, increasing umbels number/plant led to enhanced seeds yield/plant and fed in the two seasons; this clearly on 80 ppm SiO_2 NPs. These finding agreed with those of Phu *et al.* (2017) and Morteza *et al.* (2013).

Biochemical characteristics

According to Table 4, the highest percentage of chlorophyll a, b and carotenoids resulted from using 60 ppm SiO_2 nanoparticles and the lowest evaluation for this traits was achieved by soaking and spraying with 80ppm graphite NPs. Also, the same concentration of 80ppm graphite NPs recorded the highest volatile oil% higher than SiO_2 nanoparticles and the control treatment. While, the lowest volatile oil% was

achieved by 60ppm graphite NPs and 40ppm SiO_2 nanoparticles. These results revealed that nano SiO_2 could stabilize the integrality of chloroplast membrane and protect the chloroplasts from aging in contrast with the control. Therefore, with nano SiO_2 treatment spraying, the content of chlorophyll a, b and carotenoids, was higher than the control. Moreover, spraying with SiO_2 NPs increasing chlorophyll pigments and transmit of photosynthetic material from leaves to fruits at the flowering time, leaves are starting to age and the first synthetic indicator of that was changing the structure and function of the chloroplasts (Woolhouse, 1984). Yang and Hong (2006) revealed that photosynthesis improved by TiO_2 NPs may be due to stimulation of the photochemical reaction of the plant chloroplasts. These results are in agreement with those of Salama (2012), Sharma *et al.* (2012) and Razzaq *et al.* (2016).

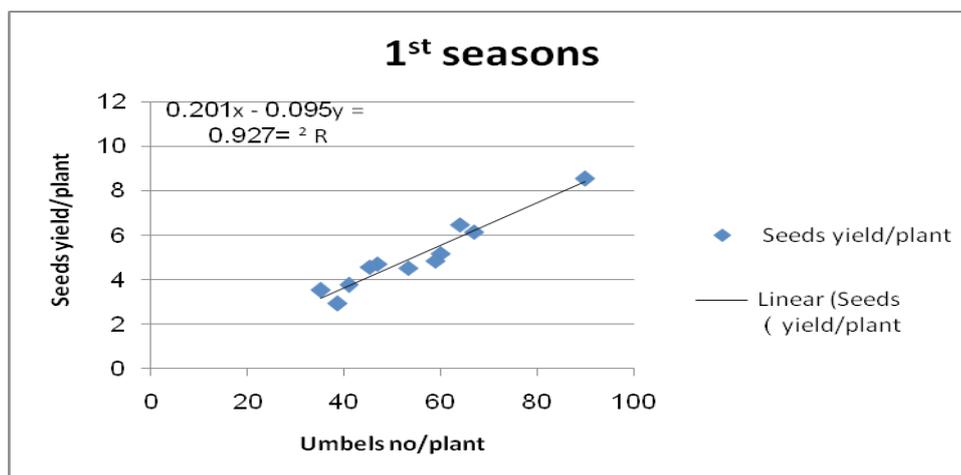


Fig.4. Correlation between umbels number/plant and seeds yield /plant as affected with Nano particles treatments

TABLE 4. Effect of graphite and silica nanoparticles on Oil components of pimpinella anisumL plants in the second seasons (2014 and 2015)

Treatments	control	G Nps 20 ppm	G Nps 40 ppm	G Nps 80 ppm	G Nps 100 ppm	Si Nps 20 ppm	Si Nps 60ppm	Si Nps 80Ppm	Si Nps 100ppm
p-cymene	0.26	0.76	1.83	0.28	0.25	0.86	4.82	0.35	0.17
Linalool	0.95	0.89	1.13	1.21	1.21	1.62	2.61	0.96	0.77
Methyl chavicol	0.44	0.60	0.28	0.23	0.22	0.18	0.15	0.29	0.15
Trans-anethole	95.86	93.61	92.41	95.73	95.33	94.28	87.49	96.28	94.59
Anise-aldehyde	1.38	2.31	1.48	1.53	2.30	1.66	1.47	1.43	2.45
Unidentified compound	1.11	1.83	2.87	1.02	0.69	1.4	3.46	0.69	1.87

Essential oil constituents

Main compounds and their relative percentage in the essential oil are shown in Table 5. Data revealed that five components were recognized as p-cymene, linalool, methyl chavicol, trans-anethole, anise aldehyde. Trans-anethole was found to be the first major compound and ranged from 96.28 to 87.49%. Its maximum content was observed in the volatile oil with 80 ppm SiO₂ nanoparticle. While, the minimum content was recorded with 60 ppm SiO₂ nanoparticle. Anise-aldehyde was recorded as the second main compound identified ranged from 2.31 to 1.38 for 20 ppm graphite nanoparticle and control, respectively. The change in the components quality occurred by using different nanoparticle treatments may be due to their effects on the metabolism and on this enzyme responsible for the components synthesis (Yousefzadeh and Sabaghnia., 2016).

Anatomical characteristics of anise root

It was reported that graphite and silica nanoparticles had a significant effect on root anatomy characteristics of *Pimpinella anisum* L. plant (Table 6 and Fig 5). A raise in the concentration of silica nanoparticles (80 ppm) has led to an improvement in root diameter, epidermis thickness and cortex thickness nevertheless, caused a decrease in vascular cylinder thickness compared with the control. In contrast, vascular cylinder thickness increased on treated plants with 80 ppm graphite nanoparticles. These results may be attributed to the mechanisms by which silicon is absorbed into the plants and interacted with polyphenols in cell walls of xylem and affected lignin deposition and biosynthesis (Parry and Kelso, 1975). These results are in harmony with previous results reported by Elfeky et al. (2013) who proved that epidermis cells of untreated plants of *Ocimum basilicum* were similar in shape and size, while the epidermal cells of the NP-treated leaves became larger in size, in addition, the thickness of mesophyll tissue was greater.

TABLE 5. Effect of graphite and silica nanoparticles on root diameter, epidermis thickness, cortex thickness and vascular cylinder thickness of root anatomy of *Pimpinella anisum* L plants in the second seasons (2015)

Characteristics Treatment	Root diameter	Epidermis thickness	Cortex thickness	Vascular cylinder thickness
Control	3875 c	20 b	562.5 b	2500 b
20 ppm nanographite	2412.3 d	10 c	500 b	1500 c
80 ppm nanographite	4125 b	20 b	550 b	3000 a
80 ppm nanosilica	4625 a	25 a	762.5 a	1025 d

Means having the same letters in the column are not significantly different at 5% level according to Duncan's Multiple Range Test.

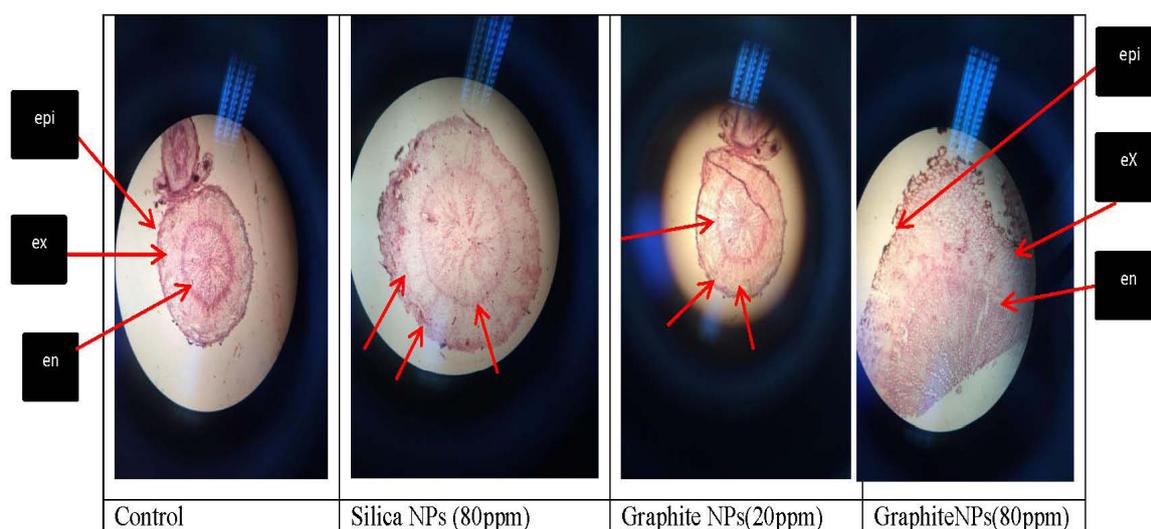


Fig. 5. Root anatomy photographed of *Pimpinella anisum* L plant using a light microscope
Abbreviations: epi, epidermis; ex, exodermis; en, endodermis.

Several studies have shown this result like Zuverza-Mena *et al.* (2016) and Keller *et al.* (2015); they found that Si deposition not only takes place in various parts of the plant such as epidermis of shoots but can also take place in the cell wall of root endodermis.

Conclusion

For improving seed germination can be soaked seeds in 20 to 40 ppm silica nanoparticles NPs. For enhancing seeds yield/plant and /fed, umbels number/plant, the diameter, epidermis, and cortex thickness of root we can be soaked anise seeds and foliar application of 60 and 80 ppm silica NPs for promoting biochemical characterization, oil components of the plant. Nevertheless, 80ppm graphite NPs enhanced volatile oil% and vascular cylinder thickness of root. In general, Applications of the nanoparticle can promote seed germination; improve yield production and chemical characters of anise plants.

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استجابة نبات الينسون للنانوجرافيت والنانوسيليكات

فردوس منيسى ، مجدى خلف الله ، ناهد راشد و أحمد معاطى

في السنوات الأخيرة كان هناك طلب متزايد على منتجات النانو ولا سيما في مجال التطبيقات الزراعية. في هذا العمل تم دراسة التأثير المثبط والمنشط لمركبات النانوسيليكات والنانوجرافيت على إنبات البذور وخصائص المحصول والتركيب الكيميائي والخصائص التشريحية للجذر لنبات الينسون. تم نقع البذور في ستة تركيزات من النانوسيليكات والنانوجرافيت (٠، ٢٠، ٤٠، ٦٠، ٨٠، ١٠٠ جزء في المليون) لمدة ٢٤ ساعة لدراسة التأثير على الإنبات في المعمل ونفس التركيزات استخدمت نقع ورش ورقى في الحقل. وأظهرت النتائج أن مركب النانوسيليكات حسنت صفات إنبات البذور بالمقارنة بالنانوجرافيت. ووجد أن تركيز ٢٠ و ٤٠ جزء في المليون أعطى أفضل صفات الإنبات لبذور الينسون وبلغت نسبة الزيادة في نسبة الإنبات إلى ٢١,٨٨٪. تركيز ٨٠ جزء في المليون زاد محصول البذور للنبات بنسبة ٦٤,٩٣٪، وزاد محصول البذور للفدان بنسبة ٦٤,٨٪، وعدد الخيام للنبات بنسبة ٥٠٪ كما زاد قطر الجذر وسك البشرة والقشرة بتركيز ٦٠ جزء في المليون زاد الخصائص البيوكيميائية بينما ٨٠ جزء في المليون من النانوجرافيت زاد نسبة الزيت العطري وسك الاسطوانة الوعائية للجذر بشكل عام كان هناك استجابة من بذور الينسون للنانوجرافيت والنانوسيليكات. مما يعرض إمكانية اتباع نهج جديد للتغلب على مشاكل إنبات البذور وزيادة نسبة الزيت.