J Nanostruct 8(1): 82-88, Winter 2018

RESEARCH PAPER

Green Synthesis of Zinc Oxide Nanoparticles Using Nigella Sativa L. Extract: The Effect on the Height and Number of Branches

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ARTICLE INFO

Article History:
Received 05 October 2017
Accepted 14 December 2017
Published 01 January 2018

Keywords:
Extract
Height
Nanoparticles
Nigella sativa L
ZnO

ABSTRACT

zinc oxide nanoparticles have been synthesized using nigella sativa L. seed extract. Nigella sativa L. is an annual herbaceous plant belonging to the Ranunculaceae family. Concentration of plant extract plays a vital role in the synthesis of nanoparticles zinc oxide. Nanostructures were characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM). This experiment was conducted in Arak University in an experiment based on randomized complete block design with four replications. Each replication consisted of one fertilizer levels including 2 per thousand of Zn-nanoparticles in one stages of growth (8 or 12 leaves). During the experiment, the height of plant, number of branches was investigated. This study showed that using spraying had significant differences in the factors like plant height number of branches. Also, using all microelement treatments had significant effects to the level of 1%. In case of using spraying treatments, the best results for number of branches and height were related to 2.perthousand of Zn-nanoparticles and the least were related to control. This formulation can be used for increasing yield, enhancing the products and removing food deficiencies.

How to cite this article
Alaghemand A, Khaghani S, Bihamta M. R, Gomarian M, Ghorbanpour M. Green Synthesis of Zinc Oxide Nanoparticles Using Nigella Sativa L. Extract: The Effect on the Height and Number of Branches. J Nanostruct, 2018; 8(1): 82-88. DOI: 10.22052/JNS.2018.01.010

INTRODUCTION

In recent years, noble metal nanoparticles have been the subject of focused research due to their unique optical, electronic, mechanical, magnetic and chemical properties that are significantly different from those of bulk materials [1]. These special and unique properties could be attributed to their small sizes and large surface areas. For these reasons, metal nanoparticles have found many applications in different areas such as catalysis, photonics and electronics [2-3]. Among noble metal nanoparticles, zinc nanoparticles have wide area of interest as they have large number of applications in such as non-linear optics, spectrally selective coating for solar energy absorption, bio-labeling, intercalation materials for electrical batteries as optical receptors, catalyst in chemical reactions and as antibacterial capacities[4-5] Hence the aim of present study is to develop a novel approach for the green synthesis

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of zinc nanoparticles using Iranian herbal plant *nigella sativa* extracts as a reducing and stabilizing agent. We have carried out a unique protocol for synthesizing of zinc nanoparticles (essential oil, temperature, time, extract preparation method and storage). Many researchers faced difficulty to collect the fine nanoparticles from plant liquids.

The World Health Organization (WHO) is providing emphasis on the exploration of medicinal plant species for the benefit of human care and systems. Emphasis have been given mainly on scientific information, on the safety, efficacy, quality control/quality assurance, dosage, toxicity description of the plant species, therapeutic uses, clinical trials, drug interactions amongst others. Effective utilization of N. sativa for therapeutic purposes as well as for trade will greatly depend upon yield (raw plant product-seeds; bioactive compounds- essential oil) and its quality.

More recently, great deal of attention has given to the seed and oils yields of black cumin. However, the crop is produced on fragmented land and soils having long cereal cropping history where crop residues are removed for various purposes without any chemical fertilizer application. Additionally, information regarding its response to fertilizer is insufficient in the country. Even though, the production and land coverage of black cumin has been increasing, the productivity is still less than 300 kg ha^{-1}. Several problems including lack of improved seed, recommended fertilizer rate, lack of knowhow on postharvest handling; improved agriculture practices and extension system, marketing system, etc.

MATERIALS AND METHODS

Scientific name and photograph of plant leaves

For the green synthesis of nanoparticles zincoxide using *nigella sativa* L. were collected from the Azarbaiejan and Yasuj and. A photograph of plants is shown in (Fig. 1). The extract is used for reducing and capping agent.

Medicinal properties

Black cumin (*Nigella sativa* L.) belongs to the family Ranunculaceae. The crop is native to the Mediterranean region and it has been used for thousands of years by various cultures and civilizations. Naturally, it grows in Mediterranean region of Turkey and Cyprus [6]. It is one of the most important medicinal plants, because it has multipurpose uses [7-9]. Black cumin is used as a whole or in crushed form for various pursues. Essential oils and oleoresins prepared by appropriate techniques to obtain value added products [8].

Black cumin seed has been widely used in folk medicine for the treatment of a number of diseases, including diarrhea, jaundice, amenorrhea, helminthiasis, ophthalmic, paralysis and asthma. One of the most important plants containing volatile and fixed oil is black cumin family Ranunculaceae is an herbaceous indigenous plant in the Mediterranean region [7].

Preparation of seed extract

The NS seeds were collected from the torbatheidarieh near mashhad in Iran. They were powdered and dried. Then, 500 g of the

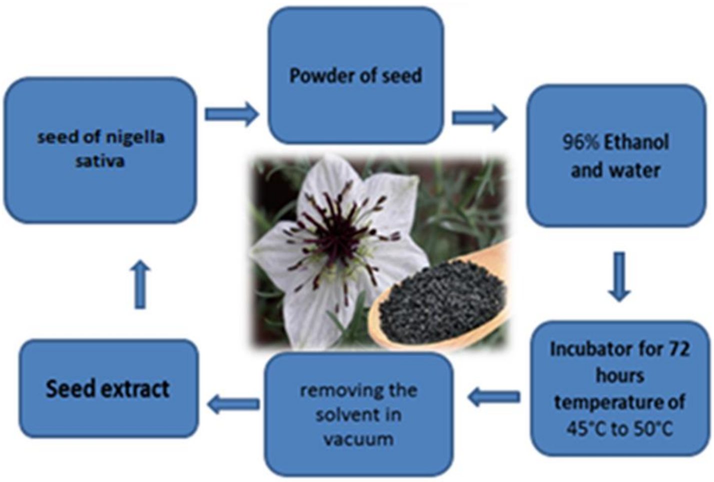

Fig. 1. Schematic of preparation of black seed extract

prepared powder was mixed with a sufficient volume of 96% ethanol and water extracted with an Incubator apparatus for 72 hours. After removing the solvent in vacuum, the extract was dried in an oven with the temperature of 45°C to 50°C. The dried extract weighed 33.3 g, and therefore, it was 33.3%. The extract was then kept in a refrigerator Finally the extract was used for the synthesis of silver nanoparticles. [10-11].

Chemical composition of black seeds

Many active compounds have been isolated, identified and reported so far in different varieties of black seeds. The most important active compounds are thymoquinone (30%-48%), thymohydroquinone, dithymoquinone, p-cymene (7%-15%), carvacrol (6%-12%), 4-terpineol (2%-7%), t-anethol (1%-4%), sesquiterpenelongifolene (1%-8%) α-pinene and thymol etc. Black seeds also contain some other compounds in trace amounts. Seeds contain two different types of alkaloids; i.e. isoquinoline alkaloids e.g. nigellicimine and nigellicimine-N-oxide, and pyrazol alkaloids or indazole ring bearing alkaloids which include nigellidine and nigellicine. Moreover, N. sativa seeds also contain alpha-hederin, a water soluble pentacyclic triterpene and saponin, a potential anticancer agent [12, 13].

Synthesize of nanoparticles zinc oxide

$Zn(NO_3)_2$ $4H_2O$ was dissolved in 30 mL of water. Then ammonia solution was then slowly added to the mentioned solution (pH was adjusted about 8) under microwave radiation (600W, 5sOn, 5s Off) for 2 minutes. The precipitate then centrifuged and rinsed with distilled water.

Statistical analysis

Zinc nitrate and NH_3 were purchased from Merck Company. All the chemicals were used as received without further purifications. X-ray diffraction (XRD) patterns were recorded by a Philips X-ray diffractometer using Ni-filtered CuK_α radiation. A multi-wave ultrasonic generator (Bandeline MS 73) equipped with a converter/transducer and titanium oscillator operating at 20 kHz with a maximum power output of 100 W was used for the ultrasonic irradiation. Scanning electron microscopy (SEM) images were obtained using a LEO instrument (Model 1455VP). Prior to taking images, the samples were coated by a very thin layer of Pt (BAL-TEC SCD 005 sputter coater) to make the sample surface conducting obtain better contrast and prevent charge accumulation.

Data were processed by the analysis of variance (ANOVA) on the basis of completely randomized design (CRD) with four replications. The data were analyzed using computer SAS software (version 9.1; CoHort Software), and the means were compared by Duncan's multiple range test ($P < 0.05$).

RESULTS AND DISCUSSION

Number of branches and height

The XRD pattern of ZnO nanoparticles is shown in (Fig. 2) and is indexed as a hexagonal phase (space group: P63mc). The experimental values

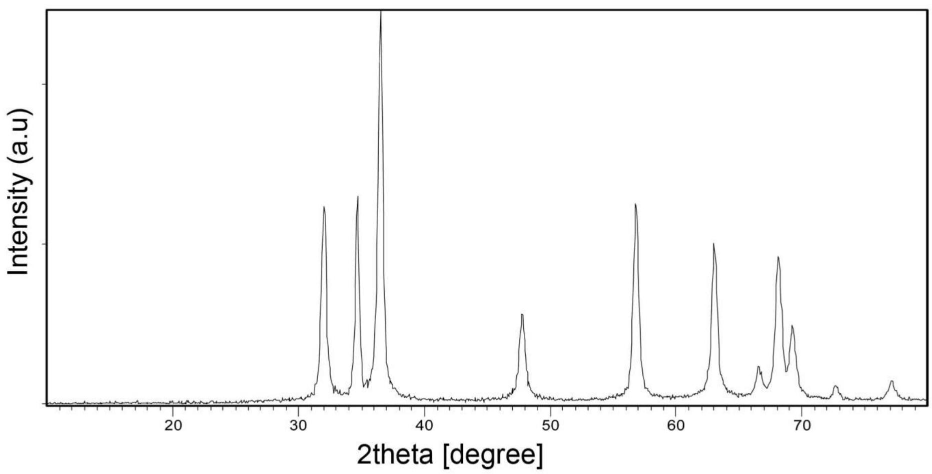

Fig. 2.XRD pattern of zinc oxide nanoparticles

are very close to the literature (JCPDS No. 79-0208). The crystallite size measurements were carried out using the Scherrer equation (Eq. 1),

$$Dc=0.9\lambda/\beta\cos\theta$$

Where β is the width at half maximum intensity of the observed diffraction peak, and λ is the X-ray wavelength (CuK_{α} radiation, 0.154 nm). The estimated crystallite size is about 20nm.

Scanning electron microscopic images of zinc oxide nanoparticles achieved by using 2 ml of extract are illustrated in (Fig. 3). Nanoparticles with average diameter about 20 nm were synthesized.

SEM images of ZnO nanoparticles achieved by using 10 ml of extract are illustrated in (Fig. 4). Nanoparticles with size less than 45 nm were prepared. Results confirm by addition of bio-compatible capping agent growth stage is preferential in comparison to nucleation stage [16-21].

Results indicated that there were significant (P <

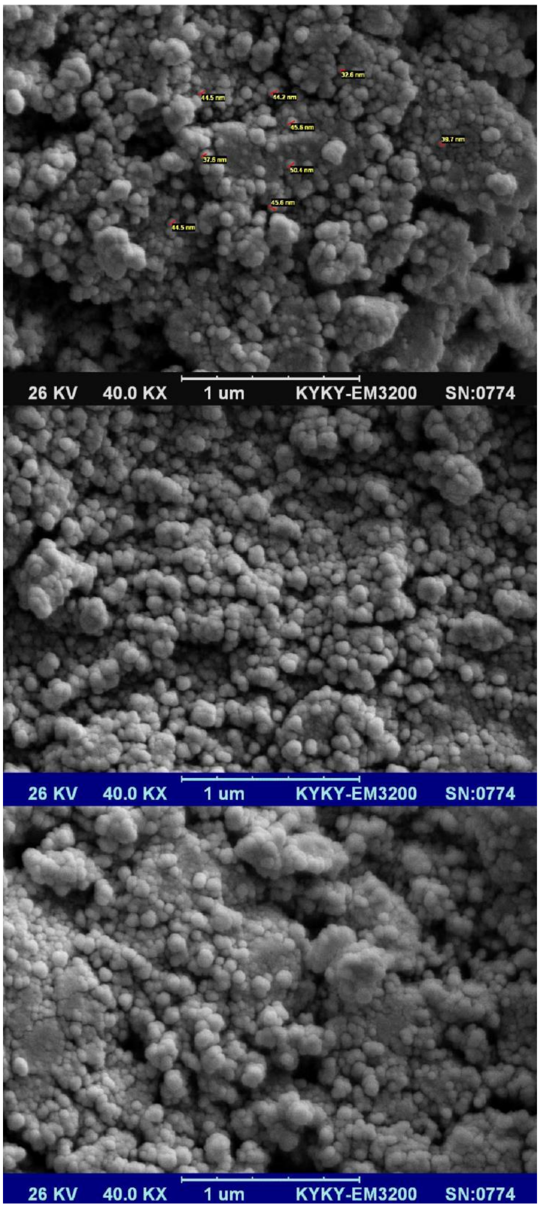

Fig. 3. SEM images of ZnO nanoparticles by 2 ml of extract

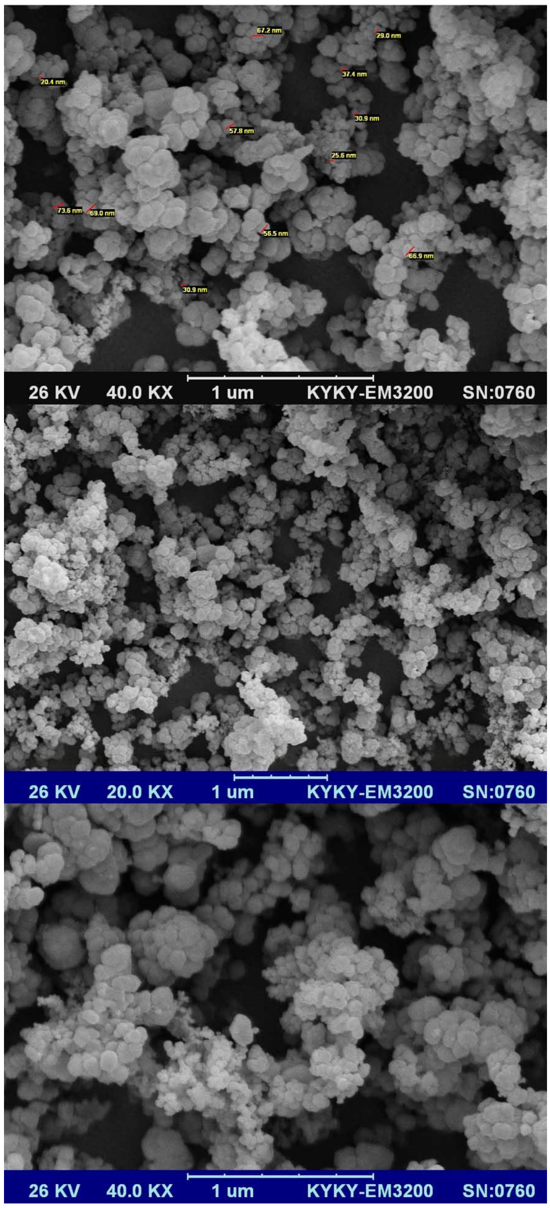

Fig. 4. SEM images of ZnO nanoparticles by 10 ml of extract

Table 1. Analysis of variance and the impact of treatments on the number of branches per nigella sativa L

Sources of variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value
Block	3	11/25	3/83	5/75*
Region	1	25	25	37/5**
Treatment	1	20/25	20/25	30/38**
The treatment × Region	1	0/25	0/25	0/38ns
Error	9	6	0/66	
cv=13.06				

* Significant at the 1% level, ** significant at the 5% level and is statistically meaningless ns

Table 2.Analysis of variance and the impact of treatments on plant height nigella sativa L.

Sources of variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value
Block	3	66/2	22/06	2/14ns
Region	1	36/3	36/3	3/53ns
Treatment	1	71/8	71/8	6/98*
The treatment × Region	1	90/7	90/7	8/82*
Error	9	92/6	10/28	
cv=13.06				

* Significant at the 1% level and is statistically meaningless ns

Table 3.compares the average orchard

Treatment	number of branches	height
AzarbaiejanRegion	5b	22/73
YasujRegion	7/5a	25/75

Posts that are shown in each column with the same letters according to Duncan's multiple range test with no significant difference (P= 0/05).

Table 4. Compare different treatments

Treatment	number of branches	height
1Treatment	5/12b	22/12b
2Treatment	7/37 a	26/36a

Posts that are shown in each column with the same letters according to Duncan's multiple range test with no significant difference (P= 0/05).

0.05) variations between fertilized plants compared to untreated controls for the investigated traits. Analysis of variance showed a significant effect on number of branches in different areas cultivated plants. The effect of treatments on the number of branches was significant at the 1% level, but the interaction between the region and treatment had no impact on the number of branches (Table. 1). The largest number of branches in the area Yasuj (5/7 = n), while in Region Azarbaiejan, 5 branches was observed (Table. 2). Most branches also were observed in treatment nanoparticles zincoxide (37/7 = n) (Table. 3) Analysis of variance showed that the effect block and cultivated area had no effect on plant height.Used significant treatment differences in plant height at 5 percent. The interaction between the treated area and plant height was significant at the 5% level (Table. 2). Means comparison showed that plants were cultivated in Region Yasuj Region Azarbaiejan had a greater height (75/25 cm) (Table. 3). The second treatment plants were also affected by higher than in the plants were treated with control (36/26 inches) (Table. 4)

Results of interaction also showed that the highest number of branches of the second zone and the second treatment (5/8), whereas the lowest number in the first zone and the first treatment plants with 75/3 number was observed (Table. 4)(Fig. 5)

The interaction effect also represents the highest plant height in Region 2 culture and treatment was second (2.30 cm), while the lowest elevation in Region 2 and was treated first (2.21 cm) (Table. 5)(Fig. 6).

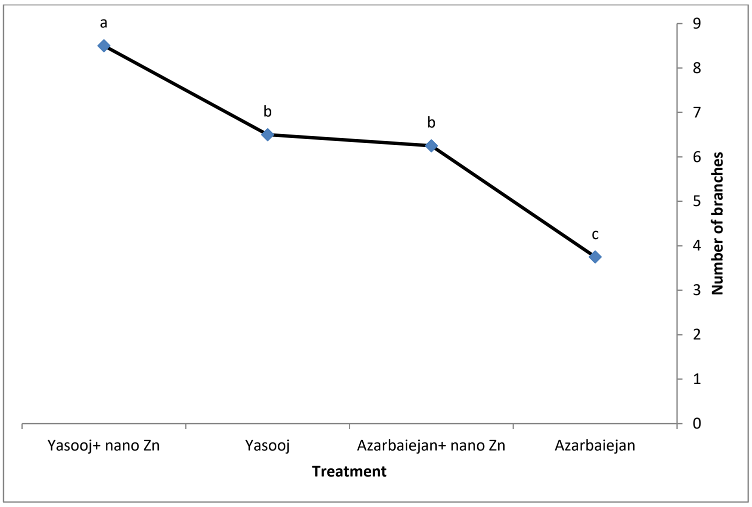

Fig. 5.The interaction of cultivated area and the number of branches treatment plant.

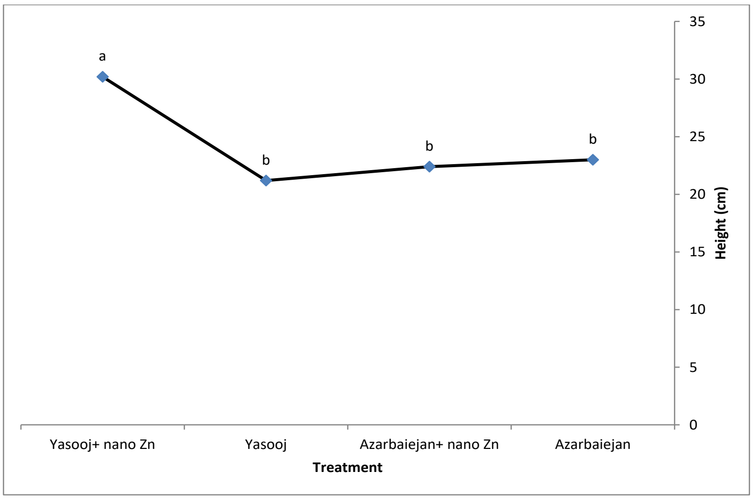

Fig. 6.The interaction of cultivated area and the height treatment plant.

Table 5. Compare the mean of the interaction between culture area and treatment

Treatment		number of branches	height
RegionAzarbaiejan	Treatment1	3/75c	23 b
	Treatment2	6/25b	22/4b
Region	Treatment1	6/5 b	21/2b
Yasuj	Treatment2	8/5 a	30/2a

Posts that are shown in each column with the same letters according to Duncan's multiple range test with no significant difference (P= 0/05).

CONCLUSION

Nigella sativa L. is an annual herbaceous plant belonging to the Ranunculaceae family. Zinc oxide nanoparticles have been synthesized using *nigella sativa* L. seed extract. Concentration of plant extract plays a vital role in the synthesis of nanoparticles zinc oxide. Nanostructures were characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM). Each replication consisted of one fertilizer levels including 2 per thousand of Zn-Nanoparticles in one stages of growth (8 or 12 leaves). During the experiment, the height of plant and number of branches was examined. This study showed that using spraying had significant differences in the factors like plant height number of branches. Also, using all microelement treatments had significant effects to the level of 1%. In case of using spraying treatments, the best results for number of branches and height were related to 2.perthousand of Zn- Nanoparticlesand the least were related to control. This formulation can be used for increasing yield, enhancing the products and removing food deficiencies.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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