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ORIGINAL PAPER

EFFECTS OF ZnO NANOPARTICULES PRODUCED BY GREEN SYNTHESIS ON GERMINATION AND SEEDLING OF *SALVIA OFFICINALIS* L. SEEDS

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Abstract

Nanotechnology is a research subject in many interdisciplinary engineering fields today. Nanoparticles have important effects on seed germination and seedling growth of plants. The aim of this study has been to produce a 54.68 nm-sized ZnO nanoparticle obtained from the *Nigella sativa* L. plant by green synthesis. The effect of medicinal sage (*Salvia officinalis* L.) on germination of plant seeds and seedling growth was investigated. The trial was set up under controlled conditions and conducted for 14 days according to a factorial trial design with 5 replications for each dose (0, 0.5, 1.5 and 2.5 mg ZnO NP kg^{-1}). As a result of ZnO NP application in different doses, germination of *Salvia officinalis* L. seeds varied between 90-94%, stem length was 1.86-2.92 cm and shoot length was between 1.01-1.98 cm. The highest root and shoot length was obtained after the application of 2.5 mg ZnO kg^{-1} NP. In the light of the results, ZnO nanoparticles are thought to be useful for seed development and agricultural applications.

Keywords: green synthesis, *Nigella sativa* L., *Salvia officinalis* L, ZnO NPs, seed germination.

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INTRODUCTION

In recent years, nanotechnology has emerged as an important research field in all aspects. Especially nano-materials play an important role in human life with their use in such areas as science, medicine, engineering, pharmacy and clinical applications (Daghan 2018, Acay et al. 2019, Anand et al. 2019, Eren, Baran 2019*a*, Keskin et al. 2021). In addition, the use of nanoparticles in various products, including agriculture, is gaining importance (Eren 2020). Nanoparticles are defined as objects ranging from 1 to 100 nm (Ahmed et al. 2016, Dogaroglu et al. 2019, Eren, Baran 2019*b*). In terms of their chemical structure and surface properties, defined as a new and rapidly developing interdisciplinary science, nanoparticles have taken their place in materials science (Hasan 2015, Gopinath et al. 2016, Saravanakumar et al. 2018, Selvakumar et al. 2018, Eren, Baran 2019*b*). Bio-reduction involves reducing metal ions or metal oxides to 0 valence metal NPs with the help of phytochemicals like polysaccharides, polyphenolic compounds, vitamins, amino acids, alkaloids, terpenoids secreted from plants (Heinlaan et al. 2008, Qu et al. 2011). Agarwal et al. (2017). In general, green nano-biotechnology means bio-synthesizing nanoparticles or nanomaterials. A green synthesis technique eliminates the use of expensive chemicals with the use of cheaper and environmentally friendly materials such as enzymes, plants, bacteria, fungi, algae and plant extracts (Figure 1.).

A biological method for nanoparticle synthesis has been identified as an environmentally friendly alternative to chemical and physical methods –

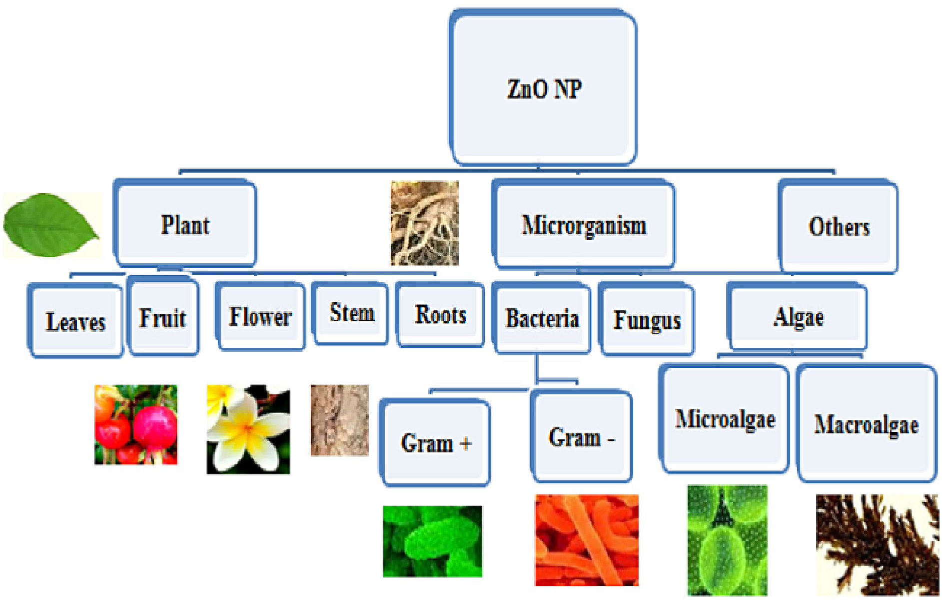

Fig. 1. Sources used in ZnO nanoparticle synthesis (Agarwal et al. 2017)

Figure 2 (Sangeetha et al. 2011, Salam et al. 2014, Ramesh et al. 2015, Suresh et al. 2015, Momeni et al. 2016, Parveen et al. 2016, Agarwal et al. 2017, Fawcett et al. 2017, Eren, Baran 2019*a*, Baran et al. 2020, Keskin et al. 2021).

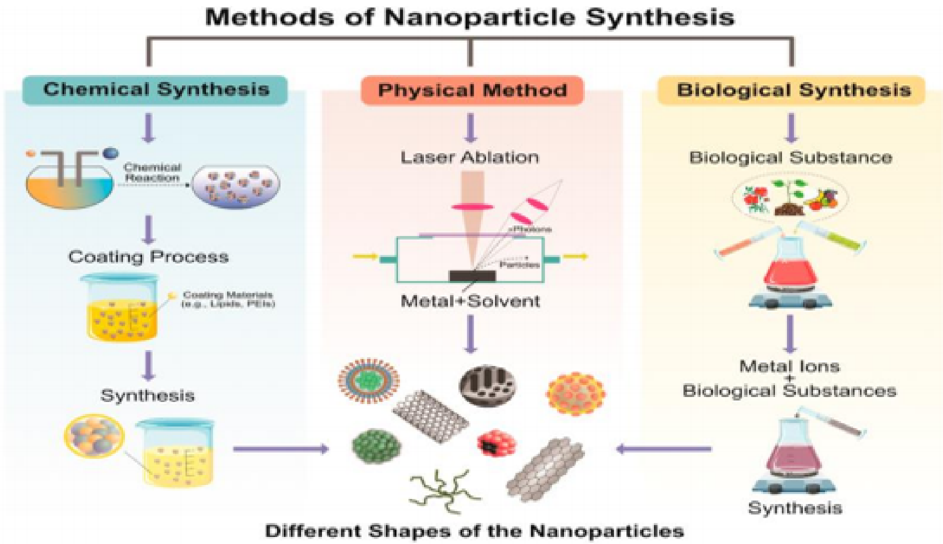

Fig. 2. Biological, chemical and physical methods commonly used for nanoparticle synthesis (Jeyaraj et al. 2019)

In addition, various metal and nonmetal nanoparticles such as silver (AgNP), gold (AuNP), selenium (SeNP), platinum (PtNP), zinc oxide (ZnONP) and copper oxide (CuONP) have been synthesized in recent years using various plants and their extracts and then studied for anti-microbial, anti-biofilm and photocatalytic activity (Anandalakshmi et al. 2016, Saravanakumar et al. 2017, Ganesh et al. 2019). Among all these metal oxides, ZnO NPs are of the greatest interest because they are inexpensive, safe and easy to prepare (Jayaseelan et al. 2012). Metallic nanoparticles such as zinc oxide (ZnO), TiO_2 and CuO are among the most common nanoparticles used in agriculture and industry (Daghan 2018). Nano fertilizers can be applied to obtain high yields at low cost by consuming a small amount of fertilizers. They increase the efficiency of fertilizers, improve the nutrient use, reduce the frequency of fertilizer application, reduce nutrient losses and minimize potential negative effects on the environment, reduce soil toxicity, increase soil fertility, enhance product quality, and increase crop yield and nutritional value by ensuring healthy growth and development of the plant (Daghan 2017). This research was carried out to determine the effect of ZnO nanoparticle obtained from *Nigella sativa* L. plant via green synthesis on the germination of *Salvia officinalis* L. seeds and the growth of seedlings.

MATERIAL AND METHOD

Different concentrations of ZnO NP solutions (0, 0.5, 1.5 and 2.5 mg L^{-1}) with an average size of 54.68 nm were prepared (Figure 3). After *Salvia officinalis* L. seeds were washed with distilled water and dried, 10 seeds were transferred to Petri dishes lined with an appropriate size filter paper. 5 mL of different ZnO NP concentrations were added to each Petri dish. Only 5 mL of pure water was added to the control group. The experiment was set up in 5 replicates according to a factorial trial design and maintained for 14 days at 18°C under controlled conditions.

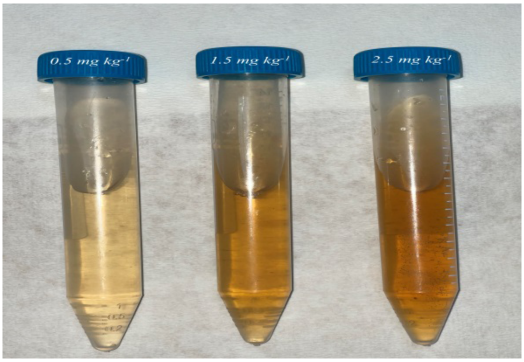

Fig. 3. ZnO NP solutions used in the experiment

Plant material

Salvia officinalis L. is a valuable medicinal and aromatic plant from the Lamiaceae family. The species in the Salvia genus are generally rich in essential oils and are therefore important in both the pharmacological and perfumery industries (Basyigit, Baydar 2017). Medicinal sage, a perennial herb, has its origins in the Mediterranean Region and coastal European countries.

Green synthesis of ZnO NPs

Having collected green leaves of *Nigella sativa* L. plant in the required amount, we washed the leaves several times with tap water and then distilled water, and then dried at room temperature in the dark. 750 g of the dried samples were taken and boiled in a beaker at 95°C for 2 h together with 1500 mL of distilled water. After that, filtration was carried out and the extract was made ready for biosynthesis. A solution of 10 mM concentration was prepared from the Merck branded Zn $(NO_3)_2$ – zinc nitrate) salt. A mixture was made in a 1 L beaker from the prepared extract and the Zn $(NO_3)_2$ salt mixed at a ratio of 8:1, while the pH was adjusted to 9.7. The mixture was left in the reaction environment at 40°C with a heated stirrer for 72 hours. Then it was centrifuged at 10.000 rpm for 5 minutes, and the upper liquid phase was removed while the remaining solid was washed several times with distilled water. The precipitated part was collected and dried in an oven at 75°C for 24 hours. The dry nano-material was ground with a glass drumstick and stored for analysis.

Characterization techniques of ZNO NPs

UV-vis spectroscopy analysis

UV-VIS spectroscopy is a frequently used method for the identification of organic molecules, ions or complexes, covering the ultraviolet and visible light regions as a wavelength range. The basis of this method is based on the increase in absorption of a beam after it is passed through a sample or reflected from the surface of a sample due to the decrease in beam intensity. Since the rays passing through or reflected from the sample are absorbed by each molecule with a specific wavelength, molecule determination is performed with reference spectra. In UV-Visible spectroscopy analysis, ZnO NP formation was observed in samples taken at different times at 5, 10, 15, 20, 25 and 30 minutes. The formation of ZnO nanoparticles was determined by measuring the wavelength scan of the samples taken at certain time intervals without mixing for 1 h at room temperature (Figure 4.).

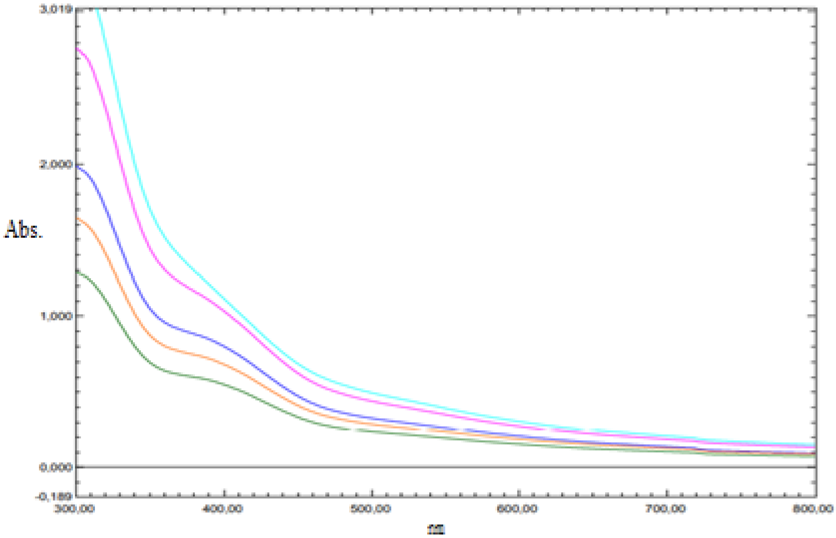

Fig. 4. Formation of ZnO NPs depending on time in UV-vis spectroscopy

FT-IR analysis

Infrared (IR) absorption spectroscopy is a type of vibration spectroscopy in which IR rays are absorbed by the vibrational movements of a molecule.

Fast and high resolution spectra are obtained without the need to scan each wavelength individually. It gives results in a short time even with a small amount of sample. In many branches of science, it is used for purposes such as identification of microbial cells, structural analysis of macromolecules, qualitative and quantitative analysis of organic substances, determination of stereo-chemical structures and purity control (Buyuksirit, Kuleasan 2014). The presence of ZnO NPs and functional groups of phytochemicals responsible for reduction were determined using Perkin Elmer One branded Fourier Transform Infrared spectroscopy (FT-IR) device (Figure 5.).

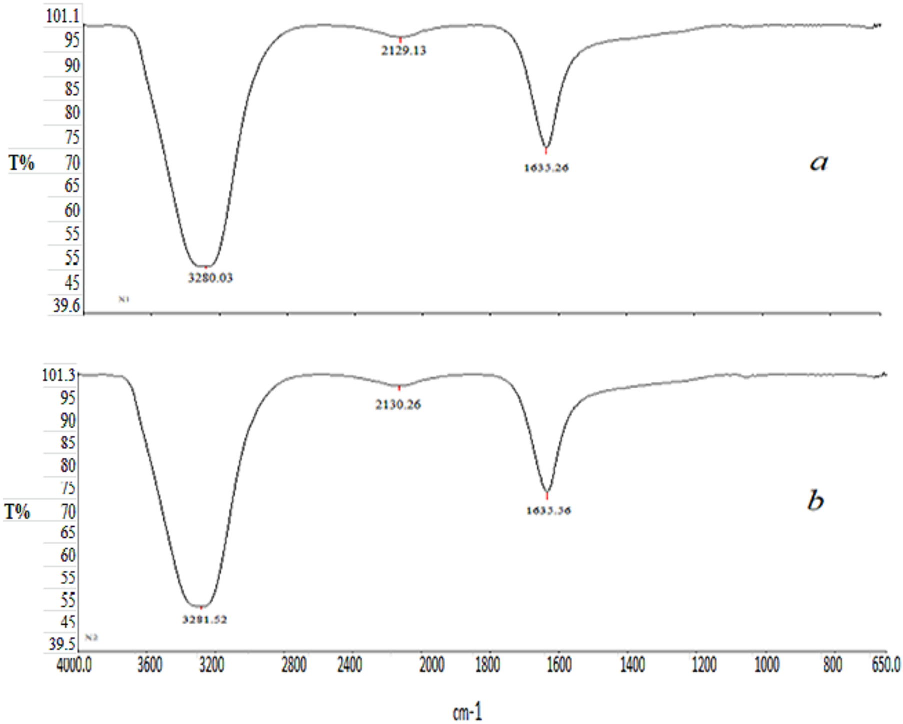

Fig. 5. FT-IR spectrum of plant extract – *a*, FT-IR spectrum of synthesized ZnO NPs – *b*

X-ray diffraction XRD analysis

The X-ray diffraction method (XRD) is based on the diffraction of X-rays in a characteristic order depending on the atomic sequences of each crystalline phase. These diffraction peaks define that crystal like a fingerprint for each crystalline phase. X-rays sent onto the crystal are diffracted according to the Bragg's law. With the X-ray diffraction method, it has been possible to better understand the physical properties of metals, polymer substances and other solids (Agarwal 2013). Certain properties of grown crystals are determined using XRD diffraction patterns. The particle size of single crystals grown was calculated using the Debye-Scherrer formula (Debye, Scherrer 1917, Holzwarth, Gibson 2011). Debye-Scherrer formula: $D=K\lambda/\beta\cos\theta$, where K=0.94 Scherrer constant, λ=1.5405 Å wavelength of x-rays, β width of half the maximum height of the diffraction peak and θ Bragg is the diffraction angle. ZnNPs synthesised from *Nigella sativa* L. plant have an average size range of 70 nm confirmed by SEM and an average size of 54.68 nm calculated using the Debye-Scherrer equation through XRD analysis results. Crystal structure and dimensions of ZnNPs were determined with Rigaku Miniflex 600 model X-ray Diptractometer (XRD) – Figure 6.

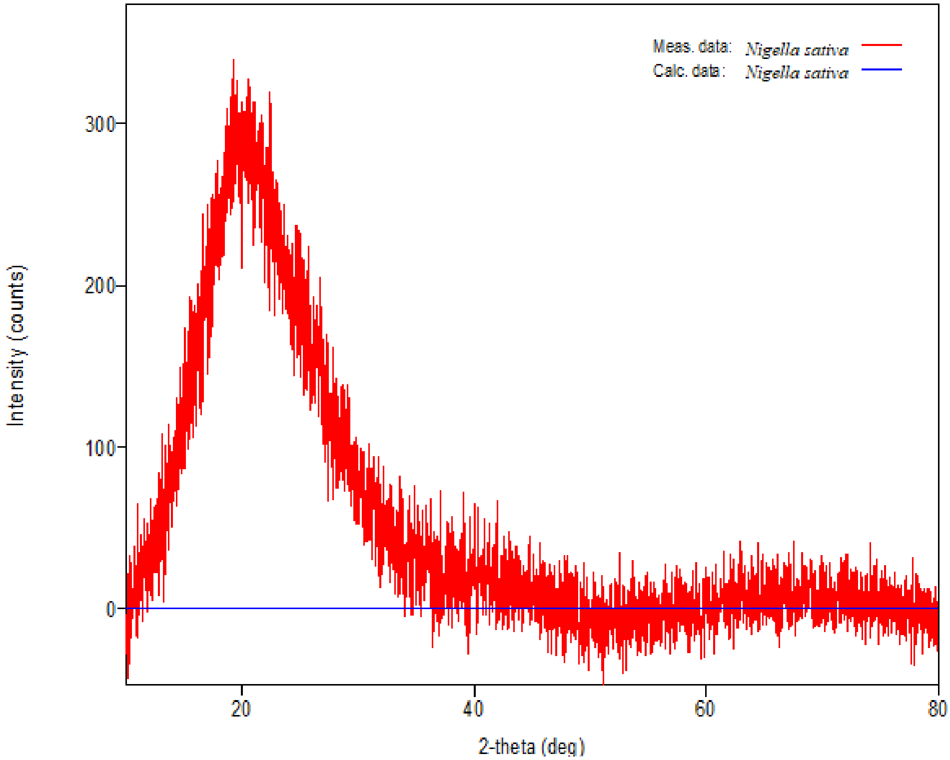

Fig. 6. XRD results of synthesized ZnO NPs

TEM analysis

Transmission Electron Microscope (TEM) is based on the imaging of high energy electrons that are passed through a very thin sample. The image created by the interaction of electrons with the sample is enlarged and focused on a sensor such as a fluorescence screen, photographic film layer or CCD camera. Synthesized ZnO NPs were determined in a JEOL 1220 JEM brand transmission electron microscope. TEM has been used to further study the particle size, crystallinity and morphology of the samples. TEM black spherical images of ZnO NP micro powders in rutile and anatase phases are given in Figure 7, respectively.

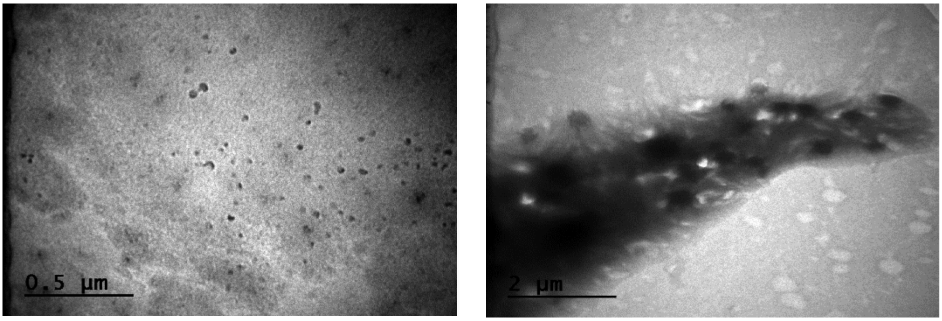

Fig. 7. TEM images of synthesized ZnO NPs

SEM analysis

Scanning electron microscope is a type of electron microscope that obtains images by scanning the sample surface with a focused electron beam. Electrons interact with atoms in the sample, producing different signals that contain information about the topography and composition of the sample surface. These signals are collected by the relevant detectors and transferred to the computer screen and an image is obtained. An EVO 40 LEQ scanning electron microscope was used to determine the morphological appearance of zinc NPs. It is clearly seen that the ZnO NPs particle in the anatase phase of the stable ZnO NPs powders mostly have spherical morphology (Figure 8).

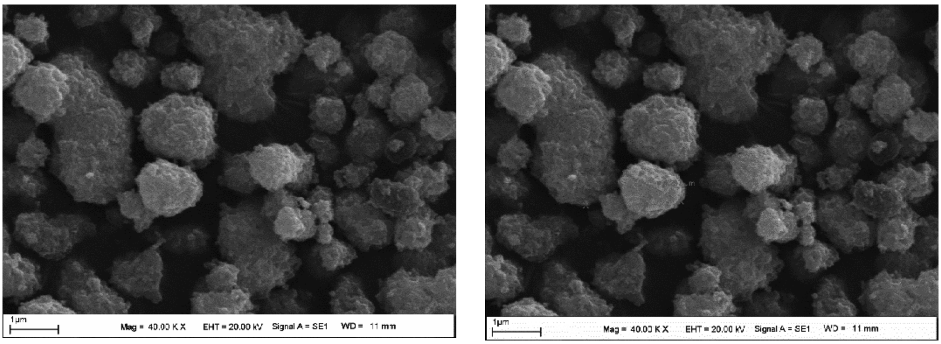

Fig. 8. SEM images of synthesized ZnO NPs

EDX analysis

EDX (Energy Dispersive X-Ray Spectrometer) is a spectrometer used to evaluate the information obtained by collecting X-rays as a result of electron bombardment. It is an analyzer that determines the percentages of each element in the sample according to its characteristic X-ray spectra. The working principle of this device is as follows: electron beams are sent on the material under study, these rays interact with the elements in the sample and are reflected back at different Kα, Lα and Mα energy levels for each element. These reflections give the different intensity of each element depending on the amount of it in the sample and the percentage of each element in the substance. The element compositions of the particles obtained by RadB-DMAX II computer controlled Energy Dispersive X-ray Diffraction (EDX) data are given in Figure 9.

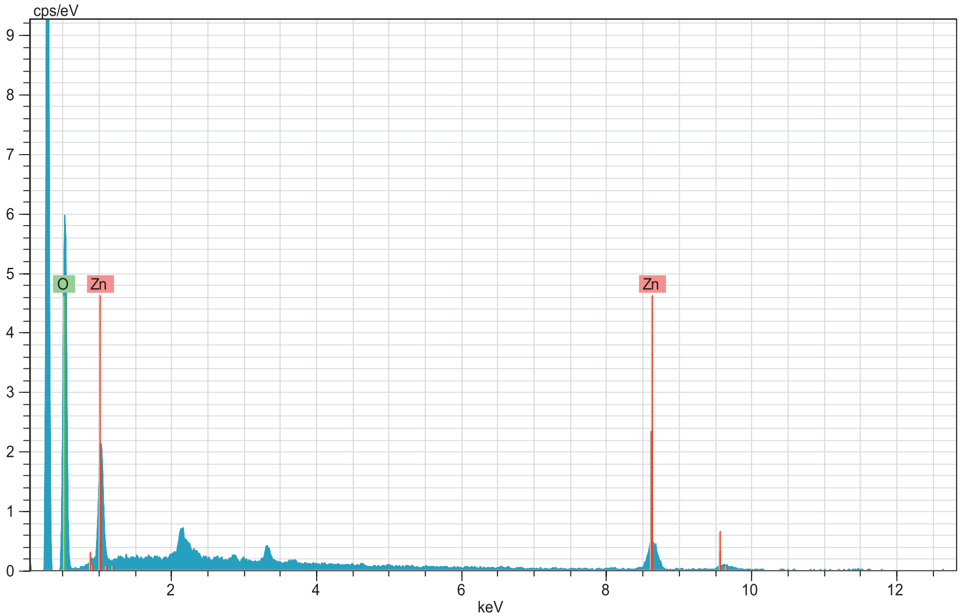

Fig. 9. ZnO NP's EDX spectrum diagram

Statistical analysis

The experiments were carried out in 5 repetitions and the data obtained as a result of the experiment were evaluated using the SPSS 22.0 statistical package program. Variance analysis was performed to determine the importance levels between values and the groups formed between values were typed by applying the Waller-Duncan test.

RESULT AND DISCUSSION

As a result of different doses of ZnO nanoparticle applications, the effect of *Salvia officinalis* L. seeds on germination was statistically significant at the level of $p \leq 0.05$, and the effect on root and shoot length was statistically significant at $p \leq 0.01$. In Table 1, averages and groups are given in letters according to the Waller-Duncan test. As a result of nanoparticle applications, the germination of *Salvia officinalis* L. seeds was between 90-94% and the highest was obtained from the application of 1.5 mg kg^{-1} ZnO NP. The root length is between 1.86-2.92 cm and shoot length is between 1.01-1.98 cm and the highest root and shoot length was obtained from

2.5 mg kg^{-1} ZnO NP application (Table 1). The effect of nanoparticle (ZnO NP) applications on *Salvia officinalis* L. seeds is given in Figure 10. In the study conducted in the presence of nano-Zn and nano-ZnO, deceleration of root elongation does not prevent the shoots from elongating to a certain extent (LIN, XING 2007). It was determined that ZnO NP applications in *Triticum aestivum* plant increased seed germination, root, shoot, leaf lengths and biomass compared to the control group (AWASTHI et al. 2017). It has been determined that the nanoscale ZnO particle increases germination, pigments, sugar and protein contents with the increased activities of antioxidant enzymes in plants (SINGH et al. 2018).

Table 1

The effect of ZnO nanoparticle applications on germination, root and shoot length in *Salvia officinalis* L. seeds.

Application	Dose (mg kg^{-1})	Germination (%)	Root length	Shoot length
			(cm)	
ZnO NP	0.0	90.0^{b}	1.86^{b}	1.01^{d}
	0.5	91.4^{ab}	1.95^{b}	1.12^{c}
	1.5	94.0^{a}	2.76^{a}	1.78^{b}
	2.5	92.0^{ab}	2.92^{a}	1.98^{a}
	F	3.28*	17.6**	321**

* $p \leq 0.05$, ** statistically significant at $p \leq 0.01$ level

Fig. 10. The effect of ZnO NP applications on *Salvia officinalis* L. seeds

CONCLUSION AND RECOMMENDATIONS

As a result of the effect of ZnO nanoparticle with 54.68 nm size obtained from *Nigella sativa* L. plant by green synthesis on germination and seedling in *Salvia officinalis* L. plant seeds; germination rate of 90-94% in seeds from the application of 1.5 mg ZnO kg^{-1} NP; root length 1.86-2.92 cm and shoot length 1.01-1.98 cm, root and shoot lengths were obtained from 2.5 mg ZnO kg^{-1} NP application. As a result of nanoparticle ZnO applications, it was determined that germination, root length and shoot length increased in *Salvia officinalis* L. seeds in general with increasing ZnO NP doses. It has been stated by many researchers that the effect of nanoparticle use on plants causes various morphological and physiological changes depending on the properties of the particles; moreover, germination, root and shoot elongation in plants can be negatively affected by using high concentrations; while in our study, it was determined that ZnO NPs used in low concentrations gave a positive response in *Salvia officinalis* L. plant seeds. Although fertilizers are very important for plant growth and development, most of the fertilizers applied become unusable by plants due to various factors. For this reason, it is thought that positive situations such as reducing nutrient losses and improving efficiency in fertilization can arise with the use and applications of new techniques through nanotechnology and NPs.

In addition, ZnNP applications obtained by the Green synthesis method increase germination, root and stem length in plants; and in the light of the data obtained as a result of testing the plants in field conditions, it is thought that the real effect of NP applications will be more reliable.

REFERENCES

Acay A., Baran M.F., Eren A. 2019. *Investigating antimicrobial activity of silver nanoparticles produced through green synthesis using leaf extract of common grape (Vitis vinifera).* Appl Ecol Environ Res, 17(2): 4539-4546.

Agarwal B.K. 2013. *X-ray spectroscopy: an introduction.* Springer, 15: 415 p.

Agarwal H., Kumar S.V., Rajeshkumar S. 2017. *A review on green synthesis of zinc oxide nanoparticles – An eco-friendly approach*. Resource-Efficient Technologies, 3(4): 406-413.

Ahmed S., Ahmad M., Swami B.L., Ikram S. 2016. *A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise.* J Adv Res, 7(1): 17- 28.

Anand G.T., Renuka D., Ramesh R., Anandaraj L., Sundaram S.J., Ramalingam G., Maria Magdalane C., Bashir A.K.H., Maaza M., Kaviyarasu K. 2019. *Green synthesis of ZnO nanoparticle using Prunus dulcis (Almond Gum) for antimicrobial and supercapacitor applications*. Surf Interfaces, 17: 100376.

Anandalakshmi K., Venugobal J., Ramasamy V. 2016. *Characterization of silver nanoparticles by green synthesis method using Pedalium murex leaf extract and their antibacterial activity.* Appl Nanosci, 6(3): 399-408.

Awasthi A., Bansal S., Jangir L.K., Awasthi G., Awasthi K.K., Awasthi K. 2017. *Effect of ZnO nanoparticles on germination of Triticum aestivum seeds*. In: Macromolecular Symposia, 376(1): 1700043.

Baran A. Keskin C., Baran M.F. 2020. *Environmentally Friendly Synthesis and Characterization of Metallic Nanoparticles*. Development and Social Researches Publications, Iksad Publications, 47-70.

Basyigit M., Baydar H. 2017. *Effects of different harvesting time on essential oil, phenolic compounds and antioxidant activity in sage (Salvia officinalis* L.*)*. Süleyman Demirel University J Natur Appl Sci, 21(1): 131-137.

Buyuksirit T., Kuleasan H. 2014. *Fourıer transform Infrared (FT-IR) spectroscopy and utilization in food*. J Food, 39(4): 235-241.

Daghan H. 2017. *Nano fertilizers*. Turk J Agric Res, 4(2): 197-203.

Daghan H. 2018. *Effects of TiO_2 nanoparticles on maize (Zea mays* L.*) growth, chlorophyll content and nutrient uptake*. Appl. Ecol. Environ. Res, 16: 6873-6883.

Debye P., Scherrer P. 1917. *Über die Konstitution von Graphit und amorpher Kohle*. Nachrichten von der Gesellschaft der Wissenschaften zu Göttingen, Mathematisch-Physikalische Klasse, 180-188.

Dogaroglu Z.G., Eren A., Baran M.F. 2019. *Effects of ZnO nanoparticles and ethylenediamine-N, N'-disuccinic acid on seed germination of four different plants*. Global Challenges, 3(9): 1800111.

Eren A. 2020. *The effect of biologically synthesized silver nanoparticles on germination of wheat (Triticum aestivum* L.*) seeds*. ISPEC J Agric Sci, 4(2): 358-365.

Eren A., Baran M.F. 2019*a*. *Synthesis, Characterization and investigation of antimicrobial activity of silver nanoparticles (AgNP) from pistachio (Pistacia vera* L.*) leaf*. Turk J Agric Res, 6(2): 165-173.

Eren A., Baran M.F. 2019*b*. *Green synthesıs, characterızatıon and antımıcrobıal actıvıty of sılver nanopartıcles (AgNPs) from maıze (Zea mays* L.*)*. Appl Ecol Envıron Res, 17(2): 4097-4105.

Fawcett D., Verduin J.J., Shah M., Sharma S.B., Poinern G.E.J. 2017. *A review of current research into the biogenic synthesis of metal and metal oxide nanoparticles via marine algae and seagrasses*. J Nanosci, 1-15.

Ganesh M., Lee S.G., Jayaprakash J., Mohankumar M., Jang H.T. 2019. *Hydnocarpus alpina Wt extract mediated green synthesis of ZnO nanoparticle and screening of its anti-microbial, free radical scavenging, and photocatalytic activity*. Biocatal Agric Biotechnol, 19: 101129.

Gopinath K., Kumaraguru S., Bhakyaraj K., Mohan S., Venkatesh K.S., Esakkirajan M., Kaleeswarran P.R., Naiyf S.A., Kadaikunnan S., Govindarajan M., Benelli G., Arumugam A. 2016. *Green synthesis of silver, gold and silver/gold bimetallic nanoparticles using the Gloriosa superba leaf extract and their antibacterial and antibiofilm activities.* Microb Pathogen, 101: 1-11.

Hasan S. 2015. *A review on nanoparticles: their synthesis and types*. Res J Recent Sci, 4: 1-3.

Heinlaan M., Ivask A., Blinova I., Dubourguier H. C., Kahru A. 2008. *Toxicity of nanosized and bulk ZnO, CuO and TiO_2 to bacteria Vibrio fischeri and crustaceans Daphnia magna and Thamnocephalus platyurus*. Chemosphere, 71(7): 1308-1316.

Holzwarth U., Gibson N. 2011. *The Scherrer equation versus the "Debye-Scherrer equation"*. Nature Nanotechnol, 6(9): 534-534.

Jayaseelan C., Rahuman A.A., Kirthi A.V., Marimuthu S., Santhoshkumar T., Bagavan A., Rao K.B. 2012. *Novel microbial route to synthesize ZnO nanoparticles using Aeromonas hydrophila and their activity against pathogenic bacteria and fungi.* Spectrochim Acta, Part A. Molecular and Biomolecular Spectroscopy, 90: 78-84.

Jeyaraj M., Gurunathan S., Qasim M., Kang M.H., Kim J.H. 2019. *A comprehensive review on the synthesis, characterization, and biomedical application of platinum nanoparticles*. Nanomaterials, 9(12): 1-41.

Keskin C., Atalar M.N., Baran M.F, Baran A. 2021. *Environmentally friendly rapid synthesis* *of gold nanoparticles from artemisia absinthium plant extract and application of antimicrobial activities*. J Inst Sci Technol, 11(1): 365-375.

Lin D., Xing B. 2007. *Phytotoxicity of nanoparticles: inhibition of seed germination and root growth*. Environ Pollut, 150(2): 243-250.

Momeni S.S., Nasrollahzadeh M., Rustaiyan A. 2016. *Green synthesis of the Cu/ZnO nanoparticles mediated by Euphorbia prolifera leaf extract and investigation of their catalytic activity*. J Colloid Interface Sci, 472: 173-179.

Parveen K., Banse V., Ledwani L. 2016. *Green synthesis of nanoparticles: their advantages and disadvantages*. In AIP Conf Proc, 1724(1): 020048. AIP Publishing LLC.

Qu J., Yuan X., Wang X., Shao P. 2011. *Zinc accumulation and synthesis of ZnO nanoparticles using Physalis alkekengi* L. Environ Pollut, 159(7): 1783-1788.

Ramesh M., Anbuvannan M., Viruthagiri G. 2015. *Green synthesis of ZnO nanoparticles using Solanum nigrum leaf extract and their antibacterial activity*. Spectrochim Acta, Part A. Molecular and Biomolecular Spectroscopy, 136: 864-870.

Salam H.A., Sivaraj R., Venckatesh R. 2014. *Green synthesis and characterization of zinc oxide nanoparticles from Ocimum basilicum* L. *var. purpurascens* Benth.-*Lamiaceae leaf extract*. Mater lett, 131: 16-18.

Sangeetha G., Rajeshwari S., Venckatesh R. 2011. *Green synthesis of zinc oxide nanoparticles by aloe barbadensis miller leaf extract: Structure and optical properties*. Mater Res Bull, 46(12): 2560-2566.

Saravanakumar A., Peng M.M., Ganesh M., Jayaprakash J., Mohankumar M., Jang H.T. 2017. *Low-cost and eco-friendly green synthesis of silver nanoparticles using Prunus japonica (Rosaceae) leaf extract and their antibacterial, antioxidant properties*. Artificial Cells, Nanomed, Biotechnol, 45(6): 1165-1171.

Saravanakumar K., Chelliah R., Shanmugam S., Varukattu N.B., Oh D.H., Kathiresan K., Wang M.H. 2018. *Green synthesis and characterization of biologically active nanosilver from seed extract of Gardenia jasminoides Ellis*. J Photochem Photobiol B: Biology, 185: 126-135.

Selvakumar P., Sithara R., Viveka K., Sivashanmugam P. 2018. *Green synthesis of silver nanoparticles using leaf extract of Acalypha hispida and its application in blood compatibility*. J Photochem Photobiol B: Biology, 182: 52-61.

Singh A., Singh N.Á., Afzal S., Singh T., Hussain I. 2018. *Zinc oxide nanoparticles: a review of their biological synthesis, antimicrobial activity, uptake, translocation and biotransformation in plants*. J Materials Sci, 53(1): 185-201.

Suresh D., Nethravathi P.C., Rajanaika H., Nagabhushana H., Sharma S.C. 2015. *Green synthesis of multifunctional zinc oxide (ZnO) nanoparticles using Cassia fistula plant extract and their photodegradative, antioxidant and antibacterial activities*. Mater Sci Semiconductor Proc, 31: 446-454.

Thema F.T., Manikandan E., Dhlamini M.S., Maaza M. 2015. *Green synthesis of ZnO nanoparticles via Agathosma betulina natural extract*. Mater Lett, 161: 124-127.