JJBS
Jordan Journal of Biological Sciences

Volume 15, Number 4,December 2022
ISSN 1995-6673
Pages 635 – 642
https://doi.org/10.54319/jjbs/150412

The Biological Effect of ZnO Nanoparticles Produced by Using *Petroselinum crispum* Extract against *Candida* spp

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Received: September 16, 2021; Revised: February 23, 2022; Accepted: April 1, 2022

Abstract

Non-thermal or cold plasma create many reactive species and charged particles when brought into contact with plant extracts. The major constituents involve reactive oxygen species, reactive nitrogen species and plasma ultra-violets. These species can be used to synthesize biologically important nanoparticles. The current study addressed the effect of the green method-based preparation approach on the volumetric analysis of Zn nanoparticles. Under different operating conditions, the traditional thermal method and the microwave method as well as the plasma generation in dielectric barrier discharge reactor were adopted as a preparation approach in this study. The results generally show that the type of method used plays an important role in determining the size of the zinc particles produced. The traditional and microwave method stimulated the formation of clusters and agglomerates of Zn nanoparticles by effect of temperature parameter. As an example, it was noted that the lowest average diameter was obtained at 50 °C, which was 18.77 nm compared with 30.07, 23, 31, and 25.27 nm in diameter for particles generated with other temperatures of 30, 60, 70, and 80 °C respectively. These formations can occur at relatively low temperature at the expense of the formation of irregular particles. However, the weights of pre-prepared *Petroselinum crispum* seeds, and the ratio of the extract of *P. crispum* seeds to the salt, are factors that may play an important role in determining the size of the Zn nanoparticles. The current study has also shown that the highest percentage of generated nanoparticles was obtained with the cold plasma method under moderate operating conditions with the advantage of the economic factor. In addition, the Zn nanoparticles synthesized by cold plasma method in 10 min in all concentrations showed more inhibition effect as antifungal against *Candida albicans*.

Keywords : Zinc nanoparticles, *P. crispum*, plasma , microwave

1. Introduction

The importance of nanoparticles (NPs) lies in the fact that the diameter of their particles is less than 100 nanometers, which gives them nanoscale properties different from the corresponding bulk materials. These unique properties are their possession of high surface energy and quantum confinement in addition to nanoparticles' specifically large surface area. Thus, this technology can have wide applications in several fields including treatment of waste/water, sensing and catalyzing, corrosion prevention, conduction, oil recovery, electronics, clean energy, and drug delivery (Singh *et al.,* 2015; Sharma *et al*., 2020 and EL-Seedi *et al.,* 2019).

The utilization of biological synthesis methods to make nanoparticles has grown increasingly popular, owing to the fact that they are non-toxic and non-polluting to the environment. Plants, yeasts, algae, bacteria, and fungi were used to make nanoparticles; however, the preparation of nanoparticles from plants had **attracted wide interest in comparison with other methods** (Daphedar and Taranath, 2018; Paul and Sinha, 2014 and Thi *et al*., 2020)

Because zinc oxide nanoparticles are distinct from other nanoparticles as a unique material, they have attracted the attention of a large number of researchers, prompting them to conduct a large number of studies on them due to the diversity of its environmentally friendly applications in a variety of fields. Anti-microorganisms and nanomedicine, UV blocking, biosensors, phytochemical activities, drug carriers, and cosmetics are examples of these applications (Gunalan *et al*., 2012, Senthilkumar and Sivakumar**,** 2014 and Thi *et al.,* 2020).

ZnNPs is an inorganic semiconducting material that has three crystalline structures: zinc blende, rock salt and wurtzite (Thi *et al.,* 2020). Its nanostructure is distinct from the rest of the metal oxides since it contains an abundance of diverse nanostructures in its shapes, properties, and attributes such as nanotubes, nanorods, and nanospheres (Yahya *et al*., 2013). The nature of ZnNPs gave it a catalytic efficiency and a high adsorption capacity, which led to its employment in a variety of promising industrial applications, including adding ingredients to paints, sunscreens synthesis, the rubber and ceramic sector, sewage treatment, and fungicides (Gunalan *et al*., 2012).

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There are many methods to prepare zinc nanoparticles. Physical methods, for example, include melt mixing, laser ablation, electric deposition, ball milling, physical vapour deposition, sputter deposition, and ion implantation (Rahayu *et al.,* 2020). While Sol-gel, chemical-mechanical synthesis, microwave method, thermal evaporation, hydrothermal technique, vapor transport synthesis, and precipitation method are some chemical methods used to make nano-zinc oxide (Sabir *et al.,* 2014). The chemical approaches necessitate the employment of organic compounds that act as redactors and capping agents, and it is one of the most widely used methods, requiring the employment of a wide range of catalytic precursors under various reaction circumstances of time, temperature, and reactant concentrations. The difference in the reaction conditions leads to the difference in the size and shape of the resulting zinc nanoparticles (Sabir *et al*., 2014 and Thi *et al*., 2020).

Recently, green synthesis has been preferred over physical and chemical methods in the manufacture of nano-zinc oxide because of its low costs and not requiring high temperatures or specialized devices or the use of toxic organic compounds as reducing agents or solvents during the synthesis process, and thus no toxic or dangerous compounds are produced from the reaction. The green method is one of the environmentally friendly methods because it reduces the risks of pollution. The properties of the resulting nano-zinc oxide depend on the exact structure and structural characteristics, which are determined according to the chosen method of manufacture. (Sabir *et al*., 2014 and Lakshmeesha *et al*., 2019).

Use of plants or plant extracts as row materials was due to its ease of work, the availability of plants in abundance, and the presence of chemical compounds, which are considered as reducing agents. In fact, (Fakhari *et al*., 2019) proved that the zinc raw material plays a key role in the internal structure and external morphology of zinc nanoparticles by using two types of primers, which are zinc nitrate and zinc acetate, and in the presence of the aqueous extract of the leaves of *Laurus nobilis* plant (Fakhari *et al*., 2019).

The precursor concentration of zinc nitrate has a significant impact on the production of nano-ZnO when *Aloe vera* extract is employed according to (Rasli *et al.,* 2020). These compounds include enzymes, proteins, flavonoids, phenols, alkaloids, tannins, soaps, and others, which play a key role in the reduction process of particles (Lakshmeesha *et al*., 2019 and Rahayu *et al.,* 2020). In addition, using zinc acetate compound as a starting material and adding *Ixora coccinea* leaf extract, (Yedurkar *et al.,* 2016) were able to generate spherical zinc oxide nanoparticles with a high degree of stability.

The species *Petroselinum crispum*, or as known locally parsley, is one of the herbal and aromatic plants that belong to the Apiaceae family. The chemical composition of parsley has revealed the presence of flavonoids, terpenes, coumarins, furanocoumarins, essential oils, and fatty acids as secondary compounds (Chaves *et al.,* 2011, Liberal *et al.,* 2020). Parsley belongs to the Mediterranean herb whose leaves have a high content of Vitamin C (ascorbic acid). This high content of vitamin C in parsley leaves can play an essential role as a strong reducing agent in this synthesis of nanoparticles (Roy *et al.,* 2015).

The present study is a serious attempt at the investigation of effective methods of chemical green synthesis on nanoparticle sizes. The current investigation was adopted on the hypothesis that says that the size of particles that can be produced with this method is restricted by the details of the preparation as well. Therefore, the conventional thermal method, the microwave method, and the plasma method have been covered in this study. The comparison was made by analyzing the size of the measured particles, using atomic force microscopy. The biological activity of ZnO NPs nanoparticles was evaluated by using well diffusion method for pathogenic fungi like *Candida albicans*, *Candida guiller*, *Candida zeylenoid* and *Candida kruse*.

2. Material and methods

2.1. Preparation of the aqueous extract of the Petroselinum crispum

P. crispum seeds were purchased from a local market in Baghdad and ground for 5-7 minutes in an electrical grinder. 10 g of *P. crispum* seeds powder was mixed with 100 mL of boiling distilled water. The mixture was placed in a magnetic stirrer for 2-3 hours and left for 24 hours. Then the mixture was filtered by using Whitman paper No.1, and the filtrate was stored at 4 °C. The methods of (Trease and Evans, 1989 and Harborne, 1973) were used to identify active phytoconstituents (Table-1).

2.2. Preparation of zinc nanoparticles

The Zn nanoparticle was prepared by three methods:

2.2.1. Preparation of zinc nanoparticles by using magnetic stirrer

ZnNPs was synthesized by adding 45 mL of $1*10^{-3}$ M of $ZnSO_4$ (Sigma-Aldrich) solution in a flask placed on the magnetic stirrer device (AL- Shaheen *et al*., 2020). After the temperature stabilized at 60 °C, added 5 mL of *P. crispum* seeds hot aqueous extract with continuous stirring for half an hour. The final solution was stored at 4 C° (Rasli *et al*., 2020).

2.2.2. Preparation of Zinc nanoparticles using Microwave:

The nanostructured zinc solution was prepared by adding 45 mL of ZnSO4 at a concentration of $1*10^{-3}$ M in a flask with the addition of 5 mL of *P. crispum* hot aqueous seeds extract. Then the mixture was placed in the microwave for 50 seconds (500-1000) watts, after which it was stored at 4 °C (Chikan and McLaurin, 2016).

2.2.3. Preparation of Zinc nanoparticles using Cold Plasma:

Dielectric barrier discharge reactor was used to generate the cold plasma. The generation system was designed in accordance with the nature of the used models and to achieve the largest plasma package that can be generated as can be seen in Figure (1). After numerous tests, the optimum distance between the electrode and the model surface was from a half to one centimeter. Four solutions of ZnNPs were prepared in four flasks, added to each solution 45 mL of $1*10^{-3}$ M ZnSO4 then added 5 mL from *P. crispum* hot aqueous seeds extract in every flask. The solutions were exposed directly to the plasma device with a difference in the exposure time of the plasma for every solution which is (1, 3, 5, and 10) minutes; then the solutions were stored at 4 °C (Primc *et al*., 2021).

2.3. Identifying the best circumstances for synthesis zinc nanoparticles

2.3.1. Determine the optimum temperature for the synthesis of ZnNPs

The optimal temperature for synthesis ZnNPs was established by mixing 45 mL of $1*10^{-3}$ M ZnSO4 solution in a flask with 5 mL of *P. crispum* hot aqueous seeds extract then put on a magnetic stirrer device at varied temperature (30, 40, 50, 60, 70 and 80 °C) every time. After that, all the solutions were kept at 4 °C (Verma and Mehata, 2016).

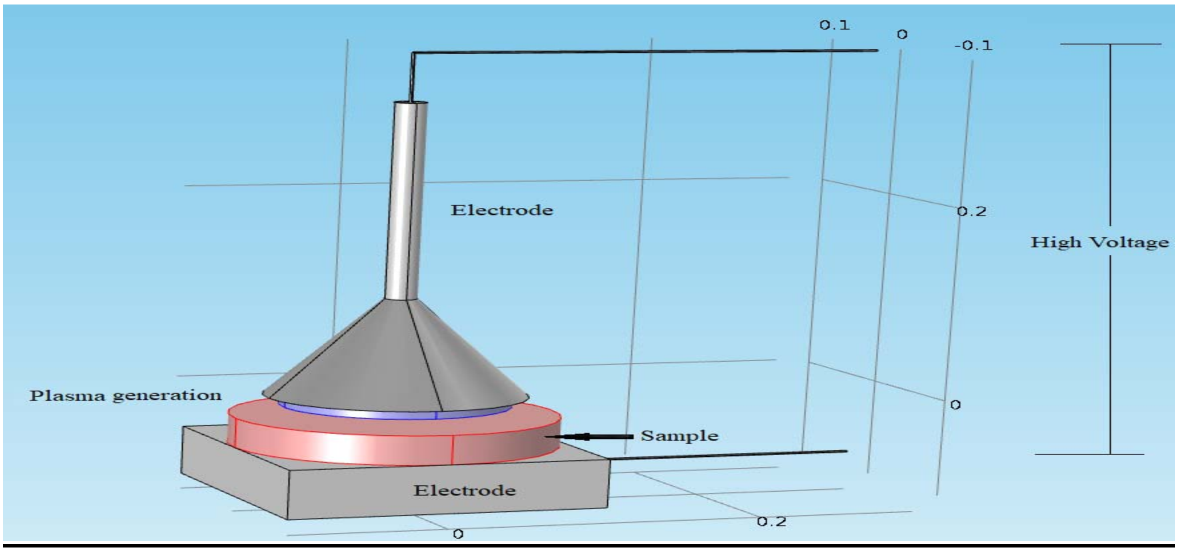

Figure 1: Schematic diagram of Plasma generation in dielectric barrier discharge (DPD) reactor used in the current study

2.3.2. The effect of variance in plant material weight in extraction of P. crispum seeds hot aqueous extract in ZnNPs synthesis:

The hot aqueous extracts were prepared using different weights of powdered *P. crispum* seeds, which are (4, 7, 10, 13, and 16 g) per 100 mL of boiling distilled water, and the extracts were placed on the magnetic stirrer device for 2 - 3 hours for homogenization then left for 24 hours. The mixtures were then filtered and kept in 4 °C (Sithara *et al*., 2017).

2.3.3. Evaluate the use of different volumes from P. crispum seeds hot aqueous extract and ZnSO4 solution in ZnNPs manufacturing.

Different volumes of seeds hot aqueous extract and ZnSO4 solution were used in manufacture of ZnNPs by adding (45, 25 and 5) mL of $1*10^{-3}$ M ZnSO4 with (5, 25 and 45) mL of seeds hot aqueous extract respectively (Ahmed *et al.,* 2016 and Javad *et al*., 2017).

2.4. Characterization of ZnNPs

2.4.1. Atomic Force Microscopy (AFM) measurement

The surface morphology of ZnNPs was investigated using AFM (Angstrom AA2000, contact mode, atmospheric circumstances, USA) pictures, which provide clear topological images of surface morphology at high magnification. A 0.5 mL sample of ZnNPs was centrifuged for 5 minutes at 10000 rpm in an eppendrof tube. Using atomic force microscopy, a few droplets of the sample were deposited on the slide, air dried, and described (Altaee *et al*., 2020).

2.4.2. UV spectrophotometer measurement

UV-vis spectroscopy was used to do preliminary characterization of the ZnNPs. The optical behavior of the biosynthesized ZnNPs was studied using a spectrophotometer (UV-Spectrophotometer - Shimadzu UV-1800) in a quartz cuvette with a 1 cm optical path at ambient temperature. Aliquots of ZnNPs were examined in the wavelength range of (200 - 700 nm) (Malarkodi *et al.*, 2014).

2.5. Biological activity of ZnNPs

The agar well diffusion assay method was used to test the antifungal activity of ZnNPs synthesized by magnetic stirrer, microwave and cold plasma in 10 minutes. Stock cultures of *Candida albicans*, *Candida guiller*, *Candida zeylenoid*, and *Candida kruse* were freshly cultured in a petri dish at 25 °C for 24 - 48 hours in an incubator (Memmert, Germany) on Sabouraud Dextrose Agar (SDA) medium (Hi Media, India) (Mahdavi *et al*., 2013). A loop full of cells were transferred from the agar to a test tube with 5 mL of Sabouraud Dextrose broth (Hi Media, India), then incubated at 30 °C with shaking for 12 - 16 hours (Bensizerara *et al*., 2013). SDA agar plates were inoculated with Candida strains under aseptic conditions and wells were filled with 50 µL with various concentrations of ZnNPs (100, 75, 50 and 25) % and incubated at 25 °C for 24 - 48 hours. The inhibition zones were measured in millimeter (well size 6 mm) (Devi and Bhimba, 2014). Antifungal nystatin (50 mcg) was selected as a positive control, by using a disc diffusion method against *Candida albicans*, *Candida guiller*, *Candida zeylenoid*, and *Candida Kruse*. The standard antifungal disc was purchased from Hi-Media, India (Deabes *et al.,* 2020).

3. Results and Discussions

The Differences in chemical and physical conditions such as weight of plant material, volumes of reactants, temperature and the kind of reaction method have significantly affected the size, form and the morphology of ZnNPs in this study.

3.1. The visual observation and pH value of Zn nanoparticles

The reaction between *P. crispum* extract and ZnSO4 solution was transparent at the beginning of the reaction; however, the change in color was noticed after that as it turned into a light yellow color after of Zn nanoparticle formation, while the pH value of ZnNPs was 7 and remained constant during the reactions and after the formation of Zn nanoparticles and did not change during the period of storage.

3.2. Characterization of the zinc nanoparticles by UV–vis spectrophotometric analysis

Zinc oxide nanoparticles were synthesized according to decided protocol using seeds aqueous extract from *P. crispum*. After the addition of seeds extract to the zinc sulphate solution, a slight color change will appear. It indicates the completion of the reaction, which is due to the excitement of plasmon vibrations in the zinc nanoparticles. Zinc sulphate solution alone without extract has no color; the intensity of color will steadily increase after the addition of seeds of the aqueous extract with an increase in the incubation period and the temperature. The ZnNPs exhibited slight yellow color, which may due to the excitement of the surface plasmon resonance (SPR) effect and the reduction of zinc sulphate. The reduction of seeds aqueous extract by zinc ions and the formation of ZnNPs was emphasized using UV–vis spectroscopy. A wavelength scanning process in the UV–vis spectra revealed an absorption peak at approximately 338 nm for ZnNPs (Figure 2).

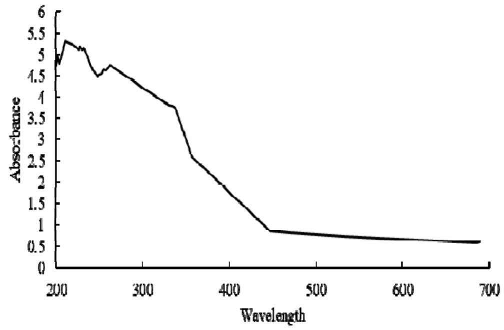

Figure 2: UV-Vis spectrum of manufactured ZnNPs by the reaction between ZnSO4 10^{-3} M with *P. crispum* seeds extract.

These results were similar to the results of (Salem *et al*., 2015) which revealed an absorption peak at 340 nm for zinc nanoparticles synthesized by green method using leaf and fruit extracts from *C. procera*. Through Table (1), which shows the detection of secondary metabolites in plant seed extract *P. crispum*, the presence of metabolic compounds in appropriate quantities such as phenols, flavonoids and tannins can interact with zinc molecules as reduction agents and help in the synthesis and stability of zinc nanoparticles.

Table 1: Qualitative Phytochemical Analysis of *P. crispum* seeds extract

Phytoconsitituents	Reagents	Aqueous extract
Alkaloids	Mayer's + Wagner's	-
Saponins	Gelatin	-
Tannins	Ferric chloride	+
Phenols	Iron chloride	+ +
Flavonoids	Alkaline Reagent	+ +
Glycosides	Borntrager's	-

3.3. The effect of experimental conditions on the formation of Zn nanoparticles

The results showed the formation of zinc nanoparticles in all different experimental conditions, such as weight of plant material, volumes of reactants, temperature as well as at different manufacturing methods such as are magnetic stirrer, microwave and cold plasma. Generally, the sizes of the Zn-nanoparticles ranged between 18.77 and 75 nm.

3.3.1. The effect of different temperatures in ZnNPs synthesis

Effect of thermal treatment was addressed in the current study. The influence of 30, 40, 50, 60, 70, and 80 °C on the preparation of zinc oxide nanoparticles (Zn-NPs) was adopted in the current investigation. Figure (3) and Figure (4) show the volume percentage of the diameters of Zn-NPs formed using Atomic Force Microscopy at different temperatures. The figures show that the temperature has a clear effect on the particle size of the Zn-NPs produced. Lowest average diameter (18.77 nm) was obtained at 50 °C. This diameter began to increase with the rise in temperature to be 23, 31, 25.27, and 30.07 nm in diameter for particles generated in temperatures of 60, 70, 80, and 30 °C respectively. In fact, these experimental data of Zn-NPs were due to the formation of clusters and agglomerates. In addition, increase in the temperature increases the acceleration of the rates of nucleation. This interpretation was also supported by (Bandeira *et al*., 2020; Parra and Haque, 2014; Dhadapani *et al*., 2014; Manzoor *et al*., 2015 and Shaziman *et al*., 2015). They emphasized that the temperature and exposure time can determine the structure and size of nanoparticles form by green method. On the other hand, the reduction in the temperature gives irregular shapes while keeping the clusting rates low. This study, and through a set of experiments, found that the temperature of 50, 60 and 80 °C gave the lowest diameter, so the average temperatures between them was 60 C°, which was adopted in the subsequent experiments. In fact, it appears that this increase in particle size with increasing temperature is not limited to zinc oxide, but many other mineral particles have had the same effect. For example, the average diameter of silver particles increases with increasing temperature, and for the same reasons above (Liu *et al*., 2020).

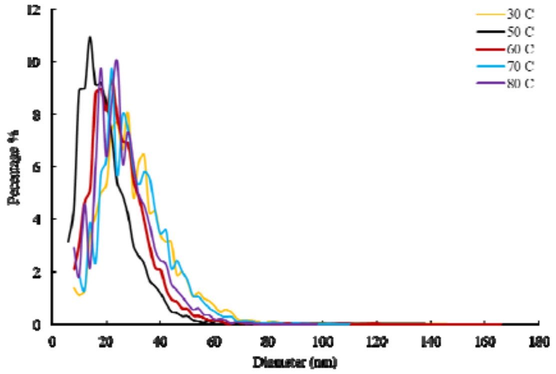

Figure 3: Granularity Distribution of Zn NPS at different temperatures (30, 50, 60, 70and 80 ºC).

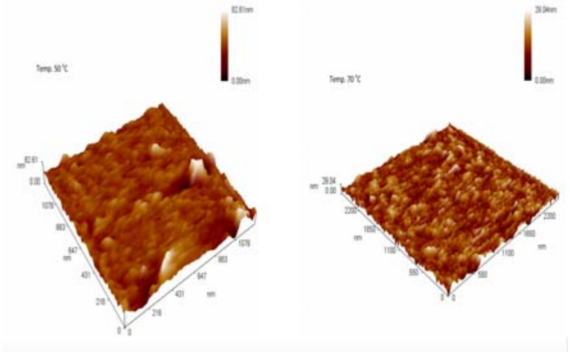

Figure 4: 3D-response of Granularity Distribution of zinc oxide nanoparticles at 50 and 70 ºC using Atomic Force Microscopy

3.3.2. The effect the different plant weights of P. crispum seeds extracts in ZnNPs synthesis

The effect of different group of weights of *P. crispum* seeds powder (4, 7, 10, 13 and 16) g, on the size of the nanoparticles generated by using green method was studied, as shown in Figure 5 and 6. The experimental data showed the variation in the average diameter of the nanoparticles and at a temperature of 60 °C and within the generated particles. The lowest diameter ratio of nanoparticles was obtained at 10 g weight compared to other weights, since the average diameter was 23.62 nm at a weight of 10 g, compared to the other weights that ranged between 64 and 75 nm.

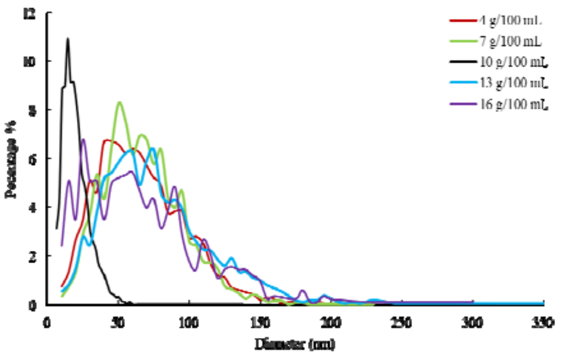

Figure 5: Granularity Distribution of zinc nanoparticles at different weight of *P. crispum* seeds (4, 7, 10, 13, and 16 g / 100 mL)

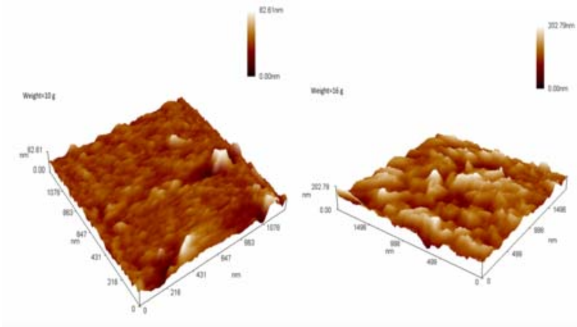

Figure 6: 3D-response of Granularity Distribution of ZnNPs at 50 and 70 ºC using Atomic Force Microscopy

3.3.3. Effect of different volume ratio of P. crispum seeds extract and ZnSO4 solution in ZnNPs synthesis

The effect of the ratio of the extract of *P. crispum* seeds to the salt on the diameter of the generated particles was verified in the present study. The results showed that the half-weights (50 % for each one) achieved the lowest possible diameter than other percentages as shown in Figure 7 and 8. 43.36 nm was achieved at 50 % volume of the extract with 50 % volume of salt pulse 50 % of the extract, while 49.62 nm and 70.16 nm were achieved at 20 % volumes of extract pulse 80 % of the extracted solution as well as 80 % volumes of extract pulse 20 % of salt, respectively. However, in the form of a single digit, the quantity that achieved less than 40 nanometers was greater in the first percentage compared to the average.

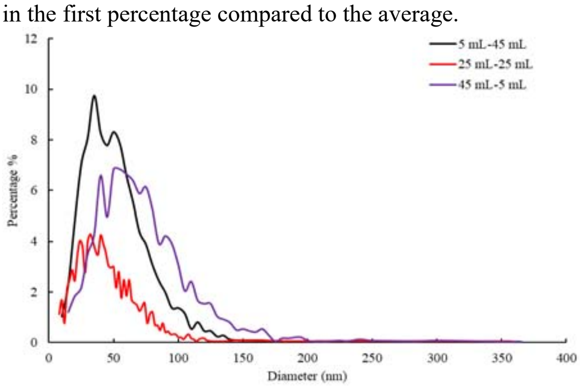

Figure 7: Granularity Distribution of ZnNPs at different volume ratio of *P.crispum* seeds extract and ZnSO4 solution

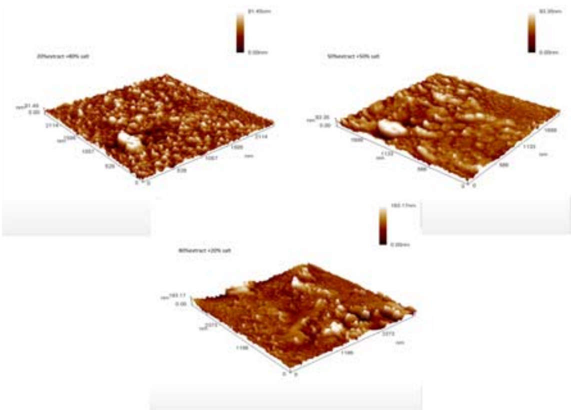

Figure 8: 3D-response of Granularity Distribution of ZnNPs at different extract solution of *P.crispum* seeds to salts ratio using Atomic Force Microscopy.

3.4. owave device in synthesis of ZnosThe effect of using cold plasma in synthesis of ZnNPs

The use of cold plasma as an alternative to the heating stage in preparing nanoparticles was also present in this investigation. Figure 9 shows a complete analysis of the size of the generated fine particles. The time factor was the important variable that was adopted in this study. The results showed that the exposure of the solution to a time of 3 minutes was the best in terms of the quantity and size of the generated particles. The reduction in time was apparently not sufficient to achieve the required particle size, whereas the increased exposure time of the models to plasma radiation may cause some of the particles to agglomerate to form larger particles. Periods 3-5 minutes led to the production of nanoparticles size. This is consistent with (AL- Azawi *et al.,* 2019) in the production of Silica NPs at a concentration of 18 nm using cold plasma for five minutes.

In fact, the cold plasma method used in the current study causes three regions to occur, which include (gas phase region, interface region, and liquid phase region). Each of these regions undergoes a specific reaction that consumes or produces electrons (Humud and Dawood, 2016 and Kaushik *et al.,* 2019). The main driver of these reactions is the plasma generated to generate hydrogen peroxides [OH^+] in the gas phase and interface regions (Milosavljevic and Micic, 1978). In the liquid phase, these generated peroxides react to produce enough electrons to form the zinc with zero ions [Zn^0].

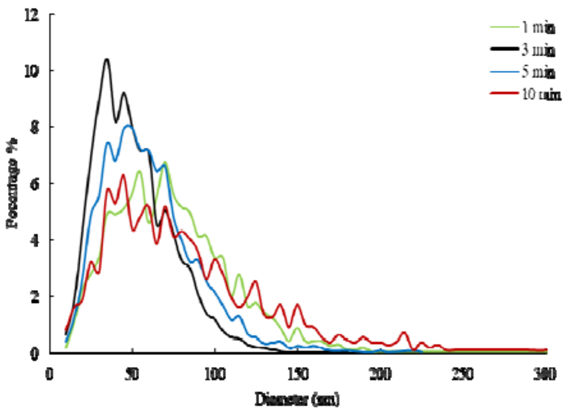

Figure 9: Granularity Distribution of zinc oxide nanoparticles using plasma approach method

3.5. The effect of using the microwave device in synthesis of Zn NPs

The use of microwave devices as another means of preparing the heat required for the formation of nanoparticles has also been adopted in the current study. The exposure time to microwave waves was selected as an independent variable in the current study. As for determining the time of exposure of the laboratory samples to those microwaves, they were restricted by the amount of heat and the temperature that the samples would reach. Fifty degrees Celsius and sixty degrees Celsius were determined after several experiments on the condition that the temperature does not reach more than the required temperature. Figure 10 shows the numerical percentage of nanoparticles formed by using this method. The results showed that this percentage of nanoparticles formed after fifty seconds is higher than that obtained after the passage of sixty seconds. The results here support previous studies that showed the contribution of heat gradient-free to improving the size of the microparticles generated (Gerbec *et al.,* 2005). More clearly, 7 % represented the numerical percentage of nanoparticles formed at 50 °C, compared to 4 % at exposure time of 60 °C. These results were consistent with what was obtained using the conventional method. However, the difference between the two methods is the short period of time for the training phase with the cost factor.

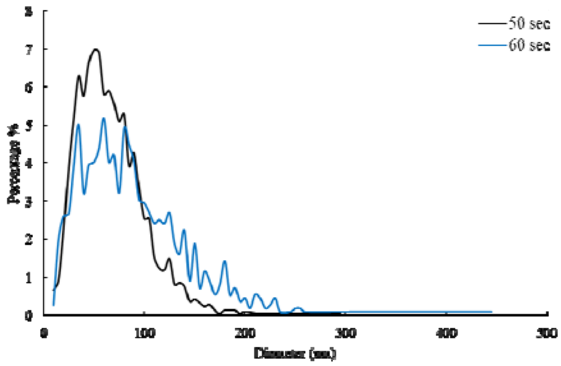

Figure 10: Granularity Distribution of zinc oxide nanoparticles using microwave approach methods.The biological activity of Zn NPs samples

Antifungal activity of Zn NPs samples was studied against four types of Candida as *Candida albicans*, *Candida guiller*, *Candida zeylenoid,* and *Candida kruse*. The results presented in table 2 show that the synthesis method of Zn nanoparticles and their concentration also the kinds of Candida under study have a great effect on the zone size of inhibition for fungi as reported by (Gunalan *et al*., 2012). The Zn nanoparticles that were synthesized by cold plasma showed a high inhibition effect unlike the ZnNPs that were synthesized by using the magnetic stirrer method, and the microwave method did not show any inhibitory effect against any *Candida* species. Table 2 shows ZnNPs synthesized by cold plasma method in the concentrations (100, 75, 50, and 25) %. The results illustrated that the highest inhibition effect against both *C. albicans* and *C. zeylenoid* was more than that with the positive control (nystatin). ZnNPs achieved a high inhibition effect in 100% concentration, compared with the positive control for *C. Kruse*. All the concentrations of ZnNPs showed a low inhibition effect than positive control of *Candida guiller*. The antimicrobial activity differences may derive from the electrostatic attraction between the negatively charged microbial cell membrane and the positively charged zinc nanoparticles (Hamouda *et al*., 2001), and this is due to difference in the synthesis method of ZnNPs. The synthesis method of zinc nanoparticles affected the interaction of zinc ions with microbial cells and inhibited their growth, as the nanoparticles formed in each way were different in terms of size and orientation (Gunalan *et al*., 2012 and Wang *et al*., 2007).

Table 2: The antifungal activity of ZnO NPs which synthesized by using three methods and the concentrations (100, 75, 50 and 25) % against Candida

Fungi	Positive Control Nystatin (mm)	Zone of inhibition (mm)				Synthesis method of ZnNPs				Concentration of ZnNPs (%)			
		Cold plasma method (10 min)				Magnetic stirrer method				Microwave method			
		100	75	50	25	100	75	50	25	100	75	50	25
Candida albicans	16	35	30	27	24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Candida guiller	18	12	17	15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Candida zeylenoid	18	25	22	20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Candida kruse	19	20	16	17	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

The Figure 11 showed that ZnNPs synthesized by cold plasma method in the concentrations (100, 75, 50 and 25) % have the highest inhibition effect against *C. albicans*., followed by *C. zeylenoid* that showed a good effect in the concentrations (100, 75 and 50) % of Zn nanoparticles; both *C. zeylenoid* and *C. kruse* have achieved almost the same inhibition effects against using the nanoparticles in the same concentrations, while the concentration 25 % of zinc nanoparticles did not show any inhibitory effect on fungi except for *C. albicans*. That may be due to proper diffusion of Zn nanoparticles in the agar medium. The results showed an increase in the percentage of fungi inhibition by increasing the concentration of zinc nanoparticles, and this is consistent with what has been achieved by both (Gunalan *et al*., 2012 and Navale *et al.*, 2015). The difference in the inhibitory effect of zinc nanoparticles on fungi may be due to difference in their individual response and their genotypic characters (Senthilkumar and Sivakumar, 2014).

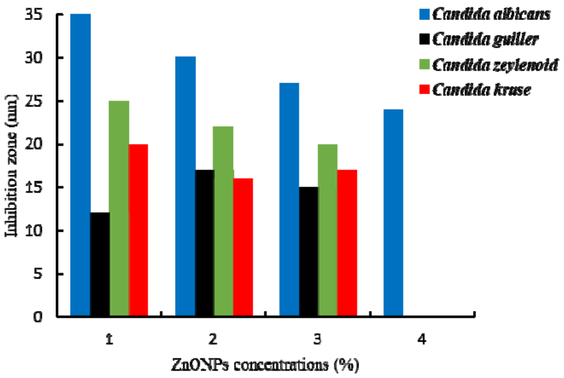

Figure 11: The effect of ZnO nanoparticles that synthesized by using cold plasma method for 10 min on the biological activity for pathogenic fungi by using different concentrations (1) 100%, (2) 75%, (3) 50%, (4) 25%.

4. Conclusions

The effect of the preparation method on the size of the zinc-nanoparticles was verified in the current study under different operating conditions. The study concluded the importance of those conditions as well as the method used in the preparation process, whether it was the traditional thermal method, microwave oven or plasma, on the size analysis of generated particles. High temperatures, in addition to being economically costly, also increase the size of zinc nanoparticles generated in the microwave and thermal method, while the use of the Plasma generation in dielectric barrier discharge (DPD) reactor has achieved the best percentage of formed particles of nano sizes with the associated operational and economic factor. The zinc nanoparticles that were prepared by using cold plasma also achieved the highest inhibition zone against fungi, especially *Candida albicans*.

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