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EFFECT OF ZnO NPS SYNTHESIZED BY OLIVE LEAVES EXTRACT IN CHLOROPHYLL CONTENT AND SOME ANTIOXIDANT ENZYMES OF *MENTHA PIPERITA* L. LEAVES

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Abstract

The Zinc oxide nanoparticles (ZnONPs) green syntheses were prepared using *olea europaea* extract as a bio-reducing agent. Zinc oxide nanoparticles have been characterized by several techniques UV-vis-spectrophotometric, FTIR, XRD, EDAX and FESEM. The antioxidant effect of catalase enzyme activity and superoxide dismutase has increased with the increase of concentration nano-zinc oxide that treated of Peppermint plant, which stimulates ZnONPs biosynthesis of the antioxidant enzymes as important components of the defense mechanisms against the toxicity of ZnONPs. The results of the present study shown a significant increase ($P \leq 0.05$) in activity of catalase and superoxide dismutase enzyme with the high concentration of ZnONPs. The content of Chl a, b and total chlorophyll levels in Peppermint plant treated with ZnONPs were increased significantly at lower concentrations of ZnONPs (5 ppm) compared with control treatment. Chl a, b contents and total chlorophyll levels decreased significantly in the higher concentrations of ZnONPs treatment was observed at 10 ppm and 15 ppm. The carotenoid in Peppermint plant treated with ZnONPs, at high concentration of ZnONPs 10 ppm and 15 ppm produce significantly a highest total carotenoid in Peppermint plant as compared with control.

Keywords**:** ZnO nanoparticles, Peppermint plant, CAT activity, SOD activity, Chlorophyll a, b, total and Carotenoids content.

Introduction

Nanotechnology could be defined as understanding and controlling a matter at rough dimensions scale about (1–100 nanometers), so novel application could be produced due to the unique phenomena of nanotechnology, nanotechnology is an incorporation of many science fields which promising platform in medicine and pharmaceutical industry (Mohanpuria *et al*., 2008). Peppermint, whose scientific name is *Mentha piperita* L. (Family: Lamiaceae), is one of the significant and oldest medicinal herb in Eastern and Western, and its first description was at 1753 by Carolus Linnaeus and around the world was at 2004 by Saller. It could be used in various applications such as flavoring agent (from chewing gum to after dinner mints), pharmaceutical products and in cosmetics. Heirba Buena, which means good herb and its scientific name is *M. piperita,* is yielding oil of peppermint and it is the most consumed volatile oils. The main component of *M. piperita* is menthol $C_{10}H_{19}OH$. Its characteristics are waxy white crystalline monoterpene substance, solid at room temperature and produced and accumulated specially in the aerial parts peltate glandular trichomes (Murray, 1995).

Traditional agricultural practices could potentially be modified by using nanomaterials, plant anatomy biochemistry, and physiology are influenced by Zinc oxide nanoparticles (ZnONPs) (Zafar *et al*., 2016).

Plant functioning is largely effected by zinc, such as in regulation of auxin tryptophan synthesis means and in the redox enzymes superoxide dismutase and dehydrogenases by being a cofactor (Narendhran *et al*., 2016).

The defense system of antioxidant is stimulated by penetration of ZnONPs into plants and seeds to be the first detection system of toxic effect (Lopez-Moreno *et al*., 2010; Ghosh *et al*., 2016). Similarly, activity of POD was noticed to be increased by the application of 500 mg L^{-1} ZnONPs in *Triticum aestivum* (Dimkpa *et al*., 2012). Siddiqui *et al* (2015) summarized different researchers findings that NPs effect on growth and development of plant could be negative and positive; depending on the physical and chemical properties, composition, size and concentration of NPs as well as species of plant.

Materials and Methods

1. Plant Collection and Identification

Olive fresh leaves from the house gardens in Hilla City, Babylon, Iraq were collected in July 2019. The plant specimen was identified in Plant Harbarium / Biology Department / College of Science / Babylon University. *Olea europaea* L's leaves. Where dried air and then ground into a fine powder with the helping of an appropriate grinder.

2. Preparing of aqueous leaves extract of olive

The extract of Olive leaves was produced by adding 0.01 kg of olive leaves powder in 0.1 l sterile purified water and heated for 30 minutes using a magnetic stirrer at 60 °C and 700 rpm, then filtered overnight utilize Whitman filter paper No.1 and stored and collected at 4 °C for additional use (Vaishnav *et al*., 2017).

3. The nanoparticles of Zinc oxide Synthesis

Prepared 100 ml of 100 mM zinc sulphate heptahydrate solution (ZnSO4.7H2O) and stored on a magnetic stirrer at around 60 °C and 700 rpm. Dropwise 25 ml of leaves extract has been added and color changes are observed. By adding 1 M NaOH solution, the pH is checked and adjusted to 12. White cloudy appearance observation marked the formation of ZnO nanoparticles. The solution is left for two hours in same condition. Then incubated Overnight at room temperature. Dispersing in sterile purified water and 700rpm centrifugation for 30 minutes three times purified the suspended particles. The particles with white color were subsequently wash by ethanol for removing the impurities from the final products. After drying for six hour in the vacuum oven at 60 °C, in order to obtain a white powder (Vaishnav *et al.,* 2017).

4. Treatment of Peppermint plant with nano - zinc oxide

Peppermint plants were grown in a pots (height 7 cm and diameter 10 cm), containing a mixture of sand soil and peatmoss at 1: 1. The plants were irrigated with 100 ml of different concentration of ZnO solution which include (2.5, 5, 10 and 15 ppm) at twice at the beginning and middle of the experiment period. The plants were irrigated with distilled water as needed for 30 days. The leaves of *peppermint* were collected after 30 days and dried in oven at 60 °C then stored at 4 °C until we used.

5. Estimation of Chlorophyll a, b, total and Carotenoids content in Peppermint leaves

Chlorophyll was measured using method of Arnon (1959).

6. Estimation of catalase enzyme activity in Peppermint leaves

The activity of catalase enzyme is measured using method demonstrate by Aebi (1983).

7. Estimation of superoxide dismutase (SOD) activity in Peppermint leaves

The efficacy of the SOD enzyme indicated by Marklund and Marklund (1974).

Results and Discussion

1. Effect of ZnO nanoparticles in catalase enzyme activity of Peppermint leaves

The CAT is notable antioxidant enzymes act as a defense in organisms which catalyze the reaction of converting H_2O_2 in to oxygen (O_2) and water and detoxification of free radicals (Ma *et al*., 2015). CAT inhibits the accumulation of H_2O_2 that produced by metabolic processes of plant (Panda and Choudhury, 2005). As shown in figure 1 the antioxidant effect of the catalase enzyme has increased with the increasing concentration of nano-zinc oxide, which indicate that ZnONps stimulates biosynthesis of the antioxidant enzymes as important components of the defense mechanisms against the toxicity of ZnONPs, the result of the present study shown a significant increase in concentrations of catalase enzyme with increasing of ZnONPs concentration. These results agreement with the impact of ZnONP treatment on CAT enzyme activity in the leaf tissue of cotton (Priyanka and Venkatachalam, 2016). Hernandez-Viezcas *et al.* (2011) demonstrate that the increase in ZnONPs concentration in mesquite plants causes increase in CAT activity. As the ZnONPs dose was increased, CAT activity increased significantly. The antioxidant enzymes activities where enhanced by ZnONPs, Some researchers indicated that ZnONPs caused increase activity of CAT in *Prosopis juliflora-velutina* (Ma *et al.*, 2015) and in *Spirodela polyrhiza* (Hu *et al*., 2013).

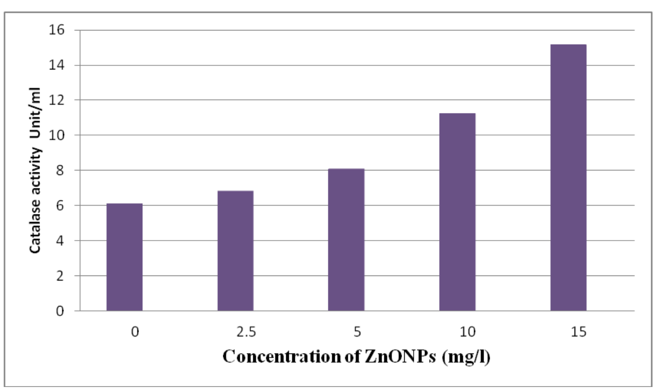

Fig 1 : Effect of ZnO nanoparticles in catalase enzyme activity of Peppermint plant leaves. L.S.D. (0.05) = 0.49

2. Effect of ZnO nanoparticles in superoxide dismutase activity of Peppermint plant leaves

The antioxidant activity of SOD enzyme that shown in figure 2 has increased with the increasing in concentration of nano-zinc oxide. The results show that SOD activity was found to increase with increasing quantity of ZnONPs up to 15 mg/L. These results showed a significant increase in concentrations of SOD enzyme with increasing in concentration of ZnONPs, which was agreed with the result of the exposure of cotton plants leaves to various doses of ZnONPs that effected on the SOD enzyme activity (Priyanka and Venkatachalam, 2016). The antioxidant defense system responsible for scavenging excess ROS can be activated to alleviate nanomaterial induced toxicity in plants. Superoxide anion radical content regulated by SOD (Mittler *et al*., 2004), where superoxide anions (O_2^-) converted in to less toxic oxygen (O2) and hydrogen peroxide (H_2O_2) by SOD which represent the first enzyme defense that catalyze the ROS (Ma *et al*., 2015). The present results are in agreement with Kouhi *et al.* (2015), and Kim *et al.* (2012), who observed increased SOD activity at various doses of ZnO NP. Hu *et al.* (2013) also demonstrated that the increase exposure of *Spirodela polyrhiza* to ZnONPs results in increase the activity of SOD enzyme and magnification the scavenging process of ROS.

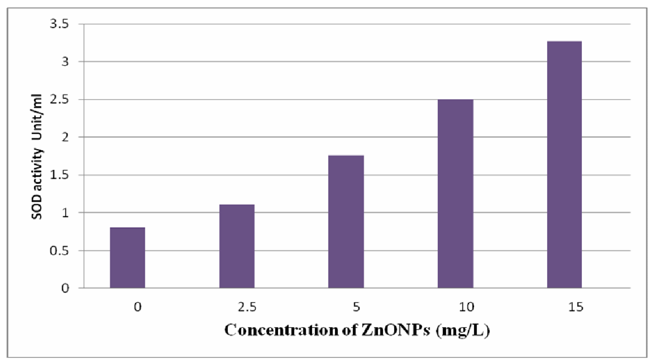

Fig. 2 : Effect of ZnONPs in superoxide dismutase of Peppermint plant leaves. L.S.D. (0.05) = 0.26

3. Effect of ZnO nanoparticles in Chlorophylls a, b, total Chlorophyll and Carotenoids of Peppermint plant leaves.

Yellow leaf color observed in ZnONPs treated plants indicates that ZnONPs affected in chlorophylls amount in the plants. The amount of Chl a measured in the Peppermint plant as shown in figure 3 is clear that the Chl a content was increased significantly at ZnONPs low concentrations 2.5 and 5 mg/L compared with control treatment. These results agreement with Behtash and colleagues results, in which the damage of chlorophyll by cadmium prevented by zinc in red beet embryo, also zinc affected on index of chlorophyll and increased chlorophyll content (Behtash *et al*., 2010).

On the other hand ZnONPs treatment decreased Chl a contents significantly in the higher concentrations was observed 10 and 15 mg/L. An inhibition of Chl a contents in Peppermint increased following the increasing of the ZnONPs concentrations. Zn is a heavy element causes toxic effects on many plants at large amount which result in chlorophyll degradation (Mohsenzadeh and Moosavian, 2017).

Zn causes toxicity to plant, prevent absorption Mg and Fe that necessary for chlorophyll biosynthesis, stimulation of chlorophyllase that causes degradation of chlorophyll, the amount of chlorophyll a is increased up to 13% when plant subjected to low level of nanoparticles (Racuciu *et al.*, 2006). The low level of chlorophyll in plant subjected to the high nano zinc oxide amount due to the effects of nano zinc oxide on the chlorophyll precursors by degradation of those precursors and prevent synthesis of chlorophyll. Lin and Xing (2008) demonstrate that there is no phytotoxicity of Zn^{2+} released from suspensions of ZnO NP on rape, ryegrass and radish. Yang *et al.* (2015) demonstrate that the effect of ZnONPs on rice and maize not depended on the Zn^{2+} but on ZnONPs itself. That agreement with (Lee *et al.*, 2013; Yang *et al.*, 2015)

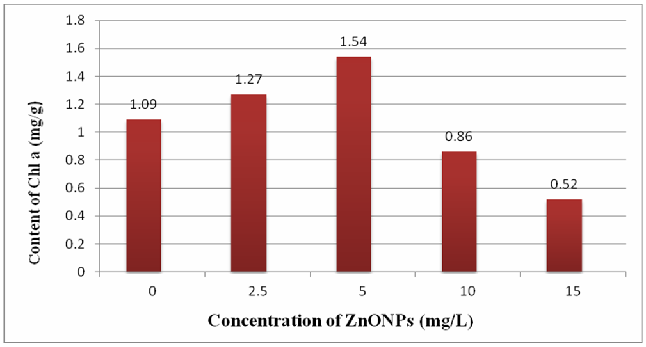

Fig. 3 : Effect of ZnO nanoparticles in Chl a content of Peppermint plant leaves. L.S.D (0.05) = 0.08

The nanoparticles are very good enhancer for efficiency of photosynthesis and causes increase light absorption by chlorophyll (Govorov and Carmeli, 2007; Mingyu *et al.*, 2007; Nadtochenko *et al.*, 2008). The presence of zinc facilitated formation of chlorophyll by affected of some substances or precursors involved in synthesis of chlorophyll or substances that are part of the chlorophyll such as magnesium and iron. In fact, The activity of some enzymes involved in biosynthesis of chlorophyll dependent on present of zinc (Lebedev and Timco, 1998). Zinc by protection of sulfhydryl groups caused synthesized chlorophyll (Cakmak, 2000). The phytotoxicity assays for NPs can be determine by measurement of photosynthetic pigments content which indicator for NPs phytotoxisity (Miralles *et al.*, 2012; Zhao *et al.*, 2013).

Contents of Chl b and total chlorophyll measured in the Peppermint plant as shown in figures 4 and 5 is clear that the Chl b and total chlorophyll contents level were increased significantly at ZnONPs low concentrations 5 mg/L compared with control treatment. These results was agree with study of Movahhedi Dehnavi (2004) on safflower in which the spraying of zinc caused increased metabolism of nitrogen and chlorophyll production. Chl b contents and total chlorophyll contents decreased significantly in the higher concentrations of ZnONPs treatment was observed at 10 and 15 mg/L. These results are confirmed with the results obtained from other studies carried out by Karthick and Chitrakala (2001). The effect of various doses of zinc oxide nanoparticles on diverse treatments on photosynthetic pigment (chlorophyll a, chlorophyll b and total chlorophyll) of sesame plant, in low concentration of ZnO nanoparticles indicates chlorophyll a chlorophyll b and total chlorophyll content had been high level, whereas the plant samples treated with higher concentration of ZnONPs showed decreased level of chlorophyll (Narendhran *et al.*, 2016). Similarly, applications of ZnO NPs at a lower concentration increased the photosynthetic pigments in cluster bean (Raliya and Tarafdar, 2013).

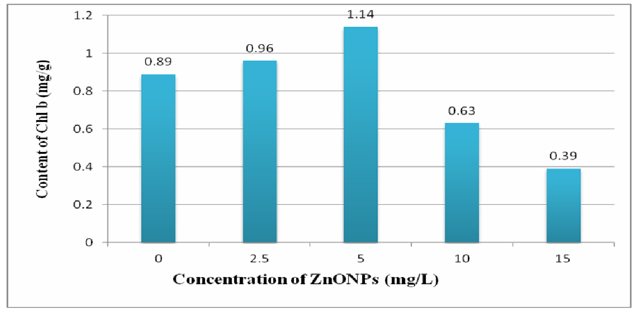

Fig. 4 : Effect of ZnO nanoparticles in Chl b content of Peppermint plant leaves. L.S.D (0.05) = 0.07

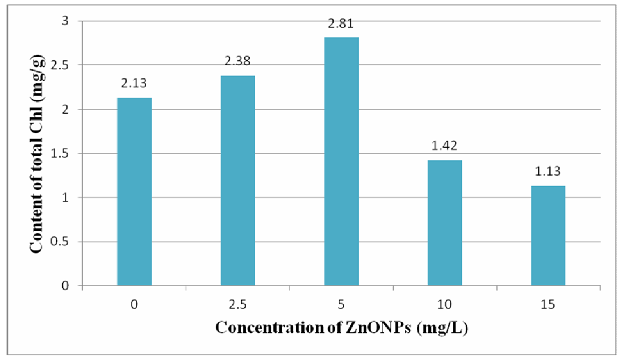

Fig. 5 : Effect of ZnONPs in total Chlorophyll content of Peppermint plant leaves. (0.05) L.S.D = 0.11

Plant cells containing soluble antioxidant compounds called Carotenoids, which play a mature role in scavenging of free radicals and prevent oxidative damage in plant. When plant exposure to environmental oxidative stress, the carotenoids which found in plast of plant tissues protect photosynthetic tissues, especially chlorophyll. When plant treated with ZnONPs, ZnONPs causes increase in synthesis of carotenoids because the zinc produces oxidative stress at certain concentration and stimulate carotenoids synthesis. The high ZnONPs concentration 10 and 15 mg/L produce significantly a highest total carotenoid in Peppermint compared with control as shown in figure 6 . Wang *et al* (2016) demonstrate that the higher concentration of ZnONPs causes decrease in chlorophylls contents and in turn decrease in photosynthesis and induce synthesis of carotenoid at high level in *Arabidopsis*. Mohsenzadeh and Moosavian (2017) also demonstrate that the higher concentration of ZnONPs causes increase total carotenoid in rosemary leaves.

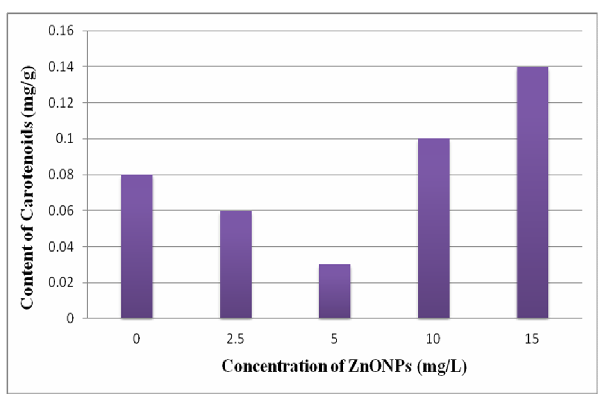

Fig. 6 : Effect of ZnO nanoparticles in Carotenoids content of Peppermint plant leaves. L.S.D (0.05) = 0.07

Conclusion

Biological method using Olive plant leaves extract showed ability to synthesis ZnO nanoparticles and acts as a reducing agent to reduce to nanosize particles. ZnONPs used antioxidant defense in Peppermint plants that caused an increase in CAT and SOD while ZnONPs reduced chlorophyll a, b and total chlorophyll but increased carotenoids in higher doses of ZnONPs-treated Peppermint plants.

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