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Effect ZnO NPs on enzyme activities in *Hordeum Vulgare L*

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Abstract---Objectives: The purpose of this study is to generate ZnO nanoparticles using environmentally friendly and low-cost materials. The production of oxide nanoparticles using plant extracts is a viable alternative to traditional and expensive chemical procedures. UV–Visible spectroscopy, transmission electron microscope (TEM), and scanning electron microscopy were used to characterize the biologically produced ZnO nanoparticles (SEM). This study examined the effects of four different ZnO nanoparticle concentrations (0, 50, 100, 150) and three different ways (foliar plant, seed soaking, and foliar + seed soaking) on enzyme activities in *Hordeum vulgare L* (Samir and Buhoth244). When foliar plants & seeds soaking treatment of the barely plant were treated with ZnO Nanoparticles, the results showed an increase in all critical indicators compared to the control treatment, which had the best effect on enzyme activities (CAT, SOD and GPX). Despite the fact that zinc is necessary for plant growth, this study discovered that its presence in nanoparticles had a significantly superior effect

Keywords--- ZnO nanoparticules, *Hordeum vulgare L*, CAT, catalase, SOD superoxide dismutase, GPX Glutathione peroxidase.

Introduction

Cereals are the primary source of nutrition for the majority of the world's population, as well as the foundation for numerous enterprises. It is no secret that cereals, particularly wheat and barley, are one of the most crucial crops in most countries throughout the world, so much so that it has become one of the most powerful and effective tools used by the powerful developed countries to put pressure on the weaker ones. The genus *Hordeum* (*Hordeum vulgare L*) belongs to the Poaceaceae family, the order Poales, and the genus *Hordeum*. Humans first

planted barley about 10,000 years ago, and they are still growing it now. Wild barley originated in the Middle East and several areas close to North Africa (Ellis, 2002). Nanotechnology is the technological revolution of the twenty-first century. The field of research and development in this area is rapidly growing all around the world (Vidya et al., 2013). This research has made significant contributions to the development of novel nanoscale materials (Sivakumar et al., 2011). These are usually particulate materials with at least one dimension of less than 100 nanometers (nm), while quantum dots could have zero dimension particles (Vidya et al., 2013). Because of their unique properties, such as catalytic, optical, magnetic, and electrical properties, metal nanoparticles have gotten a lot of interest (Singhal et al., 2011). Green nanotechnology is a way of creating nanomaterials that avoids or minimizes the use of harmful substances throughout the manufacturing process (Abdel-Azeem, et al., 2019). Green NPs can be made from bacteria, actinomycetes, fungus, cyanobacteria, macro algae, and plants, among other biological organisms (Salem et al., 2021). Green synthesis is preferred over chemical and physical techniques because of its eco-friendliness, cost-effectiveness, ease of handling, up scaling, and biocompatibility (Abdel-Azeem et al., 2019). Several NPs have recently been produced utilizing green technologies, including Ag, Au, Cu, CuO, SnO₂, Se, and others, and are employed in a variety of biological activities (Collenburg et al., 2017 and Salem *et al.* 2021).

Material and Methods

Synthesis of ZnO Nanoparticles

The precipitation method was used to make ZnO NPs, with some modifications, according to the protocol of (Hussain et al., 2017). Hibiscus subdariffa L was used as the main reducing and capping agent in the preparation of ZnO NPs. Boiling (6g) in 100 mL distilled water for 10 minutes yielded the aqueous extract. Dissolving 8.2g of zinc acetate dihydrate in 100 mL distilled water yielded a Zn(CH₃COO)₂ solution. After that, the solution was continuously boiled for 15 minutes at 50°C on a magnetic stirrer. Then, after heating to 80°C to eliminate all impurities so that laser 303 may be used, reduce step by step by adding 15 mL of plant extract and continuously boiling until the color of the solution turns light green. After that, the solution mixture was centrifuged at 3000 rpm for 10 minutes. After removing the supernatants, particles were put in deionized water and centrifuged for 10 minutes at 3000 rpm. This process was repeated four times. Centrifugation was used to separate undissolved Zn salt and plant extract, which was then washed away. The precipitate was then dried in a muffle furnace for two hours at 400 °C before being used to examine other properties of ZnO NPs. They were used to research other properties of ZnO NPs before being used to study other properties of ZnO NPs.

Preparation of ZnO NPs

A stock solution of ZnO NPs is made by dissolving 1 gram of ZnONPs in 1 liter of distilled water to a concentration of 1000 ppm. In addition, the examined concentrations (0, 50, 100, and 150) ppm were created using the titration law ($N_1 V_1 = N_2 V_2$), and quantities were deposited individually in a 1 liter flask for each treatment (Chemiasof, 2011). The experiment had three repetitions and was

totally randomized (CRD). (Khandan-mirkohi *et al.*,2017): The following nine treatments, including a control treatment, were given to each Barely variety

T1=(50+100 and150) ppm ZnONPs by seed soaking method

T2=(50+100 and150) ppm ZnONPs foliar plan method

T3=(50+100 and150) ppm ZnONPs seed soaking +foliar plant method

Plant Cultivation

The experiment was conducted out in a greenhouse at Al-Muthanna University's College of Science on November 25, 2021, for barely plant varieties using two types, Samir and Buhoth 244. Each plant pot obtained a number of twenty seeds. There are a total of 60 plant pots, three replicates for each concentration, and three different ZnO NP concentrations (0, 50, 100, and 150) ppm (seed soaking, foliar application and Seed soaking and foliar application). The experiments began on January 15, 2021, with 20 seeds planted in a pot after seeding had started to grow, and were then reduced to only 5 seeds. After two months, on January 23, 2022, the plant was sprayed with three different concentrations of ZnO NPs, and after two weeks, the plant was sprayed again. The plant was harvested on March 31, 2022, and then a practical experiment was conducted

Plant Activities Enzymes measuring

Fresh leaves were obtained from ten plants in each plot, which were chosen at random. The samples were frozen right away. The frozen leaves were then crushed to a fine powder with liquid nitrogen before being extracted under neutral pH conditions with an ice-cold 50 mM phosphate buffer (Meloni *et al.*,2003). CAT, SOD, and GPX activity were measured using the methods described by(Hadwan MH and Kadhum Ali ,2018), (, (Magnani L *et al.*, 2000), and (Ahmed AY *et al.*,2021) respectively.

Result and Discussion

Scanning Electron Microscope analysis (SEM)

SEM was used to examine the morphology of the green produced zinc oxide nanoparticles (Rajiv P, *et al.*,2013). The particles have a semispherical shape and are heavily agglomerated, as seen in Figure (1). This clearly demonstrates that the particles are present in a homogenous state, and that nanoparticle homogeneity is vital in their many functions. The particle sizes ranged from 31 to 58 nanometers.

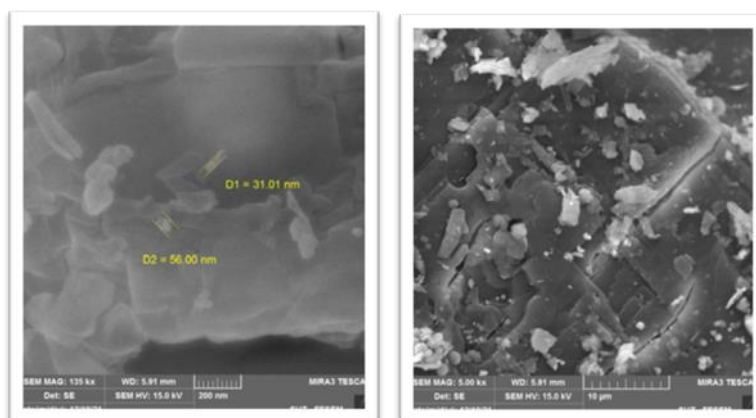


Fig. 1. SEM image of the prepared ZnO nanoparticle

Ultraviolet –Visible spectroscopy analysis (UV)

The biogenic production of ZnO NPs was confirmed by UV-visible spectroscopic investigation (Figure 2). The sample was dissolved in deionized water for this typical analysis. The UV-visible wavelength range was 200–900 nm, and the absorption spectrum of ZnO.NPs at 500 rpm is 211–314 nm in the UV spectrum of wavelength band, which is consistent with what was reported by (Gupta *et al.*, 2014). At 211 nm, the greatest absorption peaks were seen.

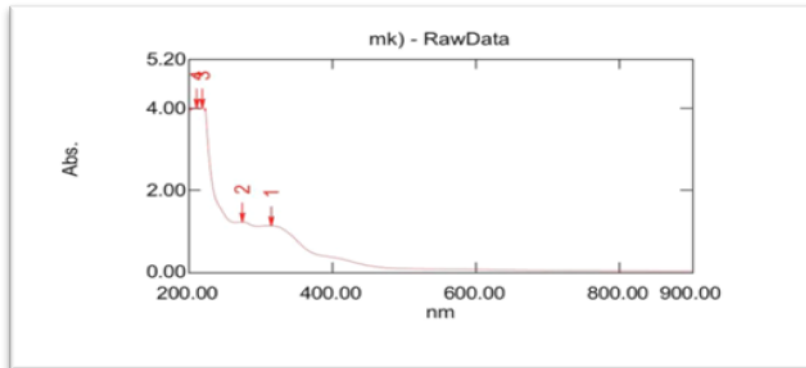


Fig. 2. Ultraviolet–Visible spectroscopy for ZnO nanoparticle

Transmission Electron Microscope analysis (TEM)

TEM micrographs were used to examine the morphology and particle size of pure zinc oxide nanoparticles, as illustrated in Figure (3). By comparing the particle size acquired through transmission electron microscopy with the particle size observed by spherical to hexagonal-shaped particles with a grain size of 35.5 nm, the development of ZnO-NPs was confirmed. As a result, the findings are consistent with earlier publications (Pillai A, et al.,2020).

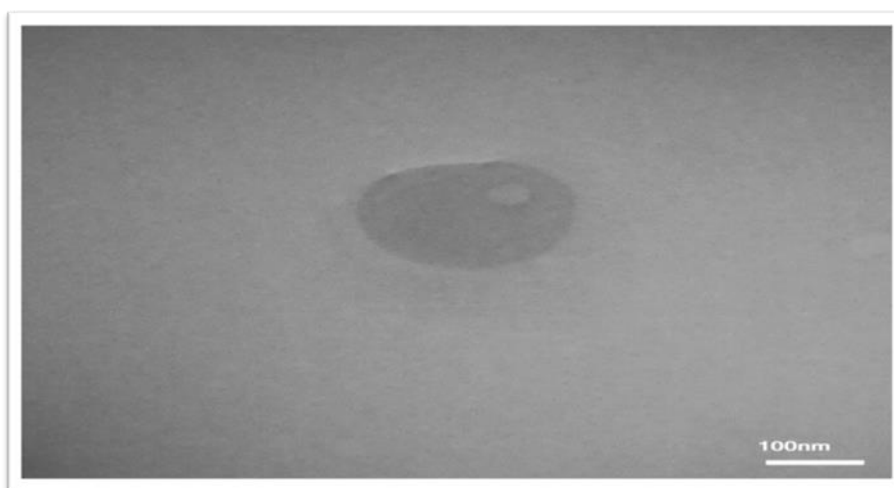


Figure 3. Transmission Electron Microscope obtained image for ZnO nanoparticles

The Effect of ZnO NPs on Catalase enzyme

The result in table (1) showed the effect of ZnO NPs on Catalase enzyme in Barely plant. The third treatment T3 (1.04) unit/ml of ZnO NPs at concentration 100 ppm is significant, followed by the second highest value for the same parameter (0.81) unit/ml at ZnO NPs concentration 150 ppm. The CAT is a well-known antioxidant enzyme that protects organisms by catalyzing the conversion of H₂O₂ to oxygen (O₂) and water, as well as free radical detoxification (Ma, et al., 2015). CAT reduces the amount of H₂O₂ produced by plant metabolic processes (Panda and Choudhury, 2005). The antioxidant impact of the catalase enzyme increases, showing that ZnONPs induces the manufacture of antioxidant enzymes as significant components of the defensive mechanisms against ZnONPs toxicity. The current study discovered that when the concentration of ZnONPs grew, so did catalase enzyme concentrations. These findings are consistent with Venkatachalam *et al.*, 2017).

Table 1
The Effect of ZnO NPs concentrations and treatments on Catalase (Unite/g f.Wt)for two varieties of *Hordeum Vulgare L*

Varieties	Treatment methods	Con. Ppm				Interference varieties × treatment	Effect of varieties	Effect of treatment
		0	50	100	150			
Samir	T ₁	0.29 s	0.49 nn	0.62 i	0.55 j	0.50 e		
	T ₂	0.41 p	0.50 ll	0.80 c	0.75 d	0.67 b		
	T ₃	0.45 o	0.50 ll	1.04 a	0.81 b	0.69 a		
Buhoth244	T ₁	0.19 t	0.49 nn	0.53 k	0.50 l	0.42 f		

	T ₂	0.31 r	0.49 n	0.71 g	0.67 h	0.51 d		
	T ₃	0.36 q	0.50 m	0.74 e	0.74 f	0.57 c		
Interference plant × Con.	<i>Samir</i>	0.47 g	0.50 e	0.69 a	0.64 b		0.57 a	
	<i>Buhoth244</i>	0.43 h	0.49 f	0.63 c	0.62 d		0.55 b	
Interference treatment × Con.	T ₁	0.35 j	0.50 hh	0.58 e	0.54 f			0.46 c
	T ₂	0.42 i	0.50 h	0.66 c	0.59 d			0.59 b
	T ₃	0.50 hh	0.51 g	0.79 a	0.78 b			0.63 a
Effect of Con.		0.50 d	0.53 c	0.66 a	0.55 b			

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test (P < 0.05).

Effect ZnO NPs on superoxide dismutase enzyme

Table 2
The Effect of ZnO NPs concentrations and treatments on superoxide dismutase Unite/g f . Wt) for two varieties of *Hordeum Vulgare L*

Varieties	Treatment methods	Con. Ppm				Interference varieties × treatment	Effect of varieties	Effect of treatment
		0	50	100	150			
<i>Samir</i>	T ₁	1.07 r	1.73 n	2.41 gg	2.41 gg	1.66 e		
	T ₂	1.26 pp	2.25 k	2.52 c	2.45 d	2.28 b		
	T ₃	1.26 p	2.36 j	2.72 a	2.61 b	2.41 a		
<i>Buhoth 244</i>	T ₁	0.97 s	1.33 o	2.40 h	2.38 i	1.48 f		
	T ₂	1.15 q	1.76 m	2.41 g	2.41 gg	1.97 d		
	T ₃	1.26 pp	2.01 l	2.44 e	2.43 f	2.20 c		
Interference plant × Con.	<i>Samir</i>	1.71 g	2.00 e	2.41 a	2.40 b		2.12 a	
	<i>Buhoth244</i>	1.26 h	1.77 f	2.28 c	2.16 d		1.88 b	
Interference treatment ×	T ₁	1.02 j	1.84 gg	1.84 g	1.74 h			1.57 c
	T ₂	1.24 i	2.19 f	2.46 c	2.43 d			2.13 b
	T ₃	1.84	2.41 e	2.53 a	2.48 b			2.30 a

Con.		gg						
Effect of Con.		1.74 d	1.84 c	2.34 a	2.08 b			

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

Table (2) showed the effect of ZnO NPs on the Superoxide dismutase in Barely plant. The third treatment (2.72) unit/ml of ZnO NPs at a concentration of 100 ppm is noteworthy is the highest, followed by the second highest value (2.61) unit/ml of the same parameter at a concentration of 150 ppm. As the concentration of nano-zinc oxide grew, so did the antioxidant activity of the SOD enzyme. These researchers discovered a considerable increase in SOD enzyme concentrations as ZnONP concentrations increased, which matched their predictions. The antioxidant defense mechanism that removes excess ROS in plants can be promoted to minimize nanomaterial-induced toxicity in plants. SOD, which is the first enzyme defense that catalyzes ROS, regulates superoxide anion radical concentration (Mittler et al., 2004). SOD converts superoxide anions (O_2^-) to less dangerous oxygen (O_2) and hydrogen peroxide (H_2O_2) (Ma et al., 2015). The current findings are consistent with those of (Kouhi et al., 2015) and (Kim et al., 2012), who found that increasing ZnO NP dosages improved SOD activity.

Effect ZnO NPs on Glutathione peroxidase enzyme

The effect of ZnO NPs on Glutathione peroxide in Barely plant is shown in Table (3). The highest result (20.71) unit/ml of ZnO NPs at a concentration of 100 ppm is significant, followed by the second highest value (20.31) unit/ml at a concentration of 150 ppm. As ZnONP concentrations grew, antioxidative enzyme activity in the seed and leaf increased. It's possible that seedling stress is causing this rise in anti-oxidative enzyme activity. Toxic free radicals and superoxide are produced as a result of metal stress, as are anti-oxidative stress enzymes. The removal efficiency of these enzymes is used to eliminate ROS and protect cells from injury (Gao, et al., 2013; Garcia-Sanchez, et al., 2015). GPX activity rose as ZnO NPS concentration increased, which is consistent with previous findings (Gowayed and Kadasa, 2016).

Table 3
The Effect of ZnO NPs concentrations and treatments on Glutathione peroxide
(Unite/g f . Wt) for two varieties of *Hordeum Vulgare L*

Varieties	Treatment methods	Con. Ppm				Interference varieties × treatment	Effect of varieties	Effect of treatment
		0	50	100	150			
Samir	T ₁	15.77 n	17.12 ll	17.33 kk	17.31 k	16.66 d		
	T ₂	16.00 mm	18.69 h	19.60 c	19.30 d	18.57 bb		
	T ₃	17.11 l	18.72 hh	20.71 a	20.31 b	19.29 a		
Buhoth244	T ₁	15.54 o	17.12 l	17.31 kk	17.31 kk	16.55 e		
	T ₂	16.00 m	18.02 j	18.84 ff	18.80 f	17.74 c		
	T ₃	16.00 mm	18.51 i	19.03 e	18.87 ff	18.53 b		
Interference plant × Con.	Samir	16.71 g	17.49 e	19.87 a	19.27 b		18.79 a	
	Buhoth244	16.00 h	17.31 f	18.74 c	17.75 d		16.98 b	
Interference treatment × Con.	T ₁	16.65 hh	17.13 g	18.06 e	17.32 f			17.56 c
	T ₂	16.65 hh	18.21 d	18.76 bb	18.35 c			17.60 b
	T ₃	16.65 h	18.26 d	19.87 a	19.78 b			18.51 a
Effect of Con.		16.65 d	17.99 c	18.81 a	18.11 b			

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test (P < 0.05).

Conclusion

ZnO NPs have a beneficial effect on barley plants by enhancing enzyme activation and so stimulating physiological and vegetative properties. The (seed soaking + foliar application) technique was shown to be the most successful in reaching the desired objectives. So, the best of the four concentrations examined, 100 ppm, had the most impact on the plant's vegetative, physiological, and productive qualities. Because zinc is a co-enzyme, the nanoscale composition of zinc particles has a significant impact on general vegetative or physiological features, particularly enzyme activity.

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