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ORIGINAL PAPER

The Effect of Zinc Oxide Nanoparticles and Salicylic Acid on Controlling Powdery Mildew of some Barley Genotypes in Egypt

Mikhail, S.Ph.[1*] ; Farroh, K.Y.[2] , and Alkolaly, A. M.[3]

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ABSTRACT

This study was carried out in two different agro- ecological zones, in terms of climatic conditions at Sakha and Giza Research Stations during season 2022/2023, on two different barley genotypes, Line 1 and Line 2, to evaluate the effectiveness of zinc oxide nanoparticle (ZnO-NPs) at three concentrations (100, 50, and 25 mg/L) and the organic acid, salicylic acid in managing powdery mildew caused by *Blumeria graminis* in barley. The systemic fungicide Raxil S was used as a reference treatment.The most effective treatment for reducing disease severity in the greenhouse was the fungicide, achieving a 92.50% for line2 and90.59% line 1 disease reduction, followed by the Zno100 ,50,25 mg/L concentrations and salicylic acid , Additionally, the highest chlorophyll levels were observed in the plants treated with the fungicide Raxil S, followed closely by ZnO100. Both treatments also led to increased levels of phenols and enzymes, further enhancing their protective effects against the disease. In field trials conducted, at Sakha and Giza Research Stations for the Line 2 genotype, the most effective treatments for achieving a good crop yield were the fungicide Raxil S, followed by the nano-zinc oxide compound at a concentrations of 100 .50 and 25ml/L and salicylic acid. The results revealed that the average disease severity percentages for the treatments, the fungicide Raxil S, Zno100 mg/L, and Zno50mg/L showed disease severities of 5, 5.4, and 5.9%, respectively, compared to control, which had a significantly higher severity of 21.8 %. Similar trends were observed for the Line 1 genotype.. Regarding genotype sensitivity, Line 1 demonstrated greater susceptibility to infection by powdery mildew compared to Line 2.

Keywords: Barley, Powdery mildew, Oxidative enzymes, Salicylic acid, ZnO NPs and Fungicides

[*]Correspondence: Sherin Ph. Mikhael
E-mail: sherymekael@yahoo.com

Sherin Ph. Mikhail
https://orcid.org/000-0001-6131-5960

1. Department of Barley Diseases Plant Pathology Research Institute, Agricultural Research Center, 9 Gamaa Street, Giza-12619, Egypt.

Khaled Y. Farroh

2. Nanotechnology and Advanced Materials Central Lab., Agricultural Research Center, Giza, Egypt.
-Regional center for Food and Feed, Agricultural Research Center, Giza, Egypt.

Asmaa M. Alkolaly

3. Department of Integrated Control Research, Plant Pathology Research Institute, Agricultural Research Centre, 9 Gamaa Street, Giza-12619, Egypt

INTRODUCTION

In Egypt, barley serves as the primary crop extensively grown in the newly reclaimed regions characterized by saline soils and limited freshwater availability, as well as the North Coastal Region. Barley is a key grain crop globally, serving as both an important source of animal and human nutrition **(Malcolmson *et al.*, 2005)[a]**. As the fourth most cultivated crop worldwide, barley is especially valuable because it can be grown in coastal areas relying on rainwater. Its significance lies in its high adaptability to climate changes, tolerance to salinity and drought, and its ability to thrive in modern reclaimed lands that are poor in soil. By using environmentally friendly materials, it is possible to enhance soil properties of the soil and boost yield while preserving a clean environment.

Powdery mildew, caused by the biotrophic fungus *Blumeria graminis* f. sp. *hordei* (**Tratwal and Bocianowski 2014**) and **Abdullaev *et al.*, 2021**), is one of the most damaging diseases in barley. In humid temperate regions, powdery mildew can lead to production losses of up to 30%, with an average loss of 5–10% (**Agostinetto *et al.*, 2014)**. Fungicide treatments are commonly used to manage barley powdery mildew; however, their effectiveness can diminish over time due to the development of resistant pathogenic strains. Additionally, fungicides can have harmful environmental and health impacts (**(Hafez and El-Baghdady, 2013)[a].**

The overarching concept is to employ the most efficient and environmentally benign techniques at specific stages of the cultivated plant's growth. One of the more straightforward and cost-effective methods to enhance the long-term sustainability of genetic resistance is the cultivation of contemporary varieties through various combinations and intricate hybrid groups, in accordance with the principles of evolutionary plant breeding. Salicylic acid, an organic compound, plays a key role in stimulating plant resistance against a wide range of plant diseases. It boosts the production of phenols, which are the plant's natural defense mechanisms against fungi, bacteria, and viruses. **Hashemi *et.al* (2019)**(**Guo *et.al.* 2020), and Soheili-Moghaddam *et.al* (2022)[b].**

Recently, there has been growing interest in studying the effects of nanoparticle compounds in agriculture, particularly as fertilizers and pesticides, due to their small size and the minimal quantities required compared to conventional compounds. In this research, nano-zinc oxide was tested at three different concentrations, and demonstrated its effectiveness in controlling a variety of mildew diseases in wheat, barley, vegetable, and fruit crops. **(Zhao *et al*., 2018andAn *et al*., 2022).**

A substance with a size ranging from a few nanometers to 500 nm is considered a nonmaterial **(Wang *et al*. 2022b)**. Nanomaterials typically have larger surfaces areas and are substantially smaller than micron-sized particles, which enhances their ability to control diseases (**Elmer and White 2018)**. Over the past decade, several nanomaterials have shown promise in improving Biological Soil Treatment (BST) management. For instance, **Paret and associates (2013)** demonstrated that light-activated titanium dioxide (TiO_2), either alone or in combination with zinc and silver, exhibited antibacterial activity. However, certain TiO_2 applications resulted in phytotoxicity, and its activation required light, which limits its commercialization potential. In another study, **Ocsoy *et al*. (2013**) developed Ag@dsDNA@GO composites, a silver nanomaterial, to reduce silver particle aggregation and enhance BST managemen.. Greenhouse trials by, **Strayer *et al.* (2016)** revealed that 100 mg/ml of Ag@dsDNA@GO significantly reduced bacterial spot of tomato severity without causing phytotoxicity. However, a higher dosage 500 mg/ml, resulted in phytotoxicity The large-scale production of silver nanoparticles is also challenging and costly, limiting their field use. To explore more affordable alternatives, **Strayer-Scherer *et al.* (2018).** evaluated core-shell, multivalent, and fixed quaternary ammonium [Quat] copper composite nanoparticles at 100 and 200 mg/ml to control bacterial spot of tomato. They found phytotoxicity when 1,000 mg/ml of these nanomaterials were applied in a greenhouse~~.~~ In addition to silver and copper, the antibacterial properties of magnesium nanoparticles have been evaluated. Magnesium is generally regarded as safe under Sections 201(s) and 409 of the Federal Food, Drug, and Cosmetic Act (**Anonymous**, 2022) and is not listed on the US Environmental Protection Agency's Toxic Release Inventory **(Anonymous2022**). Field studies have shown that magnesium oxide nanoparticles (nano-MgO) at 20 mg/m significantly reduced BST severity at 200 and 1,000 mg/ml without causing phytotoxicity or excessive elemental accumulation (**Liao *et al*. 2019a, b**). Subsequent research demonstrated that weekly or biweekly field applications of nano-MgO achieved similar disease control without notable elemental buildup in the soil **(Liao *et al*. 2021).**

The goal of this study is to investigate the impact of different concentrations of ZnO nanoparticles in comparison with systemic fungicide (Raxil S) and the environmentally friendly material salicylic acid, in order to reduce the reliance chemical fungicides for controlling barley powdery mildew. Additionally, the study aims to explore the relationship between the treatments which used and the activity levels of oxidation and reduction enzymes as well as phenol production.

MATERIALS AND METHODS

1. Barley genotypes

The barley genotypes, with their names and pedigrees detailed in Table (1), were generously provided by the Barley Research Department of the Field Crops Research Institute, Agricultural Research Centre, Egypt. These genotypes were utilized in both greenhouse and field experiments.

Table (1). Name and pedigree of (line 1 and line 2) barley genotypes used in the field and greenhouse experiments.

No.	Name	Pedigree
1	**Line 1**	117/3/Alanda/Hamra//Alanda-01
2	**Line 2**	Giza 117/6/Alanda//Lignee527/Arar/5/Ager//Api/CM67/3/ Cel/WI2269//Ore/4/ Hamra-01

2. Preparation of Zinc oxide nanoparticles (ZnO NPs)

Zinc oxide nanoparticles (ZnO NPs) were synthesized using the precipitation method as described by **Kumar *et al*. (2013).** Specifically, 7.1883 g of zinc sulfate heptahydrate (ZnSO4 7H2O, 99% purity, Sigma-Aldrich, USA) were dissolved in 50 ml of deionized water (Milli-Q, Millipore, USA) by using a magnetic stirrer. Sodium hydroxide (50 mL, 1 M, 98% purity, Sigma-Aldrich, USA) was then added dropwise to the solution. following the addition, stirring was maintained for an additional 30 min. The resulting precipitates were filtered and washed several times with deionized water then dried at 60°C for 24 hours and calcined at 500°C for two hours.

Characterization of zinc oxide nanoparticles (ZnO NPs)

Two different analyses were performed on the zinc oxide (ZnO) Nano powder to evaluate its properties: High-Resolution Transmission Electron Microscope (HR-TEM) and X-ray Diffraction (XRD) analyses. The morphology of synthesized ZnO nanoparticles was characterized by a High-Resolution Transmission Electron Microscope (HR-TEM) (Tecnai G2, FEI, Netherlands) operating at an accelerating voltage of 200 kV. To prepare the samples, a diluted ZnO NPs solution was ultrasonicated for 5 min to minimize the particle aggregation. Three drops on the sonicated solution were then placed on a carbon-coated copper grid using a micropipette and allowed to dry at room temperature. HR-TEM images of the ZnO nanoparticles on the grid were captured to evaluate their morphology. The chemical structure of the as-prepared ZnO nanoparticles was analyzed using the XRD technique. The X-ray diffraction patterns were recorded in scanning mode of an X-ray diffractometer (X 'pert PRO, PAN analytical, Netherlands) equipped with a Cu K radiation tube (= 1.54 A) and operated at 40 kV and 30 mA, the appropriate XRD pattern was captured. The standard ICCD library built into the PDF4 software was used to analyze the acquired diffraction pattern. All preparation and characterization processes were conducted at the Nanotechnology and Advanced Materials Central Lab (NAMCL), Agricultural Research Center, Egypt (Table 2).

Table (2) tested treatments:

Treatments	Active Ingredient	Rate
Raxil S (Experimental sample) Fungicide	20 g/L fluopyram (1.8% w/w) 100 g/L (8.9% w/w) prothioconazole and 60 g/L (5.4% w/w) tebuconazole	50 ml/ 100 L water
Zn ONPs	zinc sulphate heptahydrate (ZnSO4 · 7H2O	100 ,50 and 25 mg/l
Salicylic acid	Organic compound with the formula $HOC_6 H_4 COOH$.	1.1μmol

3. Greenhouse experiments:

3.1. Samples of barley powdery mildew isolates:

Samples of diseases and isolates of barley powdery mildew:

In accordance with the methods outlined by Xu et al. (2014), infected barley leaves were collected from the disease nurseries' spreading lines. A single-colony isolate of Blumeria graminis f. sp. hordei (Bgh) was generated and preserved on seedlings of the barley variety within 5 cm diameter test tubes filled with sterile soil.

3.2. Spore Production:

To propagate *Blumeria graminis* f. sp. *hordei* (Bgh) isolates, conidia from each isolate were applied to the barley seedlings of lines 1 and 2. These seedlings were grown in pots containing 400 g of sterile soil and incubated in a growth chamber at 20±2ºC under constant lighting. To prevent cross-contamination, five layers of cheesecloth were secured over the surface of a glass cylinder with diameter of 10 cm. Three to five days after the appearance of white mycelia, five-centimeter segments of the inoculated leaves were excised and placed upside-down on 1% agar plates The plates were then incubated at 18±2ºC with a 16/8-hour light/dark cycle. After five to seven days of incubation, the conidia were carefully collected onto sterile tissue paper in the laminar flow hood and transferred into 2.0 mL centrifuge tubes. The pathogenicity assay was performed using fresh conidia as described by (**Wang *et al.*, 2022 a).**

3.3. Typing in Virulence:

In the climate-controlled greenhouse of the Barley Diseases Research Department, barley grains from genotypes Line 1 and Line 2 were cultivated in 30 cm diameter clay pots for a duration of eight days. The predominant races of Blumeria graminis f. sp. hordei were artificially introduced into each pot at the 2-leaf stage by gently shaking the sporulating leaf segments while maintaining the plants in a greenhouse environment at a temperature of 20°C (Nair and Ellingboe, 1965). Twenty-four hours post-inoculation, the leaves were treated with the recommended concentrations of ZnO nanoparticles at 100, 50, and 25, along with salicylic acid and Raxil S as a chemical pesticide. For the control treatment, the plants were exclusively sprayed with distilled water. The experiment utilized three replicates for each treatment and was organized according to a completely randomized block design. Furthermore, appropriate cultural practices and irrigation methods were implemented. As per Jensen et al. (1992), the infection types (ITs) for each barley genotype, sourced from various Bgh isolates, were assessed on a scale ranging from 0 to 4, with resistance/susceptibility responses classified as follows: 0-2 indicating resistant (R) and 3-4 indicating susceptible (S). The incubation period (IP) was defined as the interval in days from inoculation to the appearance of the initial symptoms or signs of the disease, such as spots (Holliday, 2001).

4.Disease severity:

According to the method described by **Ahmed *et al.*,(2021),** disease intensity was assessed by randomly examining leaves from each treatment using a 0 – 4 scale, where 0=no disease; 1=1– 10% leaf area affected; 2=11 – 25% leaf area affected, 3=26 – 50% leaf area affected and 4≥50% leaf area affected. The percentage disease index was calculated by using the formula:

$$\mathrm{DSI} = \frac{\sum (n \times v)}{Z \times N} \times 100$$

Where:

D.S.I= Disease severity index, n = Number of leaves in each category, v = Numerical value of each category, Z= Numerical value of highest category and N = Total number of leaves in the sample.

$$\text{Reduction in disease severity \%} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

5. Field experiments (adult stage):

Field experiments were carried out at Giza Experimental Station and Sakha Station, Agricultural Research Centre (ARC), during 2022/2023 growing seasons to evaluate the efficacy of chemical fungicide treatment (Raxil S), ZnO nanoparticles, and salicylic acid against the natural infection with powdery mildew on the two susceptible Egyptian barley varieties. Using three replication plots using a randomized full block design, the seeds Line 1 and Line 2 were investigated. Each plot was 10.5 m^2 (3 × 3.5 m long) with 20 cm spacing between rows.. Customary cultural practices were implemented in accordance with the guidelines provided by the Ministry of Agriculture and Land Reclaimation. Each foliar spray treatment. including Raxil S, ZnO nanoparticles at various concentrations, and salicylic acid, was applied twice during the growing season at concentrations as the previously mentioned, the first time at the heading stage (70 days after planting), at the start of the infection, and the second time after 10 days.

6.Disease assessment:

In each trial, ten plants at the heading stage from each treatment were visually assessed for the percentage of leaf area covered by powdery mildew using a 0–10 scale as described by **Large, (2007)**. The disease scores were then converted for analysis according to **Hafez *et al.*, (2014**), using the following scale:-.0 = 0 %, 1 = 0–3 %, 2 = +3–6 %, 3 = +6–12 %, 4 = +12–25 %, 5 = +25–50 %, 6 = +50–75 %, 7 = +75–88 %, 8 = +88–94 %, 9 = +94–97 % and 10 = +97–100 %.

Disease severity index (DSI) was calculated using the following formula:

$$\mathrm{DSI} = \frac{\sum Ratings\ of\ each\ plant}{10 \times Number\ of\ plants\ rated} \times 100$$

7.Area under disease progress curve (AUDPC):

The (AUDPC) was calculated using a simple formula adopted by (**Pandy *et al.*, 1989)** as follow:

$$AUDPC = D\ [\ \tfrac{1}{2}(\ Y_1\ + Y_K\ ) + (\ Y_2 + Y_3 + \ldots\ldots\ldots + Y_{K-1})\ ]$$

Whereas,

D= days between two consecutive (time intervals)

$Y_1 + Y_K =$ sum of the first and last disease scores

$Y_2 + Y_3 + \ldots\ldots\ldots + Y_{K-1}$ = sum of all in between disease scores.

8.Yield components:

All harvested plants at the maturity stage during the growing season 2022/2023 at Giza Station and Sakha Station were recorded for biological yield (kg/plot). After harvesting, the grain yield (kg/plot) was determined based on the grains collected from the harvested plants or plots. The increase in yield component over the control was estimated using the equation adopted by **Ahmed (2013) and Hafez *et al.*, (2014**) as follow:

$$\text{Increase over control \%} = \frac{\text{Treatment} - \text{Control}}{\text{Control}} \times 100$$

9.Biochemical Analysis:

9.1. Photosynthetic Pigments:

The method described by **Lichtenthaler and Buschmann (2001**) was used to estimate the total amounts of carotenoid and chlorophyll a and b in fresh barely plant leaves. Fresh tissue was pulverized in a mortar and pestles using 80% acetone as the extraction solvent. The optical density (OD) of the solution was measured with spectrophotometer (Shimadzu UV-1700, Tokyo, Japan) at 470 nm for carotenoids and 662 and 645 nm for chlorophyll a and b, respectively. The photosynthetic pigment levels were given in milligrams per gram of fresh leaf tissue.

9.2. Total Phenol Content:

A known weight of fresh sample (1g) was extracted using 85% cold methanol (50 ml v/v) for three times at 90°C. The combined extracts were collected and made-up to a known volume with cold methanol. Then one ml of the extract was mixed with 0.5 ml Folins- Ciocalteure agent, shake, and allowed to stand for 3 min. Then 2 ml of saturated sodium carbonate (Na_2CO_3) were added to each tube followed by distilled water shaken and left for 60 min. The absorbance was determined at 750 nm using spectrophotometer (VEB Carl Zeiss) and expressed as mg tannic acid g^{-1} FW as described by **Gonzalez *et al.* (2003).**

9.3. Hydrogen peroxide (H_2O_2)

The concentration of hydrogen peroxide (H_2O_2) was determined using **Velikova *et al.* (2000**). To do so, 0.5 g of leaf tissue was homogenized with 3 mL of 1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged for 10 minutes at 10,000 rpm and 4°C. After centrifugation, 0.75 mL of 10 mM potassium phosphate buffer (pH 7.0) was mixed with 0.75 mL of the supernatant. The reaction was further treated with 1.5 mL of 1M potassium iodide (KI) solution. The H_2O_2 content was determined by measuring the absorbance of the supernatant at 390 nm and comparing it to a standard calibration curve. The concentration of $H_2O_2$was calculated based on a standard curve ranging from 100 to 1000 µmol mL^{-1}. The H_2O_2 concentration was then expressed as µmol g^{-1} dry weight (DW).

9.4.Superoxide anion radicals (O_2)

Superoxide anion radicals (O_2) were determined according to the method described by **Doke.N(1983**)

10.Statistical analysis:

The data were analyzed using the statistical software SAS. Initially, all multiple analyses were evaluated through an analysis of variance (ANOVA). The means were then compared using the least significant differences (LSD) AT P = 0.05, and Duncan's multiple range test (**Duncan, 1995**) was used to determine the results.

RESULTS

Characterization of zinc oxide nanoparticles (ZnO NPs)

The physicochemical properties of the synthesized ZnO NPs were evaluated by various techniques, presented in (Figure 1). High Resolution Transmission Electron Microscopy (HR-TEM–was employed to determine the exact particle size of ZnO NPs. The HR-TEM images, shown in Figure (1A), reveal that the nanoparticles are nearly spherical, with an average size of 13.8 nm. Figure (1B) displays the X-ray diffraction (XRD) patterns of ZnO NPs , confirming their. The diffraction peaks at 2θ = 31.77°, 34.42°, 36.25°, 56.59°, 62.85°, 67.94°, and 69.08° correspond to the (100), (002), (101), (110), (103), (112), and (201) of ZnONPs, respectively. These results indicate that the synthesized ZnONPs exhibit a hexagonal phase structure, consistent with the zincite mineral (JCPDS 04-004-2776).

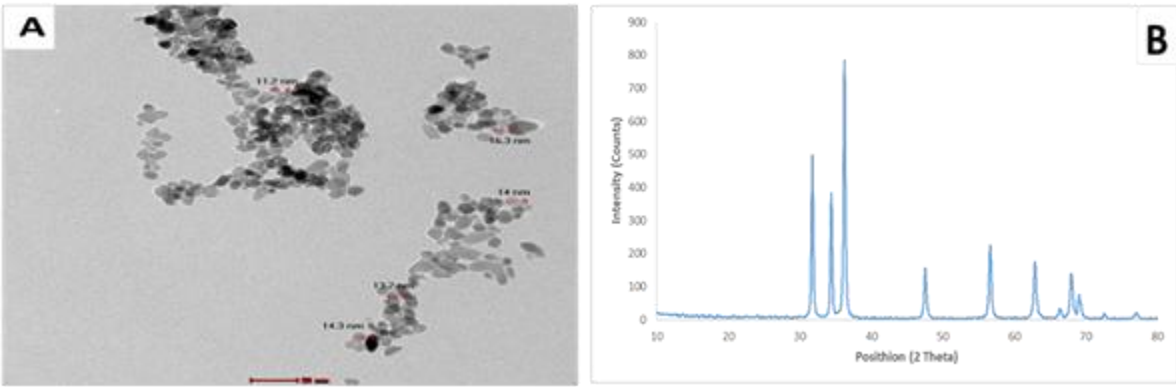

Fig 1. Characterization of ZnO NPs. **(A):** HR-TEM image showing nearly spherical shape of prepared ZnO NPs with average size 13.8 nm**. (B):** XRD pattern analysis indicating the formation of ZnO NPs.

Greenhouse studies:

Effect of zinc oxide nanoparticles (ZnO NPs), salicylic acid, and fungicide Raxil S on disease severity of barley powdery mildew under greenhouse conditions.

The results of greenhouse cultivation presented in Table (3) illustrate the effect of treatment with three concentrations of nano-zinc oxide, salicylic acid and the fungicide Raxil S, compared to the control. The results showed clear significant differences between the treatments and the control in disease severity. The most efficient treatment in reducing disease severity was the fungicide, which demonstrated an efficiency of 90.59% at line 1 and 92% at line 2 followed by a concentration of 100 of the nano Zinc oxide in reducing disease spread87.06% and

87.5%. followed by ZnO50 82.35% and 83.75% , ZnO25was 78.82% and81.25% and Salicylic acid was 76.36% and 77.50% relative to the untreated control plants .

Table (3) Effect of ZnO NPs concentration, salicylic acid, and Raxil S fungicide on disease severity% of powdery mildew in barley under greenhouse.

Treatments	Line 1		Line 2	
	Disease severity	**Reduction %**	**Disease severity**	**Reduction %**
Salicylic acid	20[b]	76.36	18[b]	77.50
ZnO100	11[d]	87.06	10[d]	87.5
ZnO50	15[c]	82.35	13[c]	83.75
ZnO25	18[bc]	78.82	15[bc]	81.25
Raxil S	8[e]	90.59	6[e]	92. 50
Control	85[a]	0.00	80[a]	0.00
L.S.D 0.05	2.78		2.25	

Results presented in Tables 4 and 5 show the estimation of chlorophyll A , B as well as the analysis of hydrogen peroxide (H_2O_2) , Superoxide anion radicals (O_2) and phenols in plants grown in two agro-ecological zones, Sakha and Giza localities . At Sakha, significant differences were observed among treatments. The highest percentage of chlorophyll A was recorded in plants treated with fungicide Raxil S and ZnO100 reaching 0.90 and 0.85 compared to control, which recorded 0.62. Similar trends were observed for chlorophyll B. and data presented inTable(4 and 5)clearly show that barley plants infected with *Blumeria graminis* accumulated higher significant amounts of reactive oxygen species namely hydrogen peroxide (H_2O_2) and singlet oxygen (O^-_2).

Regarding phenols, the highest levels were observed in plants treated with Raxil S (53.15), followed by ZnO100 (52.35) compared to the control, which recorded(40.55). Additionally, The H_2O_2 content was increased significantly in the aforementioned treatments.. Similar results were observed in Table (5) where the highest chlorophyll levels were recorded in plants treated with fungicide Raxil S, followed by ZnO100, as well as an increase in phenol, (O^-_2). and H_2O_2 content and in the treatment with zinc oxide concentration of 100,ZnO50, (ZnO25), and salicylic acid.

Field experiments:

Effect of zinc oxide nanoparticles (ZnO NPs), salicylic acid, and fungicide Raxil S on disease severity of barley powdery mildew

during 2022/2023 at Giza Station and Sakha Station. Data presented in Table (6) show the results of field experiment conducted at Sakha Research Station for Line 2 barley variety The most effective treatments that gave a good crop yield were the fungicide Raxil S, followed by the nano-zinc oxide compound at a concentration of 100 mg/L , 50 mg/L ,25 mg/L and salicylic acid. The respective yields for these treatments were: 7.4, 7 , 6.7,6.5 and 6.2 tons per fedden, respectively. The results also revealed significant differences in the average disease severity among treatments, The disease severity percentages for Raxil S, Zno100 mg/L and Zno50 mg/L,25 mg/L and salicylic acid treatments were 4.8, 5.2 and 5.4 ,6.5 and 6.59% .respectively, compared to control, which recorded a much higher severity of 20.6%. Similar trends were observed for the line 1 variety. Regarding the sensitivity of the varieties, the Line 1 was more susceptible to the disease compared to line 2. This was evident from the Area under disease progress curve (AUDPC) values, which were 467.8 for Line 1 and455 for Line 2 indicating that Line 2 was more resistant to the disease.

When studying the disease severity of the barley crop grown in Giza governorate on two varieties, the results in Table (7) indicated a higher sensitivity of the Line 1 variety to the disease compared to Line 2.

The average percentage of disease severity for them was in the order of 22.8% and 21.5%, respectively. Additionally, the area under the disease progress curve (AUDPC) values recorded for these varieties were 487.8 and 475, respectively. In the case of crop traits, the treatments showed significant differences compared to the control. The fungicide Raxil S recorded the best results, followed by nano-zinc oxide compound at a concentration of 100 mg/L , 50 mg/L ,25 mg/L and salicylic acid.

When making a comparison between data in Tables (6) and (7) in terms of the spread of the disease at a greater rate, Giza had a greater disease rate than Sakha; this is due to the availability of climatic conditions for the pathogenic fungus in Giza because Giza is hot spot for disease development.

Table (4): Effect of spraying different concentrations of nanoparticle zinc oxide, salicylic acid and Raxil S fungicide on biochemical trait chlorophyll. A, chlorophyll. B, phenol and H_2O_2 content on barley varieties (line1) under greenhouse conditions three days after the artificial infection

Treatments	chlorophyll. A		chlorophyll. B		Phenol		O_2		H_2O_2 Content	
	healthy	infected	healthy	infected	healthy	infected	Healthy	infected	healthy	infected
Salicylic acid	0.83^{b}	0.70^{a}	0.70^{b}	0.48^{a}	27.7^{a}	41.55^{b}	1.69^{a}	2.86^{b}	2.3^{b}	3.4^{b}
ZnO100	0.99^{c}	0.85^{c}	0.85^{c}	0.54^{c}	37.08^{b}	52.35^{b}	1.66^{a}	2.83^{c}	3.6^{c}	2.4^{b}
ZnO50	0.98^{c}	0.73^{c}	0.80^{c}	0.50^{d}	32.8^{b}	52.08^{c}	1.67^{b}	2.85^{b}	3.1^{b}	2.64^{b}
ZnO25	0.85	0.75^{b}	0.75^{a}	0.49^{b}	31.85^{a}	51.12^{a}	1.68^{a}	2.84^{c}	2.3^{c}	2.23^{c}
Raxil S	1.1^{b}	0.90^{a}	0.88^{b}	0.55^{a}	38.05^{a}	53.15^{a}	1.65^{a}	2.85^{a}	1.08^{a}	2.2^{c}
Control	0.80^{d}	0.62^{d}	0.57^{d}	0.44^{e}	24.124^{c}	40.55^{d}	2.96^{b}	4.33^{d}	4.9^{d}	8.21^{a}
l.s.d 0.05	0.031	0.017	0.006	0.0001	0.007	1.549	1.038	0.03	0.25	0.068

Table (5): Effect of spraying different concentrations of nanoparticle zinc oxide, salicylic acid and Raxil S fungicide on biochemical trait chlorophyll. A, chlorophyll. B, phenol content and H_2O_2 contents in the leaves of barley varieties(line2) under greenhouse conditions three days after artificial infection

Treatments	chlorophyll. A		chlorophyll. B		Phenol		O_2		H_2O_2 Content	
	healthy	infected	healthy	infected	healthy	infected	healthy	infected	healthy	infected
Salicylic acid	0.98^{b}	0.90^{b}	0.70^{b}	0.5736^{a}	33.63^{c}	50.34^{a}	1.68^{a}	2.87^{c}	3.52^{a}	4.67^{b}
ZnO100	0.83^{c}	0.75^{c}	0.60^{c}	0.4935^{b}	32.82^{d}	51.82^{c}	1.65^{c}	2.8^{e}	2.88^{c}	4.65^{c}
ZnO50	0.83^{c}	0.75^{c}	0.60^{c}	0.4983^{b}	27.92^{e}	51.62^{e}	1.64^{b}	2.83^{b}	2.82^{d}	5.66^{d}
ZnO25	1.1^{a}	0.91^{b}	0.75^{a}	0.5663^{a}	37.24^{a}	50.62^{c}	1.67^{d}	2.62^{d}	2.64^{e}	3.61^{f}
Raxil S	0.98^{b}	0.93^{a}	0.70^{b}	0.5736^{a}	36.62^{b}	52.82^{a}	1.64^{c}	2.86^{cd}	2.65^{e}	3.71^{e}
Control	0.80^{d}	0.62^{d}	0.57^{d}	0.4526^{b}	23.64^{f}	40.36^{d}	2.05^{a}	4.13^{a}	4.12^{b}	7.65^{a}
L.S.D 0.05	0.014	0.021	0.006	0.034	0.47	0.222	0.021	0.023	0.025	.004

Table (6): Effect of ZnO Nanoparticls, Salicylic acid, and Raxil S fungicide on disease severity (DS), AUDPC, and some selected yield components of Line 1 and Line 2 Egyptian barley cultivars under Sakha Station conditions in 2022/2023 season.

Treatments	Disease Severity%		AUDPC		Plant Height cm		No. Grains Spike		No. of Spikes		Grain Yield t. feddan-1	
	line 1	line 2	line 1	line 2	line 1	line 2	line 1	line 2	line 1	line 2	line 1	line 2
Salicylic acid	7.48^{b}	6.59^{b}	43^{b}	41^{b}	94^{ab}	98.2^{ab}	55.6^{ab}	57.2^{ab}	452.5^{a}	462.2^{a}	0.882^{c}	1.1^{b}
Raxil S	5^{c}	4.8^{b}	23^{c}	21^{c}	95.6^{a}	95.5^{c}	56.13^{b}	57.6^{a}	462^{a}	466^{a}	1.2^{a}	1.3^{a}
ZnO 100	5.4^{b}	5.2^{b}	25^{c}	23^{b}	91.3^{bc}	99.2^{a}	53^{b}	53.8^{c}	451^{a}	447^{a}	1.17^{ab}	1.2^{ab}
ZnO 50	5.9^{b}	5.4^{b}	25.5^{c}	23.5^{b}	91.3^{c}	97.3^{c}	52.3^{ab}	54.4^{bc}	456.5^{a}	447.3^{a}	1.13^{ab}	1.2^{ab}
ZnO 25	6.9^{bc}	6.5^{b}	26.2^{c}	28.3^{b}	95.3^{a}	99.2^{a}	57^{a}	57.2^{ab}	459^{a}	452.6^{a}	1^{b}	1.13^{ab}
Control	21.8^{a}	20.5^{a}	467.8^{a}	455^{a}	90.6^{c}	85.8^{d}	42^{c}	46.3^{d}	304^{b}	294.3^{b}	0.588^{d}	0.63^{c}
l.s.d 0.05	0.847	2.47	3.46	4.73	4.3	0.943	3.86	3	38.7	39.4	0.120	0.150

Area under disease progress curve (AUDPC)

Table (7): Effect of ZnO Nanoparticls, Salicylic acid, and Raxil S fungicide on disease severity (DS), AUDPC, and some selected yield components of Line 1 and Line 2 Egyptian barley cultivars under Giza Station conditions in 2022/2023 season.

Treatments	Disease Severity%		AUDPC		Plant Height cm		No. Grains Spikes		No. Of Spikes		Grain Yield t. Fadden -1	
	line 1	line 2	line 1	line 2	line 1	line 2	line 1	line 2	line 1	line 2	line 1	line 2
salicylic acid	8.48^{b}	7.59^{b}	44^{b}	43^{b}	93^{ab}	97.2^{ab}	54.6^{ab}	56.2^{ab}	450.5^{a}	460.2^{a}	0.84^{d}	1^{b}
Raxil S	5.3^{c}	5.1^{b}	24^{c}	22^{c}	94.6^{c}	94.5^{c}	55.13^{b}	56.6^{a}	460^{a}	464^{a}	1.17^{a}	1.2^{a}
ZnO 100	5.6^{b}	5.4^{b}	26^{c}	25^{b}	90.3^{a}	98.2^{a}	52^{b}	52.8^{c}	449^{a}	445^{a}	1.13^{ab}	1.17^{a}
ZnO 50	5.9^{b}	5.5^{b}	26.5^{c}	25.5^{b}	91.3^{c}	96.3^{c}	51.3^{ab}	53.4^{bc}	454.5^{a}	445.3^{a}	1.1^{b}	1.13^{ab}
ZnO 25	7.1^{bc}	6.6^{b}	27.2^{c}	25.2^{b}	94.3^{a}	98.2^{a}	56^{a}	56.2^{ab}	457^{a}	450.6^{a}	0.92^{c}	1.13^{ab}
Control	22.8^{a}	21.5^{a}	487.8^{a}	475^{a}	90.6^{d}	84.8^{d}	41^{c}	45.3^{d}	302^{b}	292.3^{b}	0.50^{e}	0.50^{c}
l.s.d 0.05	0.867	2.67	3.15	4.73	4.3	0.843	2.86	2.1	36.7	37.4	0.053	0.119

DISCUSSION

The primary goal of this study is to apply the methods that, at a given stage of the development of the farmed plant, are the most efficient and least detrimental to the environment. From this standpoint, alternative compounds to fungicides have been applied to reduce their remaining impact on plants and the environment.

, environmentally friendly materials can be used as an alternative to non-chemical control methods. This will increase soil fertility, keep the environment clean, and maintain sustainability development over time. (**Newton *et al.*, 2010 and Matyjaszczyk 2015).**

The integration of zinc oxide nanoparticles (ZnO-NPs) and salicylic acid in managing powdery mildew in barley represents a significant advancement in sustainable agricultural practices. The results of this study demonstrate the effectiveness of these treatments in reducing disease severity and enhancing plant health, corroborating findings by **Abdullaev *et al.* (2021),** who highlighted the increasing importance of sustainable disease management strategies in cereal crops. The efficacy of ZnO-NPs in controlling powdery mildew aligns with the literature suggesting that nanoparticles can enhance disease resistance through multiple mechanisms, such as improved nutrient uptake and enhanced plant defense responses (**An *et al*., 2022; Zhao *et al*., 2018)**. Specifically, our results indicate that the application of ZnO-NPs at 100 mg/L provided disease severity reductions comparable to the conventional fungicide Raxil S, achieving a 92% reduction in greenhouse trials. This reinforces the versatility of ZnO-NPs as a viable alternative or complement to traditional fungicides, especially in light of environmental concerns related to fungicide use **(Hafez and El-Baghdady, 2013a).** The observed increase in chlorophyll content and phenolic levels following the application of ZnO-NPs and salicylic acid further supports the notion that these treatments enhance plant physiological and biochemical responses. Salicylic acid has been well-documented to stimulate systemic acquired resistance (SAR) in plants, leading to an upregulation of defense-related metabolites **(Guo *et al*., 2020 b; Hashemi *et al*., 2019).** By enhancing the production of phenolic compounds, salicylic acid contributes to a plant's innate defense mechanism against pathogens, which aligns with previous findings by **Soheili-Moghaddam *et al*. (2022 b).** Furthermore, the differential response of the two barley genotypes examined in this study highlights the importance of genetic background in disease susceptibility. Line 1 exhibited greater susceptibility to powdery mildew compared to Line 2. This variation underscores the necessity of employing genetically resistant cultivars alongside innovative treatments like ZnO-NPs and salicylic acid. As observed by (**Malcolmson *et al*. 2005 b),** the genetic diversity within barley can be leveraged to develop improved cultivars with enhanced disease resistance, promoting both yield and sustainability. While the results are promising, there are considerations regarding the long-term implications of using nanoparticles in agriculture. Continuous application of ZnO-NPs may potentially lead to soil and water contamination, and therefore, further studies should investigate the long-term environmental impact and bioavailability of these nanoparticles in agricultural systems (**Zhao *et al*., 2018**). Moreover, the development of resistance in pathogens remains a significant challenge; thus, integrated pest management strategies that combine genetic resistance, biopesticides, and traditional fungicides could provide a more sustainable framework for managing powdery mildew in barley. In conclusion, the findings of this study provide a compelling argument for the integration of ZnO-NPs and salicylic acid in the management of powdery mildew in barley, offering an innovative approach that contributes to sustainable agricultural practices.

Author's contribution

Majority contribution for the whole article belongs to the author(s). The authors read and approved the final manuscript.

Competing interests

The author declares that he has no competing interests.

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