RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
56 (6) 2461-2470, 2024
http://doi.org/10.54910/sabrao2024.56.6.27
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

EFFECT OF ZNO-NPS ON *RHIZOCTONIA SOLANI* CAUSING ROOT AND STEM ROT ON BROAD BEAN (*VICIA FABA* L.)

R.K. MAHMOUD

College of Agriculture, Al-Qasim Green University, Babylon, Iraq
Corresponding author's email: ruisanno2010@gmail.com

SUMMARY

The presented study pursued determining the effect of biosynthesized nano-zinc oxide in managing root and stem rot on broad bean (*Vicia faba* L.) caused by the fungi *Rhizoctonia solani*. The two laboratory experiments transpired in 2022 at the Al-Qasim Green University, Iraq. The pathogenic fungal isolates cultivation on millet seeds ensued by infecting them with the *R. solani* vaccine in the laboratory. The effect of nano-zinc on *R. solani* growth suppression succeeded probing. The completely randomized design used had two factors. The first factor was bean cultivars, local and Spanish, while the second factor was the treatment of fungi *R. solani* with nano-zinc oxide six times. The results showed zinc nanoparticles considerably inhibited the fungus growth, which causes root and stem rot in broad beans. Since ZnONPs suppressed the fungal isolate growth (100%) compared to 0% in the control treatment, 20 µg/ml was optimal. In the pot experiment, the nano-zinc oxide foliar application had the lowest fungi infection (28.33%), compared with 81.67% in the control treatment. Nano-zinc oxide reduced infection severity to 6.92% versus 44.45% with fungal therapy alone. In treating the pathogenic fungus with nanoparticle-zinc oxide (ZnO) in the Spanish cultivar, polyphenol oxidase had the highest activity (0.94 unit ml^{-1}), while in the local cultivar and control, the therapy had 0.08 enzyme percentage.

Keywords: Broad bean (*Vicia faba* L.), ZnO-NPs, fungus, *R. solani***,** root and stem rot, fungus

Key findings: Zinc nanoparticles considerably suppressed the fungus development and boosted the growth of the broad bean (*Vicia faba* L.) plant.

Communicating Editor: Dr. A.N. Farhood

Manuscript received: October 17, 2023; Accepted: April 21, 2024.

Citation: Mahmoud RK (2024). Effect of ZNO-NPS on *Rhizoctonia solani* causing root and stem rot on broad bean (*Vicia faba* L.). *SABRAO J. Breed. Genet.* 56(6): 2461-2470. http://doi.org/10.54910/sabrao2024.56.6.27.

INTRODUCTION

Millions of people worldwide use legumes with their unique nutritional qualities, containing proteins, dietary fibers, carbohydrates, fatty acids, folic acid, vitamins, and minerals in their seeds (Mahmoud *et al.,* 2022). Economically, the leguminous crops are also very valuable. The widely grown annual winter crop is prominent with its nutritional values. The broad bean (*Vicia faba* L.) is distinct with its capacity to enhance soil properties via the atmospheric nitrogen fixation through root nodules collaborating with bacteria (*Rhizobium spp*.).

With its vital significance, the said crop also undergoes several stem and root rot infection, a critical fungal disease affecting the crop and causing substantial financial losses. The stem and root rot disease is one of the most critical and widespread diseases worldwide, including Iraq, caused by the fungus *Rhizoctonia solani*. The disease severely damages crops because it attacks plants at every growth stage, resulting in seed rot and seedling death before and after emergence (Nirupama *et al.,* 2017).

Consequently, various measures to stop the fungus growth employed fungicides, which are artificial chemical compounds that contain poisonous ingredients to restrain the fungal growth, reproduction, and spread in crop plants. However, a significant issue has arisen on the fungicide resistance that could occur due to frequent fungicide application. Consequently, due to the selection pressure that microorganisms experienced, resistance to several fungicides emerged, with environmental pollution also enhanced. Pesticides are also known to cause birth abnormalities, cancer in humans, and other disorders (Bustos-Obregón and Hartley, 2008**).**

Numerous studies have demonstrated the beneficial and safe use of nanomaterials in plant growth and development and germination. In various crops, including soybeans, vegetables, potatoes, tomatoes, onions, wheat, mustard, and spinach, nanomaterials like ZnO, TiO2, and FeO have improved crop quality causing higher crop growth and development (Shalaby *et al.,* 2016; Shojaei *et al.,* 2019). Laware and Raskar's (2014) findings demonstrated that modest levels of nano-zinc oxide enhance plant quality and facilitate the germination of onion seeds. Nanomaterials' capacity to absorb more nutrients and water helps boost the activity of root systems by raising their enzymatic activities, eventually improving plant development and fertilized soil quality (Dubey and Mailapalli, 2016).

The concept of green nanotechnology emerged, which became an environment-friendly, economical, and safe way to synthesize nanoparticles using biological resources, and as an alternative method by following modern technologies (Yedurkar *et al.,* 2016). In combating the phytophthora root and stem rot, the presented study planned to biosynthesize the nano-zinc oxide and investigate its effects on the growth and development of *Rhizoctonia solani* and broad beans (*V. faba* L.).

MATERIALS AND METHODS

Two experiments, comprising laboratory and potting, proceeded in 2022 at the College of Agriculture, Al-Qasim Green University, Iraq, to determine the effect of biosynthesized nano-zinc oxide on the growth and development of the fungus *Rhizoctonia solani* and broad beans (*Vicia faba* L.)**.**

Laboratory experiment

The *R. solani* isolate growing on millet seed (*Panicum miliaceum* L.) media progressed by inoculating its seeds with *R. solani* isolate vaccine individually. Five discs with a diameter of 0.5 cm and flasks placed in the incubator had a temperature of 25 °C ± 2 °C for 10 days, shaking the flasks regularly every 2–3 days to ensure distribution of the fungal inoculum and does not clump (Dewan, 1989). Then, using zinc oxide nanoparticles prepared from honey solution with the addition of zinc acetate obtained zinc nanoparticles. Their previous preparation occurred in the laboratory at the College of Agriculture, University of Kufa, Iraq. Afterward, preparing the planting media PDA followed. The 20 ml of the media placed in each Petri dish with a 9-cm diameter used three dishes for each nanomaterial test treatment. The addition of 5, 10, and 20 µg/ml of the nanomaterial to the dishes ensued before the media solidified, with stirring to ensure the nanomaterial distribution over the media. All dishes incurred inoculation with discs of the fungus, with a 5-mm diameter, from the mushroom colony grown on five-days- old PDA media. Three Petri dishes were samples for each treatment, in addition to the control treatment, which contained culture media only. The Petri dishes reached incubating at a temperature of 28 °C ± 2 °C and, after growth in the control treatment, reached the edge. The dish calculation on the percentage of fungal growth inhibition was according to the following equation (Abbott, 1925):

$$\text{Inhibition\%} = \frac{\text{Growth Rate (control)} - \text{Growth Rate (Treatment)}}{\text{Growth Rate (control)}} \times 100$$

Pot experiment

The conducted pot experiment used a CRD design that included two factors. The first factor comprised two bean cultivars, local and Spanish. The second factor consisted of six treatments of *R. solani* and nanoparticle-zinc oxide, which included healthy plants (control) (P1), plants infected with *R. solani* (P2), and plants infected with *R. solani* and treated with the chemical pesticide Swift (P3). Other treatments were plants infected with *R. solani* and treated with nano-zinc oxide (P4), healthy plants treated with nano-zinc oxide (P5), and healthy plants treated with the chemical pesticide Swift (P6).

The soil sterilization prepared for planting used a 37% commercial formalin solution, diluted to 5%. The soil received a tight polyethylene cover for seven days, and then performing aeration to evaporate the formalin. Afterward, its packing ensued in plastic pots with a capacity of 9 kg, then moistened and left for three days. Later, the superficially sterilized seeds of the two broad bean cultivars (local and Spanish) with a 1% sodium hypochlorite solution for 2–3 minutes proceeded planting in the pots at the rate of 10 seeds per pot. The pots received careful watering into two groups with three replications**.**

Plants infected (%)

After two weeks of treatment with *R. solani*, collected root samples underwent study for infection percentage at the flowering stage, with the estimation used the following formula:

$$\textbf{\textit{Plants infected}}\ \% = \frac{\textit{Number of plants infected}}{\textit{Number of total plants}} \times 100$$

Severity of injury

Broad bean root samples from two weeks after treatment with *R. solani* reached scrutiny for injury severity. The severity of injury estimated for all treatments followed the formula according to McKinny (1923). The degree of injury estimation depended on the appearance of the injury (Table 1).

$$\textit{Severity of injury\%} = \frac{\sum(\textit{Degree} \times \textit{Number of Plants})}{\textit{Total plants x highest pathological index}} * 100$$

Peroxidase enzyme

According to Hammerschmidt *et al.* (1982), estimating the peroxidase enzyme was by measuring the absorbance at a wavelength of 470 nm for the reaction mixture, consisting of 2.5 ml hydrogen peroxide solution H_2o_2 and 0.1 ml of enzymatic extract for each treatment. The enzymatic unit definition was a change in absorbance of 0.01 per minute.

Polyphenol oxidase enzyme

Approximating the polyphenol oxidase enzyme used the technique according to Mayer *et al*. (1965), measuring the absorbance at a wavelength of 470 nm for the reaction mixture, consisting of 2.5 ml of catechol solution and 0.1 ml of enzymatic extract for each treatment. The enzymatic unit occurred as the change in absorbance of 0.01 per minute.

Leaf chlorophyll content (SPAD)

The leaf chlorophyll content estimation after 50% of flowering employed the chlorophyll meter Spad-502 by randomly taking readings for three plants from each pot and then averaging**.**

Calculating the number of tillers per square meter was as an average of three plants in each pot. The calculation of dry weight of shoots comprised washing the shoot to remove the soil stuck to it and drying the plant parts in an electric oven at 60 °C until the weight was stable. Using a sensitive balance helped calculate the average dry weight of the shoots.

RESULTS AND DISCUSSION

Results showed nano-zinc oxide (ZnO) concentrations (5, 10, and 20 µg/ml) inhibited the pathogenic fungus' mycelial growth in broad bean genotypes (Figure 1). The concentration of 20 µg/ml had the most impact on the fungal isolate, and the rate of inhibition of the fungus *R. solani* reached 100%. Nanoparticles were evident in fungi cell wall destruction by creating holes in it causing the mycelium internal compounds' flow, which leads to its contraction. Nanoparticles may attack the fatty layers in the cell membrane, changing its structure (Shukla *et al.,* 2022). They also affect the germination of conidia and prevent their development. The nanoparticle compounds and their ions may cause genotoxicity due to the destruction of the fungus's genetic material (DNA). The effect of the nanoparticles may also be valid due to the release of Zn^{2+} ions that can damage the cell and its membrane and interact with cell contents, and these mechanisms lead to the death of fungal cells (Sirelkhatim *et al.,* 2015).

Injury percentage

The results revealed the effect of nano-zinc oxide (ZnO) on the infection rate (%) of two broad bean cultivars under the infection conditions with *R. solani* (Table 1). Apparently, the treatment of *R. solani* enhanced the disease infection severity in the two studied cultivars, reaching 90.00% and 73.33% for the local and Spanish cultivars, respectively. Compared with the healthy plant treated with ZnO, the healthy plant treated with the chemical pesticide, and the healthy plant only, they had 0% incidence rate. This may be due

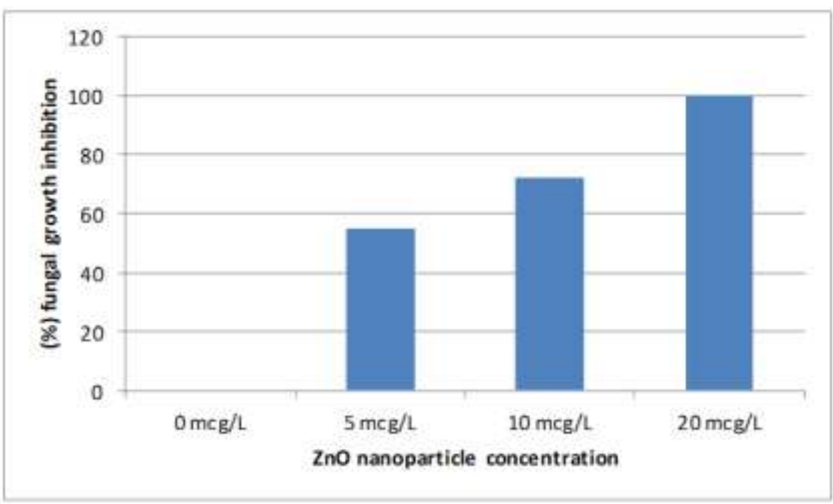

Figure 1. Inhibition of growth of *R. Solani* by using different range of concentrations of ZnO nanoparticle

Table 1. Effect of ZnO nanoparticles on the infection rate percentage of broad bean cultivars under conditions of *R. solani* infection.

Treatments	Cultivars		Means (%)
	Local	Spanish	
P1	0	0	0
P2	90.00	73.33	81.67
P3	33.33	30.01	31.67
P4	30.00	26.67	28.33
P5	0	0	0
P6	0	0	0
Means 9%)	25.56	21.67	

$LSD_{0.05}$ Treatments = 2.05, Cultivars = 3.56, Interactions = 5.03

Table 2. Effect of ZnO nanoparticles on severity of injury percentage for broad bean cultivars under conditions of *R. solani* infection.

Treatments	Cultivars		Means (%)
	Local	Spanish	
P1	0	0	0
P2	59.07	29.83	44.45
P3	21.67	11.50	16.58
P4	8.83	5.00	6.92
P5	0	0	0
P6	0	0	0
Means (%)	14.93	7.72	

$LSD_{0.05}$ Treatments = 1.63, Cultivars = 2.83, Interaction = 4.0

to the small size of the NPs, which facilitates their easy entry into the fungal cell membrane, allowing the inhibition of mechanisms that occur inside the cell (Lahuf *et al.,* 2020). The ZnO-NPs generate hydrogen peroxides that chemically interact with membrane proteins and lipid bilayers. Notably, ZnO-NPs can boost the activity of peroxidase enzymes and help remove active oxygen, effectively improving plant resistance (Wang *et al.,* 2018).

Severity of injury

The effect of nanoparticles-zinc oxide (ZnONPs) on the severity of infection (%) for two broad bean cultivars under the infection conditions of *R. solani* is available in Table 2. The increase in disease severity of treating *R. solani* and the two studied cultivars reached 59.07% and 29.83% for local and Spanish cultivars, respectively. Following the said disease severity ratios were the treatment of the chemical pesticide Swift and *R. solani,* which amounted to 21.67% and 11.50% for the local and Spanish cultivars, respectively, and 8.83% in the treatment of *R. solani* and ZnONPs. The healthy plants had an infection severity of zero, including the treatment with the ZnONPs, the chemical pesticide Swift, and the non-treatment of the healthy plant.

Peroxidase activity

The results showed zinc oxide nanoparticles (ZnONPs) affected the peroxidase activity of two broad bean cultivars under the infection conditions with *R. solani* (Table 3). The highest peroxidase enzyme activity resulted in the *R. solani* and ZnONPs treatment of the Spanish cultivar (1.19 unit ml^{-1}), followed by the healthy plant and ZnONPs in the Spanish cultivar (0.99 unit ml^{-1}) and the fungus *R. solani* treatment of the Spanish cultivar (0.98 unit ml^{-1}). Meanwhile, the lowest percentage of this enzyme appeared in the control treatment of the local broad bean cultivar (0.23 unit ml^{-1}).

Table 3. Effect of ZnO nanoparticles on peroxidase activity for broad bean cultivars under conditions of *R. solani* infection.

Treatments	Cultivars		Means (unit ml^{-1})
	Local	Spanish	
P1	0.23	0.44	0.34
P2	0.77	0.98	0.87
P3	0.56	0.76	0.66
P4	0.98	1.19	1.08
P5	0.78	0.99	0.89
P6	0.31	0.51	0.41
Means (unit ml^{-1})	0.61	0.81	
$LSD_{0.05}$ Treatments = 0.03, Cultivars = 0.05, Interactions = 0.07			

Table 4. Effect of ZnO nanoparticles on polyphenol oxidase enzyme activity in broad bean cultivars under conditions of *R. solani* infection.

Treatments	Cultivars		Means (unit ml^{-1})
	Local	Spanish	
P1	0.08	0.19	0.13
P2	0.63	0.74	0.68
P3	0.41	0.52	0.47
P4	0.82	0.94	0.88
P5	0.62	0.73	0.86
P6	0.15	0.26	0.20
Means (unit ml^{-1})	0.45	0.56	
$LSD_{0.05}$ Treatments = 0.01, Cultivars = 0.03, Interactions = 0.04			

Polyphenol oxidase enzyme effectiveness

The findings indicated the effect of nanoparticles-zinc oxide on the activity of the polyphenol oxidase enzyme for two broad bean cultivars under conditions of infection with *R. solani* (Table 4). The maximum activity of the polyphenol oxidase enzyme occurred in the treatment of *R. solani* and ZnONPs in the Spanish cultivar (0.94 unit ml^{-1}) and local cultivar (0.82 unit ml^{-1}). Following them were the fungus *R. solani's* treatment on the Spanish cultivar (0.74 unit ml^{-1}) and the treatment of the healthy plant and ZnONPs in the Spanish cultivar (0.73 unit ml^{-1}). However, the minimum percentage of this enzyme emerged in the control treatment of the local bean cultivar (0.08 unit ml^{-1}).

Noticeably from the results, the induction of peroxidase and polyphenol oxidase enzymes was highest in the treatment of *R. solani* and nanoparticle-zinc oxide because every pathogenic fungus is an induction agent in broad bean. Additionally, the high induction of peroxidase and polyphenol oxidase enzymes manifested in healthy plants treated with ZnONPs. The supreme effectiveness of these enzymes results from the inducing factor of ZnONPs. ZnO nanoparticles work to induce resistance in the plants, causing many resistance factors to appear in the plant's response to the infection, including pathogenicity-related proteins (PRP), the plant's natural response to the infection with plant diseases (Lahuf *et al.,* 2019).

The enzyme's high efficiency coupled with the higher level of resistance in broad bean plants, as the peroxidase enzyme works with hydrogen peroxide (H_2O_2) to break down the pathogen's enzymes. It included the pectinase enzyme (which is responsible for decomposing pectin in the cell walls of the crop plants), and thus, inhibits the penetration of the pathogen into the plant host. The peroxidases also interact with some cell wall proteins to form cross-links and multiple compounds, increasing the cell wall's rigidity (Hibar *et al.,* 2007). However, the healthy plants appeared to be free of induction factors; hence, these enzymes were low.

Leaf chlorophyll content

The data indicated that the two factors of the study significantly influenced the chlorophyll percentage in broad bean leaves (Table 5). The nanoparticle-zinc oxide showed to be most influential, recording the highest leaf chlorophyll content (55.17) in the local cultivar, followed by the chemical pesticide (47.90). Moreover, the fungi affected the leaf chlorophyll percentage in beans, and it was 34.40 in the presence of mushrooms. Concerning the Spanish cultivar, the significant increase in leaf chlorophyll content resulted from using ZnONPs. It may refer to the fact that zinc is considerably a catalyst for the oxidation process in plant cells. The importance of this process comes in regulating sugar consumption and increasing the energy needed to produce the chlorophyll pigment. It may also be because zinc contributes indirectly to the chlorophyll manufacturing process by directly modifying the formation of carbohydrates, energy compounds, and amino acids (Mogazy and Hanafy, 2022).

Zinc is also a catalyst for the carbonic anhydrase enzyme, which regulates the pH inside the chloroplast, and leads to protecting proteins from losing their nature and vitality, contributing to increased chlorophyll (Escudero-Almanza *et al.,* 2012). The superiority of the foliar application of the ZnONPs may be due to the nanoparticles fast entering plant leaves through the stomata, penetrating more easily and transferring into the transport vessels more efficiently (Wang *et al.*, 2013). It is worth noting that zinc is an essential element in plants. Increasing zinc levels in broad beans will enhance photosynthesis and its efficiency. Many scientists believed that high photosynthesis production is necessary to achieve high grain yields (Dou *et al.,* 2021**).**

Tillers per plant

By treating with nanoparticle-zinc oxide, the broad bean local cultivar provided with the topmost total tiller number (9.50 tillers m^{-2}), followed by the chemical pesticide (8.30 tiller m^{-2}) (Table 6). Furthermore, fungi affected the tiller number, with 5.83 tiller m^{-2} for the local cultivar and 5.30 tiller m^{-2} observed in the Spanish cultivar**.** The reason for the significant increase in the number of tillers with ZnONPs refers to the positive role zinc plays in forming auxins, which participates by interacting with gibberellin in dividing cambium cells and differentiating its cells into xylem and phloem tissues. Thus, vascular differentiation occurs in the lateral shoots connected to those in the main stem, positively increasing the tiller number (Roy *et al.,* 2022).

The increase in the total tiller number in the treatments sprayed with nanomaterials may be because these materials help speed up cell division and elongation. It also affects the vital processes, including enzymatic reactions, photosynthesis, and protein synthesis. It is a role similar to hormones that reduce the plant's need to make hormones in plant parts and transports them to places where they need to carry out their physiological functions. In addition to zinc's role in the metabolic processes inside the plants and in the formation and division of meristematic cells, the plant has the highest ability to enhance the number of tillers (Gurmani *et al.,* 2003).

Shoot dry weight

The superiority of broad bean plants emerged with nanoparticle-zinc oxide treatment by recording the shoot's ultimate dry weight (63.13 and 59.56 g $plant^{-1}$) for the local and Spanish cultivars, respectively (Table 7). This may be due to the stimulating effect of the induction agent, ZnONP, which was evident through a decrease in the severity of the pathogen infection. Based on vegetative growth and productivity indicators, it was notable that vegetative growth indicators, such as plant height and dry vegetative weight, were superior (Mogazy and Hanafy, 2022; Merhij *et al*., 2024; Sarhan *et al*., 2024). This could refer to the effect of ZnONPs in inhibiting the pathogen infection through the high values of these indicators in the healthy plants and ZnONP treatment compared with the lowest values in the *R. solani* treatment only. The

Table 5. Effect of ZnO nanoparticles and the chemical pesticide Swift on the broad bean cultivars under conditions of *R. solani* infection for leaf chlorophyll content.

Treatments	Cultivars		Means (SPAD)
	Local	Spanish	
P1	52.13	50.00	51.07
P2	35.67	34.40	35.03
P3	43.00	41.17	42.08
P4	41.93	42.63	42.28
P5	55.17	53.40	54.28
P6	47.90	47.37	47.63
Means (SPAD)	45.97	44.83	
$LSD_{0.05}$ Treatments = 0.44, Cultivars = 0.77, Interaction = 1.09			

Table 6. The effect of ZnO nanoparticles and the chemical pesticide Swift on the broad bean cultivars under conditions of *R. solani* infection for tillers per plant.

Treatments	Cultivars		Means (tillers $plant^{-1}$)
	Local	Spanish	
P1	5.20	4.90	5.05
P2	5.83	5.30	5.56
P3	7.50	6.93	7.21
P4	6.66	6.20	6.43
P5	9.50	8.86	9.18
P6	8.30	7.20	7.75
Means (tillers $plant^{-1}$)	7.16	6.56	
$LSD_{0.05}$ Treatments = 0.23, Cultivars = 0.40, Interactions = 0.57			

Table 7. Effect of ZnO nanoparticles and the chemical pesticide Swift on the broad bean cultivars under conditions of *R. solani* infection for dry weight.

Treatments	Cultivars		Means (g $plant^{-1}$)
	Local	Spanish	
P1	58.20	57.16	57.68
P2	28.40	26.80	27.68
P3	34.16	33.80	33.98
P4	47.13	44.40	45.76
P5	63.13	59.56	61.35
P6	57.16	56.23	56.70
Means (g $plant^{-1}$)	48.03	46.32	
$LSD_{0.05}$ Treatments = 0.25, Cultivars = 0.44, Interaction = 0.62			

fungi *R. solani* possesses digestive enzymes that break down plant tissue and mycotoxins that inhibit various plants' vital activities. Therefore, when inducing the resistance and suppressing the disease, plant growth parameters and vegetative growth improve by inhibiting the harmful effects on broad bean plants caused by the pathogen (Lahuf *et al.,* 2019)**.**

CONCLUSIONS

This study demonstrated that zinc nanoparticles could inhibit the growth of the fungus *R. solani*, which causes the root and stem rot in broad bean (*Vicia faba* L.) plants. The results also revealed the superiority of treatment with nanoparticle-zinc oxide (ZnONPs) in most of the vegetative growth characteristics. Nanoparticle zinc oxide showed 100% inhibition rate to the fungus. It was also evident that the higher induction of peroxidase and polyphenol oxidase enzymes appeared in the treatment of *R. solani* and ZnONPs.

REFERENCES

Abbott WS (1925). A method of computing the effectiveness of an insecticide. *J. Entomol*. 18: 265-267.

Bustos-Obregón E, Hartley R (2008). Ecotoxicology and testicular damage (Environmental chemical pollution). A review. *Int. J. Morphol.* 26(4): 833-840.

Cooper C (1977). The Tools of Biochemistry. John Wiley and Sons, Inc., USA.

Dewan MM (1989). Identify the frequency of occurrence of fungi in the roots of wheat and ryegrass and their effect on take-all – and host growth. Ph.D. Thesis. Univ. West Australia, pp. 210.

Dou Z, Li Y, Guo H, Chen L, Jiang J, Zhou Y, Zhang H (2021). Effects of mechanically transplanting methods and planting densities on yield and quality of Nanjing 2728 under rice-crayfish continuous production system. *Agronomy* 11(3): 479-488.

Dubey A, Mailapalli DR (2016). Nanofertilizers, nanopesticides, nanosensors of pest and nanotoxicity in agriculture. *Sustain. Agric. Rev.* 307-330.

Escudero-Almanza DJ, Ojeda-Barrios DL, Hernández-Rodríguez OA, Chávez ES, Ruíz-Anchondo T, Sida-Arreola JP (2012). Carbonic anhydrase and zinc in plant physiology. *Chilean J. Agric. Res.* 72(1): 131-140.

Gurmani AR, Khan MQ, Bakhsh A, Gurmani AH (2003). Effect of various micro elements (Zn, Cu, Fe, Mn) on the yield and yield components of paddy. *Sarhad J. Agric.* 4-11.

Hammerschmidt R, Nuckles EM, Kuc J (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiol. Plant Pathol*. 20(1): 73-82.

Hibar K, Edel-Herman V, Steinberg C, Gautheron N, Daami-Remadi M, Alabouvette C, El-Mahjoub M (2007). Genetic diversity of *Fusarium oxysporum* populations isolated from tomato plants in Tunisia. *J. Phytopathol.* 155(3): 136-142.

Lahuf AA, Abdullah KM, Mohammadali MT (2020). Assessment of the nanosized particles of ZnO and MgO and some cultivars in control of *Alternaria solani* causing tomato early blight. *Ecol. Environ. Conserv. J.* 26: 89-95.

Lahuf AA, Kareem AA, Al-Sweedi TM, Alfarttoosi HA (2019). Evaluation of the potential of indigenous biocontrol agent *Trichoderma harzianum* and its interactive effect with nanosized ZnO particles against the sunflower damping-off pathogen, *Rhizoctonia solani*. *In IOP Conf. Ser: Earth and Environ. Sci*. 365(1): 1-12.

Laware SL, Raskar S (2014). Influence of zinc oxide nanoparticles on growth, flowering and seed productivity in onion. *Int. J. Curr. Microbiol. Sci.* 3(7): 874-881.

Mahmoud R, Casadebaig P, Hilgert N, Alletto L, Freschet GT, De Mazancourt C, Gaudio N (2022). Species choice and N fertilization influence yield gains through complementarity and selection effects in cereal-legume intercrops. *Agro. Sustai. DeveloP. 42*(2): 12-27.

Mayer AM, Harel E, Shaul RB (1965). Assay of catechol oxidase acritical comparison of methods. *Phytochemistry* 5(4): 783-789.

McKinny HH (1923). Influence of soil temperature and moisture on infection of wheat seedling by Helminthosporium sativum. *J. Agric. Res*. 26, 195-217.

Merhij MY, Hanoon MB, Karbul MA, Atab HA (2024). Genotypic and phenotypic variations and genetic gain in faba bean with influence of nano-silicon. *SABRAO J. Breed. Genet.* 56(4): 1484-1491. http://doi.org/10.54910/sabrao2024.56.4.14.

Mogazy AM, Hanafy RS (2022). Foliar spray of biosynthesized zinc oxide nanoparticles alleviate salinity stress effect on *Vicia faba* plants. *J. Soil Sci. Plant Nut.* 22(2): 2647-2662.

Nirupama RK, Devi BS, Devi S (2017). Native trichoderma for the management of wire stem of mustard (*Brassica* spp.) caused by Rhizoctonia solani. *Int. J. Curr. Microbiol. Appl. Sci.* 6(9): 2319-2328.

Roy S, Das S, Saha KK, Rahman MR, Sarkar SK, Rashid MH, Paul SK (2022). Growth and seed yield of faba bean (*Vicia faba* L.) as influenced by zinc and boron micronutrients. *Fundamental and Appl. Agric*. 7(2): 139-149.

Sarhan IA, Yousif MD, Cheyed SH (2024). Growth and physiological properties of faba bean genotypes affected by zinc. *SABRAO J. Breed. Genet*. 56(2): 838-845. http://doi.org/10.54910/sabrao2024.56.2.34.

Shalaby TA, Bayoumi Y, Abdalla N, Taha H, Alshaal T, Shehata S, El-Ramady H (2016). Nanoparticles, soils, plants and sustainable agriculture. *Nanosci. Food Agric.* 1: 283-312.

Shojaei TR, Salleh MAM, Tabatabaei M, Mobli H, Aghbashlo M, Rashid SA, Tan T (2019). Applications of nanotechnology and carbon nanoparticles in agriculture. In *Synthesis Tech. Applications of Carbon Nanom.* 247-277.

Shukla S, Sharma M, Yadav S, Raghupathy A, Shukla K, Varma A, Mishra A (2022). Synthesis and applications of nanoparticles: State of the art and future perspective. *Nanoscience Nanotechnol. Asia*. 12(1): 2-15.

Sirelkhatim A, Mahmud S, Seeni A, Kaus NHM, Ann LC, Bakhori SKM, Mohamad D (2015). Review on zinc oxide nanoparticles: Antibacterial activity and toxicity mechanism. *Nano-Micro Letters* 7(3): 219-242.

Wang WN, Tarafdar JC, Biswas P (2013). Nanoparticle synthesis and delivery by an aerosol route for watermelon plant foliar uptake. *J. Nanom. Res.* 15(1): 1-13.

Wang XP, Li QQ, Pei ZM, Wang SC (2018). Effects of zinc oxide nanoparticles on the growth, photosynthetic traits, and antioxidative enzymes in tomato plants. *Biol. Plant.* 62(4): 801-808.

Yedurkar SMM (2016). Synthesis of nanoparticles by green chemistry process and their application in surface coatings: A review. *Arch. Appl. Sci. Res*. 8(5): 55-69.