Al-Muthanna J. For Agric Sci

Online ISSN:2572-5149
Print ISSN: 2226-4086

Vol. 12 , Issue 2. **2025**

https://muthjas.mu.edu.iq/

http://doi.org/10.52113/mjas04/12.2/3

Induction of Systemic Resistance in Shrub Rose (Rosa hybrida) Using Biosynthesized Zinc and Copper Nanoparticles Against the Powdery Mildew Pathogen (*Podosphaera pannosa*)

Istabraq adnan[1] , Mena waleed[2]

[1]Researcher Istabraq.adnan2304m@coagri.uobaghdad.edu.iq

[2]Assistant Professor mena.waleed@coagri.uobaghdad.edu.iq

Department of Plant Protection, College of Agricultural Engineering Sciences, University of Baghdad

Abstract

The experimentation was carried out in the greenhouse in the fields of the College of Agricultural Engineering Sciences / University of Baghdad / Department of Plant Protection through the winter and spring season of the year (2024-2025). This study aimed to induce systemic resistance in Rosa hybrida (rose plants) using biologically synthesized zinc oxide and copper oxide nanoparticles (prepared using curcumin) as a preventive treatment against the fungal pathogen *Podosphaera pannosa*, the causal agent of powdery mildew. The study investigated the effects of these materials on disease severity and biochemical responses through the estimation of PAL and peroxidase enzyme activity under greenhouse conditions. The results demonstrated the superior performance of biologically synthesized copper oxide nanoparticles (Cn) at a concentration of (300) mg/L, which achieved the lowest disease severity rate of (6.21)% and enhanced systemic resistance by increasing PAL and peroxidase enzyme activity. Similarly, biologically synthesized zinc oxide nanoparticles (Zn) at (300) mg/L and curcumin extract showed significant effectiveness in reducing disease severity to (14.46)% and (13.64)%, respectively, while enhancing enzymatic activities compared to other treatments.

Keywords: Powdery mildew, Shrub Rose Zinc Nanoparticles ,Copper Nanoparticles.

Introduction

Shrub roses are unrivaled among ornamental flowers, widely cultivated for their aesthetic appeal, charm, and fragrant scent. The primary global producers of shrub roses include the Netherlands, Colombia, Kenya, Israel, Italy, the United States, and Japan [7]. Powdery mildew, caused by the obligate biotrophic pathogen *Podosphaera pannosa*, is one of the most significant fungal diseases affecting roses in greenhouses [8], [11].

Numerous studies highlight the role of nanoparticles in managing plant diseases [3]. Notably, copper oxide and zinc oxide nanoparticles positively influence the growth and yield of leguminous plants under abiotic and biotic stress conditions. These nanoparticles enhance symbiotic interactions with bacteria, photosynthesis, nutrient uptake, and nitrogen fixation [12], [14].

Plants possess an intrinsic immune system comprising both constitutive and inducible defense mechanisms. As noted by [5],[6], these defenses can be augmented through exposure to biotic or abiotic elicitors, leading to a state of enhanced protection against pathogens and pests, a phenomenon termed induced resistance.

Nanotechnology necessitates alternative, non-toxic, clean, and environmentally friendly methods to replace conventional chemical approaches. Green synthesis methods for nanomaterials present a promising alternative to other preparation techniques, utilizing fungi, plant extracts, yeast, and bacteria [10].

In green synthesis, a plant extract is prepared by collecting desired plant parts such as fruits, branches, and leaves [1], [2].

A study by [17] demonstrated the efficiency of curcumin as a reducing agent and advanced its use as an eco-friendly reducing agent in the synthesis of metal nanoparticles.

Material and methods

Biological Synthesis of Zinc and Copper Oxide Nanoparticles Using Curcumin Extract:

The experiment was conducted in the Nanotechnology Laboratory / Department of Plant Biotechnology / College of Science / University of Baghdad during the academic year 2024-2025. Curcumin extract was (2)g of dissolved in (200)ml of deionized water. This solution was placed in an ultrasonic bath for two hours at a temperature of (70) °C to ensure dissolution and homogeneity of the mixture. The ultrasonic waves facilitated increased solubility of the extract in water and improved mixing.

The dissolved extract was then distributed into (10) ml tubes and placed in a centrifugal device at (400) rpm for (20) minutes to separate the precipitate from the supernatant. The precipitate was discarded, and the supernatant was collected. To each (200) ml portion of the supernatant, (20) g of copper chloride and zinc nitrate were added separately. A color change in the supernatant indicated the transformation into nanoparticles. The mixtures were then placed in flasks, sealed with cotton and Parafilm, and agitated in a shaker for (24) hours to enhance miscibility.

Subsequently, the mixtures were distributed into 10 ml tubes and placed in a centrifugal device for (20) minutes to precipitate the nanoparticles. The supernatant was discarded, and the precipitate, representing nano copper oxide and nano zinc oxide, was collected. The resulting quantities were pooled in dishes and placed in an oven for a full day. After removal from the oven, the precipitate was scraped off and transferred to tubes for washing with an ethanol solution. These tubes were then centrifuged for (20) minutes to remove residual copper and zinc. The supernatant was discarded, and the washed precipitate was placed in a dish in the incubator for (10) minutes to dry. Following drying, the scraping process was performed [1].

Verification of the nanoscale sizes was conducted using an AFM microscope at the Chemistry Department laboratories / College of Science / University of Baghdad.

Evaluation of the Effectiveness of Micro and Nano Zinc and Copper Oxides in Protecting Shrub Rose Plants from Powdery Mildew Infection Under Plastic House Conditions.

The experiment was conducted in the fields of the College of Agricultural Engineering Sciences / University of Baghdad / Al-Jadriya, specifically within the Plant Protection Department, during the winter season of the academic year 2024-2025. The land was prepared, leveled, cleared of weed residues, and sterilized with the fungicide (Beltanol) on October 15, 2024.

The plastic house was prepared one month after the sterilization period. Shrub rose plants (Baby Rose), approximately one and a half years old, growing in (22) cm diameter pots, were procured on November 16, 2024, from a nursery located in the Al-Basatin area, Baghdad governorate.

The plastic house included three rows for prophylactic treatment plants, (22) meters long. The distance between treatments was (2) meters. Each row comprised (22) experimental units, with three seedlings per replicate. A drip irrigation system was installed for watering the plants. Treatments were distributed randomly within each section, with continuous irrigation and regular weeding of the field, as illustrated in Figure (1), which shows the arrangement of treatments in the field.

Foliar application of the treatments, detailed in Table (1), was performed once to the point of run-off. Two days later, on January 14, 2025, artificial inoculation of the plants with the pathogen was carried out using a spore suspension. The inoculum was prepared by collecting infected parts (flowers, leaves, stems) from rose plants. Conidial spores, the reproductive parts, were then collected using a clean, sterile brush with sterile water, which served as the primary spore solution for preparing the fungal suspension. The suspension was adjusted

to a concentration of $1x10^6$ spores/ml, which was counted using a Hemocytometer.

Inoculation of the shrub rose plants with the fungal suspension was performed to evaluate the efficacy of the materials used in disease prevention.

Figure (1) illustrates the arrangement of treatments in the field.

Disease severity was calculated weekly according to the formula by [15].

$$\text{Disease Severity} = \frac{\text{Total number of infected leaves per category} \times \text{category code}}{\text{Total Number of Leaves} \times \text{Highest Score}}$$

No.	Treatment Code	Treatment Name	Concentration (mg/L)
1	T1	Control (Plant only)	0
2	T2	Control (Pathogen only)	0
3	T3	Chemical Fungicide (Vitra) + Pathogen	3 gm/L
4	T4	Curcumin Extract	10 gm/L
5	C1	Micro Copper Oxide	100
6	C2	Micro Copper Oxide	200
7	C3	Micro Copper Oxide	300
8	CN1	Commercial Nano Copper Oxide	100
9	CN2	Commercial Nano Copper Oxide	200
10	CN3	Commercial Nano Copper Oxide	300
11	Cn1	Biosynthesized Nano Copper Oxide	100
12	Cn2	Biosynthesized Nano Copper Oxide	200
13	Cn3	Biosynthesized Nano Copper Oxide	300

14	**Z1**	**Micro Zinc Oxide**	100
15	**Z2**	**Micro Zinc Oxide**	200
16	**Z3**	**Micro Zinc Oxide**	300
17	**ZN1**	**Commercial Nano Zinc Oxide**	100
18	**ZN2**	**Commercial Nano Zinc Oxide**	200
19	**ZN3**	**Commercial Nano Zinc Oxide**	300
20	**Zn1**	**Biosynthesized Nano Zinc Oxide**	100
21	**Zn2**	**Biosynthesized Nano Zinc Oxide**	200
22	**Zn3**	**Biosynthesized Nano Zinc Oxide**	300

Table (1) shows the treatments used in laboratory and field experiments and their concentrations.

Effect of Spraying on PAL Enzyme Activity for Prophylactic Treatments:

PAL enzyme activity was measured at the laboratories of the Ministry of Agriculture / Department of Plant Protection / Abu Ghraib, at 7, 14, and 21 days after treatment with the substances listed in Table (1). Absorbance readings were recorded using a spectrophotometer at a wavelength of (290) nm. Enzyme activity was quantified based on micrograms of cinnamic acid per hour per gram of fresh weight [16], according to the following equation:

Y=0.070X-0.049

In this context, if:

Y =represents the absorbance value

X=represents the cinnamic acid concentration in the sample

Effect of Spraying on Peroxidase Enzyme Activity for Prophylactic Treatments:

Peroxidase enzyme activity was measured at the laboratories of the Ministry of Agriculture / Department of Plant Protection / Abu Ghraib at 7, 14, and 21 days after treatment with the substances listed in Table (1). Light absorbance was directly measured using a spectrophotometer at a wavelength of (420) nm every (30) seconds (six readings were taken). The rate of change in absorbance was then calculated [19], according to the following equation:

$$\text{Rate of Change in Absorbance} = \frac{\overline{\Delta t}}{\text{Gram fresh weight}}$$

ΔA = Change in absorbance

Δt = Change in time/minute

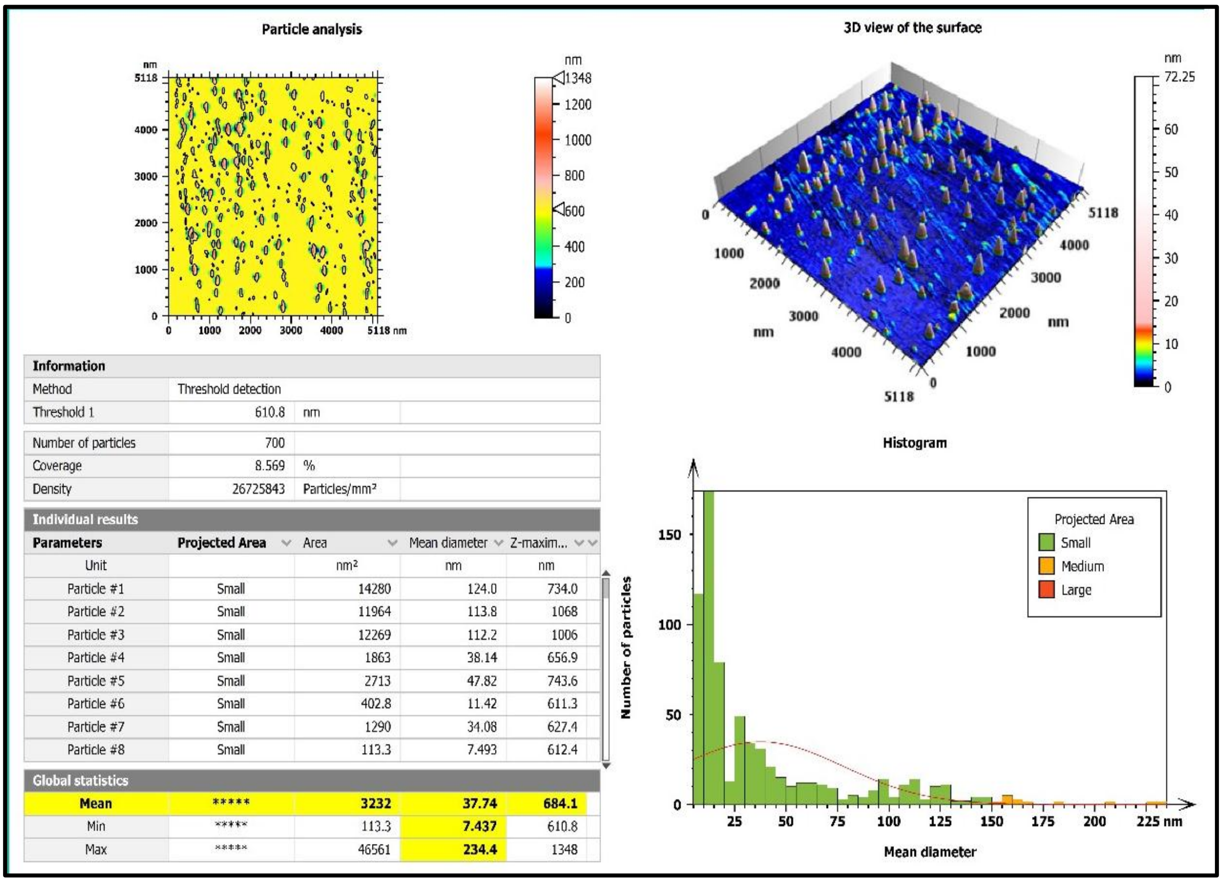
Results and Discussion

Evaluation of the Effectiveness of Micro and Nano Zinc and Copper Oxides in Protecting Shrub Rose Plants from Powdery Mildew Infection Under Plastic House Conditions.

Effect of Spraying on Disease Severity for Prophylactic Treatments

Atomic Force Microscope (AFM) analysis confirmed the nanoscale sizes of the synthesized materials. The biosynthesized copper oxide nanoparticles had an average size of (37.74) nm, as shown in Figure (2). The biosynthesized zinc oxide nanoparticles measured (82.81) nm, as presented in Figure (3).

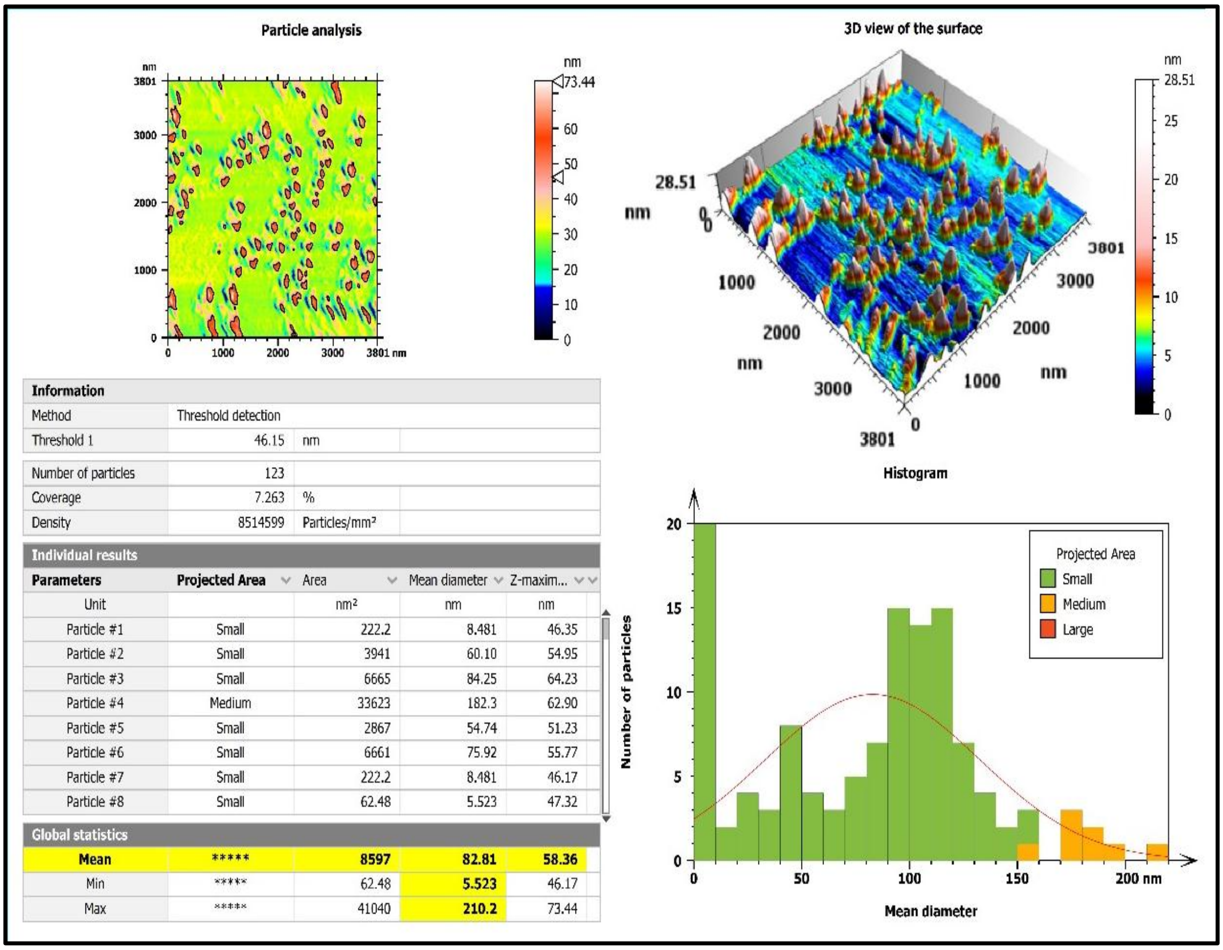
Table (2) highlights the effectiveness of both micro and nano copper and zinc oxides in reducing *Podosphaera pannosa* infection severity over a five-week treatment period. Biosynthesized nano copper oxide (Cn) demonstrated the highest initial reduction in disease severity during the first week, reaching (2.21)%. This reduction intensified in subsequent weeks, ranging between (4.41)% and (6.21)% in weeks two and three, and further improved to (7.53)% and (10.67)% in weeks four and five.



Information		
Method	Threshold detection	
Threshold 1	610.8	nm
Number of particles	700	
Coverage	8.569	%
Density	26725843	Particles/mm²

Parameters	Projected Area	Area	Mean diameter	Z-maxim...
Unit		nm²	nm	nm
Particle #1	Small	14280	124.0	734.0
Particle #2	Small	11964	113.8	1068
Particle #3	Small	12269	112.2	1006
Particle #4	Small	1863	38.14	656.9
Particle #5	Small	2713	47.82	743.6
Particle #6	Small	402.8	11.42	611.3
Particle #7	Small	1290	34.08	627.4
Particle #8	Small	113.3	7.493	612.4
Global statistics				
Mean	*****	3232	37.74	684.1
Min	*****	113.3	7.437	610.8
Max	*****	46561	234.4	1348

Figure (2) illustrates the AFM analysis result of biosynthesized nano copper oxide.



Information		
Method	Threshold detection	
Threshold 1	46.15	nm
Number of particles	123	
Coverage	7.263	%
Density	8514599	Particles/mm²

Parameters	Projected Area	Area	Mean diameter	Z-maxim...
Unit		nm²	nm	nm
Particle #1	Small	222.2	8.481	46.35
Particle #2	Small	3941	60.10	54.95
Particle #3	Small	6665	84.25	64.23
Particle #4	Medium	33623	182.3	62.90
Particle #5	Small	2867	54.74	51.23
Particle #6	Small	6661	75.92	55.77
Particle #7	Small	222.2	8.481	46.17
Particle #8	Small	62.48	5.523	47.32
Global statistics				
Mean	*****	8597	82.81	58.36
Min	*****	62.48	5.523	46.17
Max	*****	41040	210.2	73.44

Figure (3) illustrates the AFM analysis result of biosynthesized nano zinc oxide.

Statistical analysis Table (2) of the average disease severity across all treatments showed that biosynthesized nano copper oxide (Cn) was highly effective in controlling powdery mildew. The (300) mg/L concentration of Cn significantly outperformed the (200) mg/L and (100) mg/L concentrations, achieving an average disease severity of (6.21)%. Following this, biosynthesized nano zinc oxide (Zn) also proved highly effective, with its (300) mg/L concentration leading to an average disease severity of (14.46)%, surpassing the lower concentrations.

Furthermore, micro copper oxide (C) treatments exhibited superior disease control compared to commercial nano copper oxide (CN), micro zinc oxide (Z), and commercial nano zinc oxide (ZN). The (300) mg/L concentration of micro copper oxide reduced disease severity to an average of (17.49)%, while (200) mg/L and (100) mg/L concentrations resulted in average severities of (19.87)% and (21.93)%, respectively.

Treatments involving curcumin extract (10) gm/L and the chemical fungicide (3) gm/L also provided notable disease suppression, with average disease severities of (13.64)% and (23.61)%, respectively. These results are significantly better than the control treatments (plant only and pathogen only), which showed much higher average disease severities of (36.15)% and (71.46)%, respectively. A statistically significant difference was observed among all treatments.

Table (2) shows the Effect of spraying on Disease Severity for Prophylactic Treatments.

Treatment	Concentration (mg/L)	Week 1 (%)	Week 2 (%)	Week 3 (%)	Week 4 (%)	Week 5 (%)	Average (%)
Micro Copper Oxide (C)	100	10.67	19.53	22.62	27.06	29.78	21.93
	200	9.73	17.72	20.41	23.51	27.97	19.87
	300	9.77	15.06	18.21	22.62	21.77	17.49
Micro Zinc Oxide (Z)	100	13.31	17.76	22.21	28.87	30.21	22.47
	200	10.67	15.52	19.97	24.41	29.31	19.98
	300	12.41	14.62	19.51	23.92	26.21	19.33
Commercial Nano Copper Oxide (CN)	100	10.17	15.06	20.87	31.07	39.52	23.34
	200	11.97	15.53	19.52	27.52	39.52	22.81
	300	12.41	14.62	18.17	26.18	38.16	21.91
Biosynthesized Nano Copper Oxide (Cn)	100	4.87	9.77	11.51	14.61	21.72	12.5
	200	6.63	8.82	10.62	13.32	17.72	11.42
	300	2.21	4.41	6.21	7.53	10.67	6.21
Commercial Nano Zinc Oxide (ZN)	100	18.67	23.11	25.31	29.31	43.06	27.89
	200	14.61	19.52	22.21	28.88	39.62	24.97
	300	11.11	15.92	20.41	25.76	36.21	21.88
Biosynthesized Nano Zinc Oxide (Zn)	100	8.41	15.52	17.72	20.87	25.73	17.65
	200	7.97	11.52	15.06	19.11	23.52	15.44
	300	9.31	11.52	14.21	16.41	20.87	14.46
Control (Plant only)		21.31	27.97	34.62	44.87	51.97	36.15
Chemical Fungicide (3 gm/L)		14.21	20.41	25.31	26.18	31.92	23.61
Pathogen (Control)		50.48	62.11	72.42	80.12	92.18	71.46
Curcumin Extract (10 gm/L)		7.97	11.52	13.27	14.62	20.82	13.64
LSD 5%		0.016	0.016	0.016	0.016	0.016	0.007

Effect of Spraying on PAL Enzyme Activity for Prophylactic Treatments

The results presented in Figure (4) demonstrate the efficacy of the applied treatments and their significant impact on PAL enzyme activity.

Biosynthesized nano copper oxide (Cn) exhibited superior performance in increasing PAL enzyme activity. The (300) mg/L concentration of Cn significantly outperformed the (200) mg/L and (100) mg/L concentrations, yielding an average activity of (501.53) mg cinnamic acid/minute/gm fresh weight.

This was followed by biosynthesized nano zinc oxide (Zn) treatment, where the (300) mg/L concentration showed high enzyme activity compared to the (200) mg/L and (100) mg/L concentrations, with an average of (481.59) mg cinnamic acid/minute/gm fresh weight.

Commercial nano copper oxide (CN) treatments also surpassed commercial nano zinc oxide (ZN), micro copper oxide (C), and micro zinc oxide (Z) treatments in enhancing enzyme activity. For CN, the (300) mg/L concentration resulted in an average enzyme activity of (476.70) mg cinnamic acid/minute/gm fresh weight, while the (200) mg/L concentration showed (474.06) mg cinnamic acid/minute/gm fresh weight, and the (100) mg/L concentration yielded (471.15) mg cinnamic acid/minute/gm fresh weight.

These results are notably higher compared to the control plant and pathogen-only treatments, which recorded average PAL enzyme activities of (386.72) and (365.97) mg cinnamic acid/minute/gm fresh weight, respectively. A significant difference was observed among the treatments.

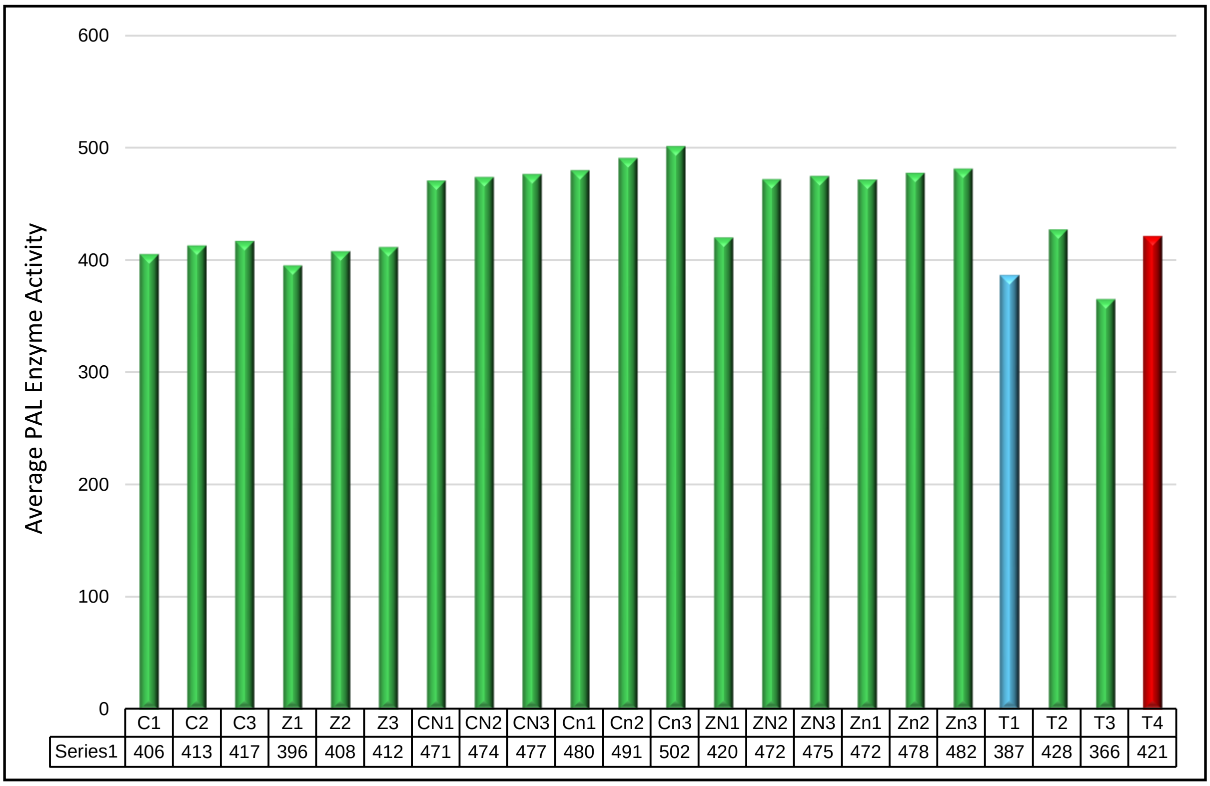


Figure (4) shows the Average PAL Enzyme Activity.

Effect of Spraying on Peroxidase Enzyme Activity for Prophylactic Treatments

Figure (5) illustrates the efficacy and significant impact of the applied treatments on peroxidase enzyme activity.

Biosynthesized nano copper oxide (Cn) demonstrated superior performance in increasing peroxidase enzyme activity across all treatments. The (300) mg/L concentration of Cn significantly outperformed both (200) mg/L and (100) mg/L concentrations, yielding an average activity of (16.31) (change in absorbance/minute/gm fresh weight).

This was followed by biosynthesized nano zinc oxide (Zn) treatment, where the (300) mg/L concentration also showed higher enzyme activity compared to the (200) mg/L and (100) mg/L concentrations, with an average of (9.47) (change in absorbance/minute/gm fresh weight).

Furthermore, commercial nano copper oxide (CN) treatments surpassed commercial nano zinc oxide (ZN), micro copper oxide (C), and micro zinc oxide (Z) treatments in enhancing enzyme activity. The average enzyme activities for CN were (8.74) (change in absorbance/minute/gm fresh weight) for the (300) mg/L concentration, (5.47) (change in absorbance/minute/gm fresh weight) for (200) mg/L, and (4.72) (change in absorbance/minute/gm fresh weight) for 100 mg/L.

These results are markedly higher when compared to the control plant and pathogen-only treatments, which recorded average peroxidase enzyme activities of (2.85) and (2.73) (change in absorbance/minute/gm fresh weight), respectively.

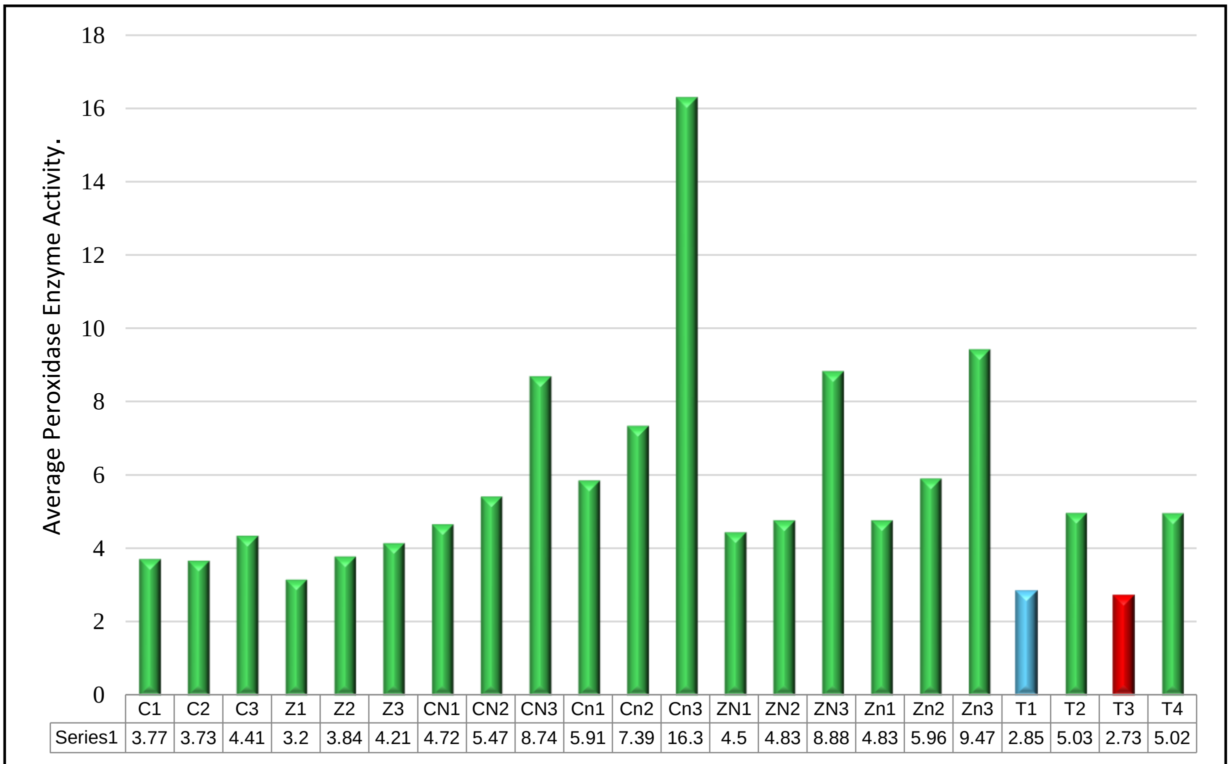


Figure (5) shows the Average Peroxidase Enzyme Activity.

These results align with the findings of [9], [18], who demonstrated that nano copper oxide, extracted from Ziziphus spina-christi leaves at concentrations of (50, 100, and 250) mg/L, modulated the enzymatic activity of peroxidase. When used against Fusarium solani, the causal agent of tomato root rot, peroxidase enzyme activity increased by (350, 386, and 762)%, respectively. This was in comparison to healthy, untreated and infected tomato plants, which showed peroxidase enzyme activities of (0.13)% and (0.048) units/minute/gram, respectively.

Our findings are also consistent with the study by [13] on copper oxide nanoparticles for controlling cucumber root rot caused by Fusarium solani. They found that Cu_2ONPs significantly increased peroxidase enzyme activity, measuring (1.397 ± 0.23) compared to control treatments at (0.329 ± 0.21).

Furthermore, these results are in agreement with [4], who used copper oxide nanoparticles synthesized with coffee powder against Fusarium oxysporum in 7-day-old chickpea seedlings. They observed a significant increase in PAL enzyme activity, with increases of (21.53, 12.30, and 7.69)% for concentrations of (50, 25, and 10) ppm, respectively, compared to fungal-infected seedlings, which exhibited (54.54)% activity.

Conclusion

1.Biosynthesis of zinc and copper nanoparticles using curcumin showed promising efficacy in preventing Podosphaera pannosa, the causal agent of powdery mildew.

2.The applied control agents played a role in inducing systemic resistance in the plant by increasing the activity of PAL and peroxidase enzymes. Notably, the biosynthesized nano copper oxide (Cn) treatment was superior to all other treatments in reducing disease severity.

Acknowledgement

We would like to thank and appreciate all the employees of the Ministry of Agriculture / Plant Protection Department / Technical Center who assisted us in achieving this research.

References

1. **Abdul-Karim**, E. K., & Hussein, H. Z. (2022). The biosynthesis of nanoparticles by fungi and the role of nanoparticles in resisting of pathogenic fungi to plants: a review. Basrah Journal of Agricultural Sciences, 35(1), 243–256.
2. **Al-Jubouri**, Ali Kadhum Mohammed. 2020. Study of the Efficacy of Alcoholic Extract and Nanoparticle Preparation from Dodonaea viscosa L. Leaves on Certain Biological Aspects and Causing Mortality in the Greater Wax Moth, Galleria mellonella L. (Lepidoptera: Pyralidae). p. 64.
3. **BAKER**, S., VOLOVA, T., PRUDNIKOVA, S. V., SATISH, S. & PRASAD, N. 2017. Nanoagroparticles emerging trends and future prospect in modern agriculture system. Environmental toxicology and pharmacology, 53, 10-17.
4. **BHATT,** D., TIWARI, V., BHATT, M. D., BAMBHAROLIYA, K. S., NATH, M., ARORA, S. & DOBRIYAL, A. K. Application of Green Synthesized Copper Oxide Nanoparticles for Effective Mitigation of Fusarium Wilt Disease in Roots of Cicer Arietinum. Available at SSRN 4559752.
5. **Castellanos-Barriga,** L.G., Santacruz-Ruvalcaba, F., Hernández-Carmona, G., Ramírez-Briones, E. and Hernández-Herrera, R.M., 2017. Effect of seaweed liquid extracts from Ulva lactuca on seedling growth of mung bean (Vigna radiata). *Journal of Applied Phycology*, *29*(5), pp.2479-2488.
6. **DE KESEL,** J., CONRATH, U., FLORS, V., LUNA, E., MAGEROY, M. H., MAUCH-MANI, B., PASTOR, V., POZO, M. J., PIETERSE, C. M. & TON, J. 2021. The induced resistance lexicon: do’s and don’ts. Trends in plant science, 26, 685-691.
7. **DESTA,** B., TENA, N. & AMARE, G. 2022. Response of rose (Rosa hybrida L.) plant to temperature. Asian J. Plant, Soil Sci, 7, 93-101.
8. **DOMíNGUEZ-SERRANO,** D., GARCíA-VELASCO, R., MORA-HERRERA, M. E., SALGADO-SICLAN, M. L. & GONZALEZ-DIAZ, J. G. 2016. The rose powdery mildew (Podosphaera pannosa). Agrociencia, 50, 901-917.
9. **EL-ABEID,** S. E., MOSA, M. A., EL-TABAKH, M. A., SALEH, A. M., EL-KHATEEB, M. A. & HARIDY, M. S. 2024. Antifungal activity of copper oxide nanoparticles derived from Zizyphus spina leaf extract against Fusarium root rot disease in tomato plants. Journal of Nanobiotechnology, 22, 28

10. **GUDKOV,** S. V., BURMISTROV, D. E., SEROV, D. A., REBEZOV, M. B., SEMENOVA, A. A. & LISITSYN, A. B. 2021. A mini review of antibacterial properties of ZnO nanoparticles. Frontiers in Physics, 9, 641481.
11. **Hateem,** M. W., & Khazal, J. (2021). Effectiveness of Foliar Spray and Root Treatment with Regular and Nanoparticle Silicon in Controlling Powdery Mildew on Squash in Greenhouse. Ind. J. Ecol, 17, 399–402.
12. **Hatem,** M. W. (2021). The Effect Of Nanoparticles Of Zinc Oxide And Titanium Oxide In Controlling Plant Pathogens. Int. J. of Aquatic Science, 12(2), 4647–4654.
13. **KAMEL,** S. M., ELGOBASHY, S. F., OMARA, R. I., DERBALAH, A. S., ABDELFATAH, M., EL-SHAER, A., AL-ASKAR, A. A., ABDELKHALEK, A., ABD-ELSALAM, K. A. & ESSA, T. 2022. Antifungal activity of copper oxide nanoparticles against root rot disease in cucumber. Journal of Fungi, 8, 911.
14. **KAUR,** S., GARG, T., JOSHI, A., AWASTHI, A., KUMAR, V. & KUMAR, A. 2024. Potential effects of metal oxide nanoparticles on leguminous plants: Practical implications and future perspectives. Scientia Horticulturae, 331, 113146.
15. **McKinney,** H. H. (1923). Influence of soil temperature and moisture on infection of wheat seedlings by Helminthosporium sativum.
16. **Narwal,** S. S., Bogatek, R., Zagdanska, B. M., Sampietro, D. A., & Vattuone, M. A. (2009). Plant biochemistry. Studium Press Texas.
17. **PATRA,** D. & EL KURDI, R. 2021. Curcumin as a novel reducing and stabilizing agent for the green synthesis of metallic nanoparticles. Green Chemistry Letters and Reviews, 14, 474-487.
18. **Sharma,** S. H. S., G. Lyons, C. McRoberts, D. McCall, E. Carmichael, F. Andrews, R. Swan, R. McCormack, and R. Mellon. "Biostimulant activity of brown seaweed species from Strangford Lough: compositional analyses of polysaccharides and bioassay of extracts using mung bean (Vigno mungo L.) and pak choi (Brassica rapa chinensis L.)." *Journal of Applied Phycology* 24, no. 5 (2012): 1081-1091.
19. **WHITAKER,** J.R. and Bernhard, R.A., 1972. Experiments for: an introduction to enzymology.