

# Natural and Engineering Sciences

NESciences, 2025, 10 (2): 552-562 doi: 10.28978/nesciences.1759477

# Evaluation of the Effectiveness of Biologically Synthesized Copper and Zinc Nanoparticles in Controlling Powdery Mildew on Rose Hybrida Caused by the Fungus Podosphaera Pannosa



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#### **Abstract**

The use of biologically synthesized nanomaterials in controlling pathogens is considered one of the promising and environmentally safe methods. Therefore, this study intended to evaluate the effectiveness of regular and nanoparticle zinc and copper oxides, both biosynthesized by green synthesis using curcumin, in controlling the fungus Podosphaera pannosa, which causes powdery mildew on Rosa hybrida. Furthermore, studying the effect of these materials on growth parameters, calculating the control effectiveness, infection severity, and area under disease progress curve (ADPC) under greenhouse conditions. The findings demonstrated that using a concentration of 300 mg/L of biologically produced copper oxide nanoparticles (Cn) was more effective than the other concentrations at lowering the severity of the disease, which reached 12.23%, and the AUDPC, which reached 72.73%, and it was superior in control effectiveness of 81.71%. The utilization of zinc oxide nanoparticles produced biologically at a 300 mg/L concentration showed superiority in reducing the infection severity, which reached 17.76%, and the AUDPC reached 74.45%, and gave an increase in the control effectiveness, which reached 80.91% compared to the treatment of the control plant, 15.51% and the pathogenic fungus, 0.00.

#### **Keywords:**

Powdery mildew, podosphaera pannosa, curcumin, biologically synthesized copper oxide nanoparticles.

### **Article history:**

Received: 28/04/2025, Revised: 31/05/2025, Accepted: 19/07/2025, Available online: 30/08/2025

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#### Introduction

Roses are the most significant flowering ornamental plants in the world. They are also regarded as one of the most popular decorative plants and a major source of commercial cut flowers. Rose hybrida is classified as one of the most significant decorative species in the Rosaceae family and achieves the highest global production among commercial cut flowers (Khyade & Wanve, 2018; El-Naggar et al., 2023). The fungus Podosphera pannosa is the cause of powdery mildew, one of the most prevalent harmful diseases affecting greenhouse roses (Pillai & Bhatia, 2024; Maharjan et al., 2015). Suitable temperature and relative humidity in greenhouses are the main reasons for the spread of powdery mildew (20, Hateem & Khazal, 2021; Fatima et al., 2024). Studies are moving towards using effective and environmentally friendly methods to control plant diseases and pests while minimizing negative influences on human health and the environment, given the growing global need for food security and sustainable agriculture (Cooke et al., 2006). Nanotechnology has been found to have the possible to radically transform plant protection methods (Abdul-Karim & Hussein, 2022, Kim et al., 2018; McKinney, 1923). Research by (Pariona et al., 2019; Ali, 2016) indicated that copper (Cu) nanoparticles prepared by green synthesis can be an effective fungicide against Fusarium solani, Neofusicoccum sp., and Fusarium oxysporum, as they presented morphological fluctuations in the mycelium and damage to the cell membranes of the pathogens (Krishnan & Iyer, 2024, Pai et al., 2025). A study on zinc nanoparticles showed a positive effect at concentrations of 100, 150, and 200 mg/L against powdery mildew disease on pepper plants, compared to a fungicide. Nanozinc led to an important decrease in disease severity at a concentration of 200 mg/L (Ismail et al., 2021). Curcumin is one of the most important non-toxic natural components, extracted from the turmeric plant Curcuma Longa, which is currently being developed for use as a biocompatible reducing agent in the synthesis of nanoparticles. It was first isolated by Vogel in 1842 (Khyade & Wanve, 2018; Patra & El Kurdi, 2021).

#### Materials and Methods

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

The experimentation was carried out in the Nanotechnology Laboratory / Department of Plant Biotechnology / College of Science / University of Baghdad for the academic year 2024-2025. (2 g) of curcumin extract was taken and liquified in (200 ml) of deionized water ++8and placed in an ultrasonic water bath in the (Ultra sonic Bath) device for two hours at a temperature of (70 °C) to dissolve and homogenize the mixture, as the ultrasonic waves work to increase the solubility of the extract in the water and homogenize the mixture (Abdi, 2017). The extract dissolved in water was distributed into 10 ml tubes, and these tubes were placed in a centrifuge (400 rpm) for 20 minutes to obtain a precipitate and a filtrate. The precipitate was ignored, and the filtrate was taken, and 20 grams of copper chloride and zinc nitrate were added to each 200 ml of it, each separately, as the color of the filtrate changed, which is evidence of the transformation into nanoparticles. The mixture was placed in Flasks and closed with a piece of cotton and cellophane, and placed in the Sheaker device for 24 hours to increase the mixing. The mixture was distributed in 10 ml tubes, and these tubes were placed in a centrifugal device for 20 minutes to settle the nanoparticles. A precipitate and a filtrate were obtained, where the filtrate was ignored, and the precipitate, which represents nano copper oxide and nano zinc oxide, was taken. The resulting quantity was collected in dishes and placed in the incubator (oven) for a whole day. The precipitate was skimmed after removing it from the incubator and placed in a (Tube) to carry out the washing process using ethanol solution and placed in a (Center fugal device) for (20 minutes) to get rid of the remaining copper and zinc, where the filtrate was neglected and the precipitate was taken and placed in a plate in the incubator for (10 minutes) to dry and then it was skimmed (Al-Jabouri, 2020, Debener &

Byrne, 2014) and the examination was conducted out in the laboratories of the Chemistry Department / College of Science / University of Baghdad to confirm the nano sizes and using an AFM microscope.

Evaluation of the effectiveness of micro zinc and copper oxides nanoparticles as treatments in monitoring powdery mildew on Rose Hybrida plants under greenhouse environments.

#### Preparing treatments in the field:

The research was carried out in the fields of the College of Agricultural Engineering Sciences, University of Baghdad, Al-Jadriya, affiliated with the Department of Plant Protection, during the winter season of the academic year (2024-2025). The land was prepared and leveled, cleaned of weed residues, and sterilized with the fungicide (Platanol) on October 15, 2024.

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

Seq.	Treatment code	Treatment name	Concentration (mg/L)
1	T1	Comparison (plant only)	0
2	T2	Comparison (pathogenic fungus)	0
3	Т3	Vitra pesticide + (pathogenic fungus)	3 g/L
4	T4	Curcumin extract	10 g/L
5	C1	Micro-copper oxide	100
6	C2	Micro-copper oxide	200
7	C3	Micro-copper oxide	300
8	CN1	Commercial copper oxide nanoparticles	100
9	CN2	Commercial copper oxide nanoparticles	200
10	CN3	Commercial copper oxide nanoparticles	300
11	Cn1	Commercial copper oxide nanoparticles	100
12	Cn2	Commercial copper oxide nanoparticles	200
13	Cn3	Commercial copper oxide nanoparticles	300
14	Z1	Micronized zinc oxide	100
15	Z2	Micronized zinc oxide	200
16	Z3	Micronized zinc oxide	300
17	ZN1	Commercial zinc oxide nanoparticles	100
18	ZN2	Commercial zinc oxide nanoparticles	200
19	ZN3	Commercial zinc oxide nanoparticles	300
20	Zn1	Biological copper oxide nanoparticles	100
21	Zn2	Biological copper oxide nanoparticles	200
22	Zn3	Biological copper oxide	300

#### Preparing the spore suspension for infection:

The inoculum was prepared by collecting infected parts of rose plants (flowers, leaves, and stems). The reproductive parts (conidia) were taken using a clean brush sterilized with sterile water, which is the basic spore solution for the spore suspension, at a concentration of 1 x 106 spores/ml. This concentration was calculated using a hemocytometer. An infection was carried out on the Rose Hybrida plant with the spore suspension to estimate the efficiency of the materials used in monitoring the disease. After the symptoms began to appear on the plants, foliar spraying was carried out with the materials as in Table 1 for the infected

plants until moisture, with 5 sprays between each spray (7) days apart. The effectiveness of control was calculated for the treatments according to the following equation (24):

$$= \frac{Infection\ severity\ in\ comparative\ treatment\ -\ Infection\ severity\ in\ treatments}{Infection\ severity\ in\ comparative\ treatment} \times 100$$

The effect of spraying with micro zinc and copper oxides nanoparticles on the infection severity of Rose Hybrida class (Baby Rose) Plants in a greenhouse with powdery mildew infections: 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). The field was prepared inside the greenhouse, where the plants were placed on (16/11/2024) while continuing the watering operations and cleaning the greenhouse and pots from weeds. Artificial infection was carried out on all plants on (14/1/2025), and the treatments mentioned in Table 1 were sprayed foliarly until moisture, with (5) sprays between each spray (7) days for the treatments.

The infection severity was calculated according to equation (11).

$$Infection \, Severity \, \% = \frac{Total \, number \, of \, infected \, leaves \, per \, class \times class \, code}{Total \, number \, of \, leaves \, \times highest \, score} \times 100$$

The ADPC is calculated according to the following equation (25):

AUDPC = 
$$\frac{1}{2}$$
 (2S1 + 2S2 + 2S3 + 2S4 + 2S5 + ··· . SX)

S1 = disease severity in the first reading.

S2 = disease severity in the second reading.

# **Results and Discussion**

Evaluation of the effectiveness of micro zinc and copper oxides nanoparticles as treatments for the control of powdery mildew on Rose Hybrida plants under greenhouse conditions. The results of examining the nanomaterials with an Atomic Force Microscope (AFM) showed that the sizes of the materials were nano, as the size of the biological copper oxide nanoparticles reached 37.74 nm (Figure 1), and the size of the biological zinc oxide nanoparticles reached 82.81 nm, as shown in Figure 2.

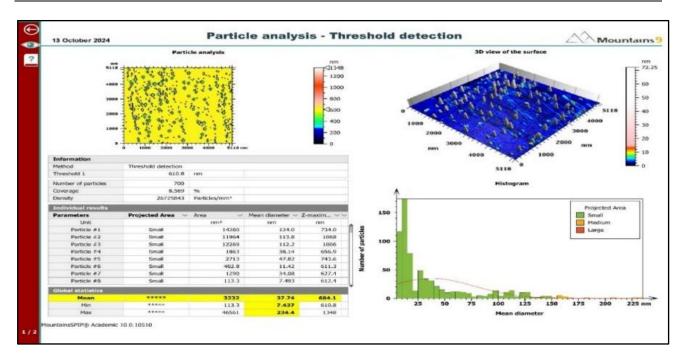


Figure 1.Shows the results of examining the biological copper oxide nanoparticles using an AFM microscope.

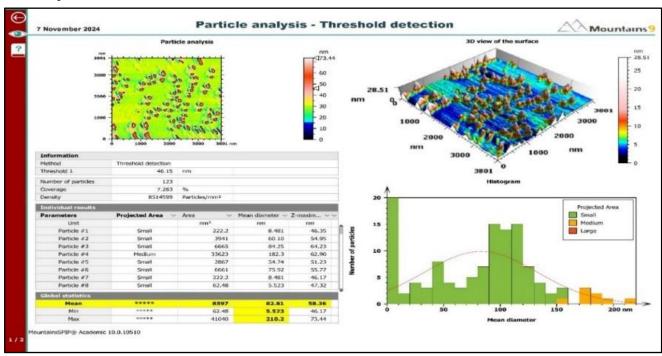


Figure 2. Shows the results of examining the biological zinc oxide nanoparticles using an AFM microscope.

The statistical analysis's findings in Table 2 show that the treatment of biological copper oxide nanoparticles Cn at a concentration of 300 mg/L was superior to the concentrations of 200 and 100 mg/L in increasing the effectiveness of control, as its effectiveness of control reached 81.71%. Followed by the treatment Zn, with a concentration of 300 mg/L superior to concentrations of 200 and 100 mg/L, giving an effective control of 80.91%, in contrast to the control plant treatment of 15.51% and the pathogenic fungus

treatment of 0.00%. Then, the CN treatment exceeded the C, Z, and ZN treatments in controlling the disease, as it caused the percentage of controls to rise at a concentration of 300 mg/L, which was 75.02%, at a concentration of 200 mg/L, it reached 72.34% and at a concentration of 100 mg/L, it reached 70.31%. Curcumin extract (10 g/L) and chemical pesticide (3 g/L) treatments enhanced the control effectiveness, achieving 77.51% and 71.21%, respectively, compared to the control treatment. These findings are consistent with the study of (Hatem, 2021; Feyzi Laeen et al., 2024; Patra & El Kurdi, 2021) in which zinc oxide, copper oxide, and silicon dioxide nanoparticles were used to control grape rot (Vitis vinifera cv. Shahroodi) brought on by the fungi Aspergillus niger, Botrytis cinerea, and Penicillium expansum. The findings of this study indicated that the rot of grapes infected with A. niger and B. cinerea was significantly controlled by spraying grape clusters with nano zinc oxide, with a control of 33 and 30%, respectively, and nano silicon dioxide, with a control of 25 and 32%, respectively. Control of rot in grapes infected with P. expansum was also achieved by spraying grape clusters with zinc oxide nanoparticles, which amounted to 16%, and copper oxide nanoparticles, which amounted to 25%. (6) In a study on copper nanoparticles prepared in aqueous media, their effectiveness in opposition to Fusarium solani, Neofusicoccum sp., and Fusarium oxysporum was evaluated. It was found that copper nanoparticles at a concentration of 50 mg/ml increased the effectiveness of controlling F. oxysporum by 97% and Neofusicoccum sp. by 95%, and that F. solani is the fungus most affected by low concentrations of copper nanoparticles. The control reached 55% at 10 mg/ml.

Table 2. Control effectiveness (the effect of spraying with biological micro zinc and copper oxides nanoparticles, curcumin extract (10 g/L), and chemical pesticide (3 g/L) on the control effectiveness of Rose Hybrida plants (Baby Rose) infected with powdery mildew disease in greenhouse conditions.

Treatment	Concentration (mg/L)	Control Percentage%
Micronized copper oxide	100	61.81
(C)	200	67.04
	300	70.61
Micronized zinc oxide	100	60.53
(Z)	200	65.81
	300	69.81
Commercial copper oxide nanoparticles (CN)	100	70.31
	200	72.34
	300	75.02
Biological copper oxide nanoparticles (Cn)	100	70.71
	200	80.41
	300	81.71
Commercial zinc oxide nanoparticles (Zn)	100	64.91
	200	69.31
	300	73.51
Biological zinc oxide nanoparticles (Zn)	100	72.21
	200	78.41
	300	80.91
Comparison plant		15.51
Chemical pesticide (3 g/	71.21	
Pathogenic fungus	0.00	
Curcumin extract (10 g/	77.51	
LSD 5% 0.01		
There is a significant difference with the	control plant T1 at the 5%	probability level.
There is a significant difference with the pa	thogenic fungus T3 at the	5% probability level.

The impact of spraying with micro zinc and copper oxides nanoparticles on the infection severity of Rose Hybrida plants (Baby Rose) infected with powdery mildew in greenhouse conditions. The findings of Table 3 show the efficiency of micro zinc and copper oxides nanoparticles in reducing the average infection severity with the fungus Podosphaera Pannosa for the treatments. The treatment of Cn achieved high efficiency in reducing the infection severity with powdery mildew at a concentration of 300 mg/L, as it exceeded the concentrations of 200 and 100 mg/L and gave an average of 12.23%. Followed by the treatment of Zn, which had an average infection severity of 12.86% at a concentration of 300 mg/L, and 14.45% and 18.64% at concentrations of 200 and 100 mg/L, respectively. Then, the CN treatment exceeded the C, Z, and ZN treatments in reducing the average infection severity at a concentration of 300 mg/L by 16.78%, at a concentration of 200 mg/L by 18.56% and at a concentration of 100 mg/L by 19.90%. The treatments of using curcumin extract (10 g/L) and chemical pesticide (3 g/L) showed control over the disease and a reduction in the average infection severity for them, which reached 15.08% and 19.30%, respectively, in contrast to the control treatment and the pathogenic fungus, which had an average infection severity for them of 56.71% and 67.12%, respectively, and there was a significant difference.

Table 3. The effect of spraying with biological micro copper and zinc oxides nanoparticles, curcumin extract (10 g/L), and chemical pesticide (3 g/L) on the infection severity of rose hybrida plants (baby rose) infected with powdery mildew disease in greenhouse conditions

Treatments	Concentrations	Infection severity %						
	(mg/L)	First	Second	Third	Fourth	Fifth	Sixth	Average
		week	week	week	week	week	week	
Micronized copper	100	33.75	29.72	28.43	23.96	21.31	16.41	25.60
oxide	200	29.31	28.87	23.06	19.52	17.31	14.62	22.12
(C)	300	24.08	23.97	21.77	18.21	16.41	13.77	19.70
Micronized zinc oxide	100	27.96	29.72	27.51	26.21	24.86	22.62	26.48
(Z)	200	25.73	25.31	23.96	21.31	21.31	20.10	22.95
	300	25.33	23.06	21.72	19.11	16.82	15.53	20.26
Commercial copper	100	21.74	20.87	20.41	19.53	18.63	18.21	19.90
oxide nanoparticles	200	27.53	22.21	18.16	17.31	13.74	12.43	18.56
(CN)	300	22.21	20.41	16.82	15.53	13.31	12.41	16.78
Biological copper oxide	100	34.61	23.96	19.52	15.93	12.41	11.52	19.66
nanoparticles (Cn)	200	27.06	19.96	14.21	9.72	4.41	3.51	13.15
	300	30.21	19.52	11.52	8.21	2.63	1.31	12.23
Commercial zinc oxide	100	28.87	28.87	26.16	23.06	18.68	15.53	23.53
nanoparticles (Zn)	200	31.06	25.31	21.31	17.32	14.63	13.72	20.56
	300	26.21	20.87	18.21	16.43	13.31	11.52	17.76
Biological zinc	100	29.77	23.11	17.76	15.92	12.88	12.42	18.64
oxidenanoparticles (Zn)	200	29.26	20.71	13.31	9.72	7.53	6.17	14.45
	300	23.52	19.80	12.41	9.07	7.07	5.31	12.86
Comparison	plant	35.52	44.87	54.62	62.63	70.63	71.97	56.71
Chemical pestici	25.31	22.21	19.92	16.87	15.97	15.52	19.30	
Pathogenic f	50.61	56.87	59.06	69.72	77.31	89.16	67.12	
Curcumin extrac	23.52	19.93	16.87	14.62	8.47	7.06	15.08	
LSD 5%	0.031	0.016	0.016	0.027	0.016	0.039	0.011	
There is a significant difference with the control plant T1 at the 5% probability level.								
There is a significant difference with the pathogenic fungus T3 at the 5% probability level.								

The effect of spraying with micro zinc and copper oxides nanoparticles on the AUDPC in Rose Hybrida plants (Baby Rose) infected with powdery mildew in greenhouse conditions. The statistical analysis results in Table 4 show the value of the (AUDPC) on the rose plant in the greenhouse. The study results showed that the lowest value in the AUDPC was in the Cn treatment at a concentration of 300 mg/L, which exceeded the concentrations of 200 and 100 mg/L, reaching 72.73. Followed by the Zn treatment, and the concentration of 300 mg/L was superior to the concentrations of 200 and 100 mg/L, and gave a value of 74.45 in the AUDPC, compared to the treatment of the control plant, 304.26, and the pathogenic fungus 358.15, with a significant difference. Then, the CN treatment exceeded the C, Z, and ZN treatments in controlling the disease, as it led to reducing the AUDPC at concentrations of 300 mg/L, reaching 94.45. At a concentration of 200 mg/L, it reached 105.13, and at a concentration of 100 mg/L, it reached 110.25. The treatments of curcumin extract (10 g/L) and chemical pesticide (3 g/L) reduced the AUDPC values to 86.80 and 108.02, respectively, compared to the control treatment and the pathogenic fungus.

Table 4. Stages of AUDPC (according to what was mentioned in the analysis results of the treatments)

Treatments	ts Concentr	Infection severity %						
	ations (mg/L)	Infection severity in the first week %	Infection severity in the second week %	Infection severity in the third week %	Infection severity week 4% 11/2	Infection Severity week 5% 18/2	Infection severity week 6% 25/2	Average
		21/1	28/1	4/2				
Micronized copper	100	33.75	29.72	28.43	23.96	21.31	16.41	145.30
oxide	200	29.31	28.87	23.06	19.52	17.31	14.62	125.31
(C)	300	24.08	23.97	21.77	18.21	16.41	13.77	111.11
Micronized zinc	100	27.96	29.72	27.51	26.21	24.86	22.62	147.51
oxide	200	25.73	25.31	23.96	21.31	21.31	20.10	127.61
(Z)	300	25.33	23.06	21.72	19.11	16.82	15.53	113.81
Commercial	100	21.74	20.87	20.41	19.53	18.63	18.21	110.25
copper oxide	200	27.53	22.21	18.16	17.31	13.74	12.43	105.13
nanoparticles (CN)	300	22.21	20.41	16.82	15.53	13.31	12.41	94.45
Biological copper	100	34.61	23.96	19.52	15.93	12.41	11.52	112.18
oxide nanoparticles	200	27.06	19.96	14.21	9.72	4.41	3.51	77.13
(Cn)	300	30.21	19.52	11.52	8.21	2.63	1.31	72.73
Commercial zinc	100	28.87	28.87	26.16	23.06	18.68	15.53	133.42
oxide nanoparticles	200	31.06	25.31	21.31	17.32	14.63	13.72	116.48
(Zn)	300	26.21	20.87	18.21	16.43	13.31	11.52	100.76
Biological zinc	100	29.77	23.11	17.76	15.92	12.88	12.42	105.61
oxide nanoparticles	200	29.26	20.71	13.31	9.72	7.53	6.17	83.61
(Zn)	300	23.52	19.80	12.41	9.07	7.07	5.31	74.45
Comparison p	Comparison plant		44.87	54.62	62.63	70.63	71.97	304.26
Chemical pesticide (3 g/L)		25.31	22.21	19.92	16.87	15.97	15.52	108.02
Pathogenic fungus		50.61	56.87	59.06	69.72	77.31	89.16	358.15
Curcumin extract (10 g/L)		23.52	19.93	16.87	14.62	8.47	7.06	86.80
LSD 5%		0.031	0.016	0.016	0.027	0.016	0.039	0.052
Th	There is a significant difference with the control plant T1 at the 5% probability level.							
There is a significant difference with the pathogenic fungus T3 at the 5% probability level.								

These results are consistent with (Azim et al., 2022) who used Vernonia cinerea leaves for the green synthesis of zinc oxide nanoparticles and their effect on the growth of tomato seedlings that zinc oxide

nanoparticles (ZnO-NPs) at a concentration of 50 mg/L enhance the morphological and physiological properties by 22% compared to raw zinc and control treatments when using lower doses, and that they give better results compared to high doses. Moreover, high doses showed inhibition of the growth and development of tomato seedlings. (Mekhail et al., 2025) evaluated the effectiveness of zinc oxide nanoparticles at three concentrations: 100, 50, and 25 mg/L, and the fungicide Raxil, in controlling powdery mildew caused by the fungus Blumeria graminis in barley. The average infection severity and the AUDPC were found to be lower for the fungicide, followed by concentrations of ZnO100 mg/L and ZnO50 mg/L, with infection severity reaching 5, 5.4, and 5.9%, respectively, compared to the control treatments, which had a much higher severity of 21.8%. The AUDPC was 23 for the fungicide, followed by ZnO100 mg/L and ZnO50 mg/L, which were 25 and 25.5, respectively, compared to the control treatments of 467.8. (7) evaluated the antifungal effect of different concentrations of 100, 150, and 200 mg/L of MgONPs and ZnONPs against powdery mildew of pepper Capsicum annuum caused by the fungus Oidiopsis sicula compared to the traditional fungicide Penconazole (0.25 ml/L). It was found that ZnONPs at a concentration of 200 mg/L resulted in a significant decrease in the mean infection severity of 11.90 compared to the pesticide 10.28, and the (AUDPC) gave a mean of 650.52 compared to the pesticide 508.99. These results are consistent with (Abou-Salem et al., 2022) in a study on copper oxide nanoparticles against the fungi Fusarium oxysporum, Macrophomina phaseolina, and Pectobacterium carotovorum and their effect on vegetative growth and infection rate of sugar beet plants. The results showed that a concentration of 150 µg/ml of copper oxide nanoparticles led to the greatest reduction in the infection rate, with efficacy values reaching 24.53, 13.25, and 23.59% for F. oxysporum, M. phaseolina, and P. carotovorum, respectively. These results are consistent with (El-Abeid et al., 2024) who derived copper oxide nanoparticles from the extract of Sidr leaves in a study and showed great effectiveness in controlling fungi when used against Fusarium solani, the fungus that causes tomato root rot disease, as there was a significant decrease in the infection severity with root rot disease by a percentage ranging from 72.0-88.6% compared to the infected sample of 80.5%. Three different concentrations of copper oxide nanoparticles: 50, 100, and 250 mg/L, were used. However, the 250 mg/L concentration achieved the highest reduction in infection severity compared to the other concentrations.

# **Conclusions**

- Curcumin was used in the biosynthesis of zinc and copper oxide nanoparticles, which demonstrated encouraging antifungal efficacy against the powdery mildew-causing Podosphaera pannosa fungus.
- Biological copper oxide nanoparticles (Cn) treatment was superior in increasing the control
  effectiveness and reducing the infection severity and AUDPC under greenhouse conditions.

#### **Conflict of Interest**

The authors declare that they have no competing interests.

# **Author Contributions**

All authors' contributions are equal for the preparation of research in the manuscript.

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