العـــدد 20A1 آذار 2026
No.20A1 Mar. 2026
المجلة العراقية للبحوث الإنسانية والإجتماعية والعلمية
Iraqi Journal of Humanitarian, Social and Scientific Research
Print ISSN 2710-0952 Electronic ISSN 2790-1254

Effect of *Ruta graveolens* and *Dianthus caryophyllus* extractions and their nanoparticles of ZnO on the resistance of *Fusarium incarnatum* and *Rhizoctonia solani* in vitro

Noor Tariq Abed , Najm Abdullah Alzubaidy, Rabab Majead Abed
Faculty of Education for Pure Sciences, University of Diyala, Iraq

Corresponding author:

Abstract

A lab experiment was conducted in the private lab, the Phi Nanoscience Center, during 2024 to evaluate the impact of *Ruta graveolens*, *Dianthus caryophyllus* extracts, and zinc nanoparticles against *Fusarium incarnatum* and *Rhizoctonia solani* in vitro. According to the results, all treatments resulted in an increase in the inhibition percentages of *F. incarnatum* and *R. solani* compared to the control treatment. The treatment of *Dianthus caryophyllus* + ZnO NPs with concentrations of 5, 4, 3, 2, and 1% achieved the highest percentage of inhibition of *F. incarnatum*, which amounted to 93.3, 92.2, 91.1, 86.6, and 84.4%, respectively, whereas the treatment of *Ruta graveolens* + ZnO NPs with a concentration of 5% was outstanding in recording the highest percentage of inhibition of the fungus *R. solani*, which amounted to 93.3%.

Keywords: zinc nanoparticles, *Ruta graveolens*, *Dianthus caryophyllus* *Fusarium incarnatum*, and *Rhizoctonia solani*

Introduction

The most common soil-borne fungi that cause dangerous diseases in a range of vegetable crops are Rhizoctonia and Fusarium, as *Rhizoctonia solani* is a non-obligate parasite that causes damping off and necrosis on a range of host plants. Because it lacks conidia, it occurs naturally as

vegetative hyphae and sclerotia. The Fusarium fungus causes Fusarium wilt, one of the most effective and damaging soil-borne diseases. It produces significant economic losses by infecting host roots and colonizing xylem vessels, which results in plant wilting **(Salim and Simon, 2015; Salim et al., 2016).** Reducing the use of chemical compounds is essential to limiting environmental pollution and promoting plant growth, so it is necessary to select environmentally friendly methods to improve the health and validity of crops; therefore, botanical extracts are the best substitutes for these dangerous chemicals **(Salim et al., 2023).** *Ruta graveolens*, a plant belonging to the Rutaceae family, has the ability to produce furanocoumarins, which are phytoalexins. These phytoalexins may be part of the natural composition of the species and play a role in the activity of antifungal and antimicrobial **(Al- Bawani and Eltayeb, 2004; Milesi, 2001).** Carnation (*Dianthus caryophyllus* L.) belongs to the Caryophyllaceceae family. Phytochemical analysis of Dianthus caryophyllus showed that it contained triterpenes, alkaloids, coumaruns, cyanogenic glycoside, cyanidin, pelargonidin, the yellow isosalipurposide, essential oil, volatile oil and many other chemical contents, and the plant possessed antibacterial, antifungal, antiviral, insecticidal and repellent (**Al-Snafi, 2017)**. Also, the other substitutes for these hazardous chemicals are nano compounds. Due to their diminutive size, expansive surface area, and heightened reactivity, nanoparticles exhibit promise for applications as bactericides, fungicides, nanopesticides, and nanofertilizers **(Elmer and White, 2016).** Zinc-based nanomaterials, with their targeted antimicrobial capabilities and low activity for potential phytotoxic effects, can achieve antifungal, antibacterial, antiviral, and anti-toxigenic activities against a range of phytopathogens **(Khan et al., 2020).** The main aim of the study was to evaluate the effect of *Ruta graveolens*, *Dianthus caryophyllus*

extracts and their nanoparticles of ZnO against *Fusarium incarnatum* and *Rhizoctonia solani* in vitro**.**

Materials and Methods

The study included the laboratory experiment in the Laboratory of Plant Pathology, Diyala Agriculture Directorate for the season 2024.

The pathogen fungi

The pathogen fungi *F. incarnatum* and *R. solani* were obtained from the Plant Pathology Lab, Directorate of Diyala Agriculture, which were molecularly diagnosed by PCR technique that included the extraction and multiplication of DNA and electrophoresis under recording codes *F. incarnatum* (OQ357846-OQ357847) and *R. solani* (OQ357844-OQ357845).

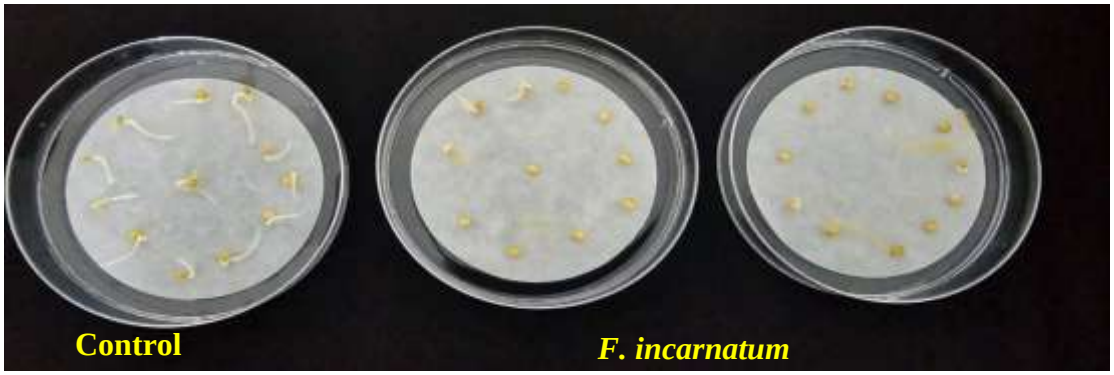
Pathogenicity test on eggplant seeds

Ten ml of sterile distilled water was added to each petri dish of fungi, *R. solani* and *F. incarnatum*, then the spores and mycelium were harvested by scraping them with a glass slide and filtering through three layers of muslin cloth. Then, 1 ml of each fungus suspension was put in 9 ml of sterile distilled water to prepare a dilution of 0.1, then sprayed on the ten eggplant seeds (cv. Barchilona) placed on the blotting paper, while the control seeds were sprayed with distilled water and incubated at room temperature. After that, the germination percentage was assessed (Table 1 and Figure 1).

Table 1. Germination percentage of eggplant seeds

Days	Germination%			
	F. incarnatum	Control	*R. solani*	Control
1	0	0	0	0
2	0	0	0	0

3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	10	0	10
8	0	10	0	10
9	0	20	0	20
10	0	40	0	40
11	0	60	0	60
12	0	80	0	80
13	10	100	10	100
14	20	100	20	100
15	20	100	20	100
16	30	100	30	100
17	40	100	30	100
18	40	100	30	100



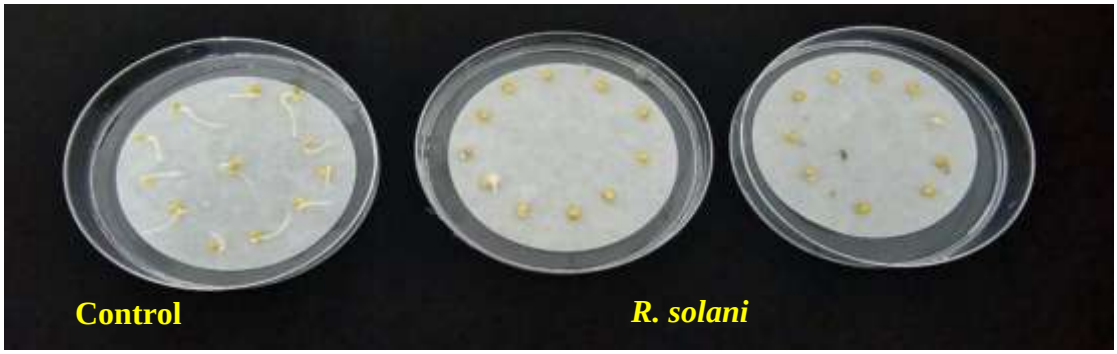


Figure 1. Germination percentage of eggplant seeds

Preperation of *Ruta graveolens* and *Dianthus caryophyllus* extracts

Ten grams of *Ruta graveolens* and *Dianthus caryophyllus* powders, each of them, were mixed with 150 ml of deionized water (DW) and 350 ml of ethanol. The mixture was then put on a magnetic stirrer and heated to 70 °C for three hours. The glass flask had to be sealed with aluminum foil. After that, the precipitation was separated by filter papers (Whatmann No. 1, pore size 25 µm), and then the extract is kept in the refrigerator.

Preparation of ZnO nanoparticles

The preparation of ZnO NPs was carried out according to **(Suresh et al., 2015, Ahmed et al., 2017, Alharbi et al., 2023, Sivasankarapillai et al., 2023)** with minor modification. 10 g of $Zn(NO_3)_2.6H_2O$ was dissolved in 150 ml of D.W. under stirring for 30 min. Then add 45 ml of *Ruta graveolens* or *Dianthus caryophyllus* with stirring for 60 min. Next, 2.5 g of sodium hydroxide (NaOH) was added in 20 ml of D.W. drop by drop, and the mixture was stirred for 10 minutes until a milky precipitate formed. The precipitate was washed with D.W. and ethanol four times to remove any impurities and then dried in an oven at 150°C for 1 hour. Finally, the white powder was calcination at 550°C for 3 hour (Figure 2).

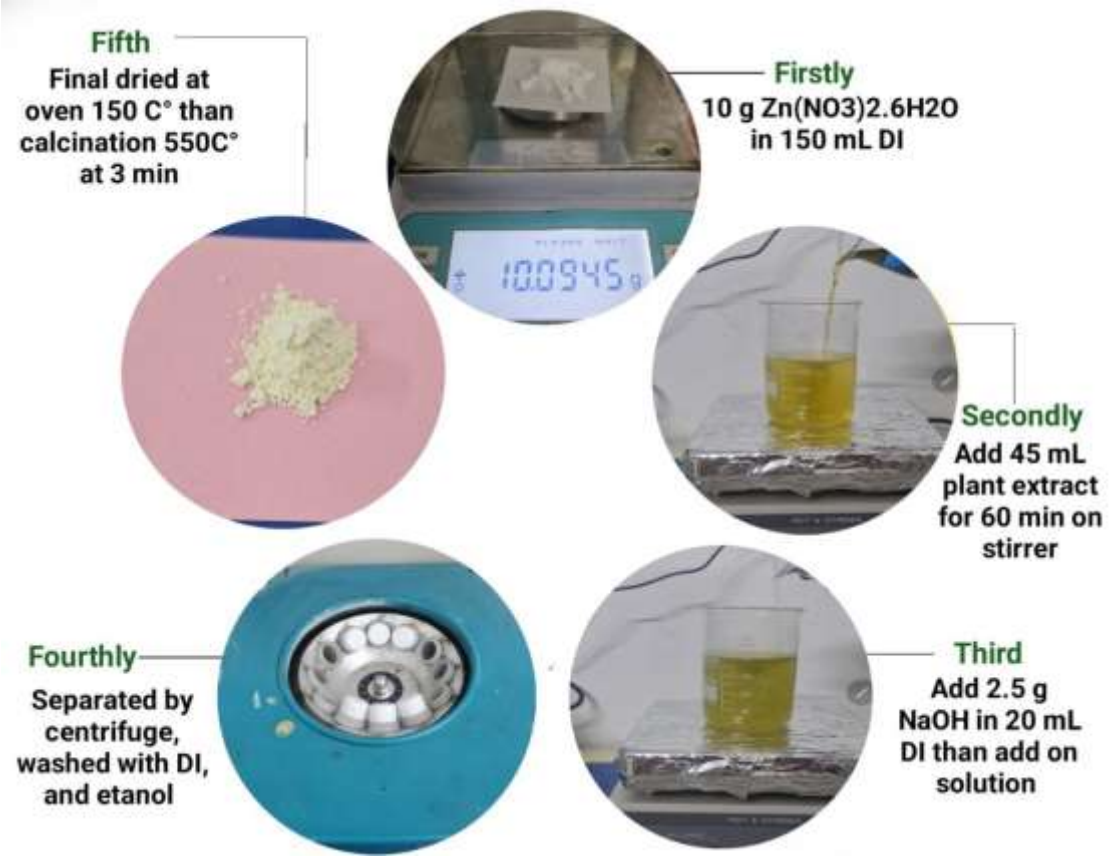


Figure 2. Steps of preparation ZnO nanoparticles

Estimation of the inhibition percentage %

The treatments of *Ruta graveolens* extract, *Dianthus caryophyllus* extract, ZnO NPs from *Ruta graveolens*, ZnO NPs from *Dianthus caryophyllus*, *Ruta graveolens* extract with ZnO NPs, and *Dianthus caryophyllus* extract with ZnO NPs at five concentrations (1, 2, 3, 4, and 5%) were prepared to inhibit the growth of *F. incarnatum* and *R. solani* on PDA medium. Each concentration was added to the dishes before solidifying the PDA medium, ensuring homogeneity by moving the dish in a circular motion. The dishes were inoculated by 6 mm-diameter inoculum discs of *F. incarnatum* and *R. solani* separately and then placed in an incubator at 26°C, and the fungi were allowed to grow. After four days, the inhibition percentage was calculated according to the following formula:

% inhibition = (C-T)/C × 100

C = diameter of the fungal colony in the control

T = diameter of the fungal colony in the treatment

Statistical analysis

The statistical data were analyzed using Complete Randomized Design (CRD) in laboratory experiment using the statistical program SPSS to analyze according to Duncan's multinomial test at a probability level of 0.05 **(Al-Rawi and Khalaf Allah, 2000).**

Results

The table (2) and figures (3 to 8) showed an increase in the inhibition percentage of *F. incarnatum* in all treatments compared to the control treatment, where the treatment of *Dianthus caryophyllus* + ZnO NPs with concentrations of 5, 4, 3, 2, and 1% were characterized by recording the highest percentage of inhibition, which amounted to 93.3, 92.2, 91.1, 86.6, and 84.4%, respectively, followed by the other treatments, while the treatment of *Ruta graveolens* with concentrations of 1 and 2% and ZnO NPs from *Dianthus caryophyllus* with a concentration of 1% recorded the lowest percentage of inhibition, which amounted to 52.2, 53.8, and 52.7%. It is clear from these results that there is a direct relationship between the rate of inhibition and the concentrations of the treatments; the higher the concentration, the higher the inhibition percentage.

The table (3) and figures (3 to 8) revealed an increase in the inhibition percentage of *R. solani* in all treatments compared to the control treatment, where the treatment of *Ruta graveolens* + ZnO NPs with a concentration of 5% was superior to the other treatments in recording the highest percentage of inhibition of the fungus *R. solani*, which amounted to 93.3%, followed by the other treatments, while the treatment of ZnO NPs from *Dianthus caryophyllus* with a concentration of 1% recorded the lowest percentage of inhibition, which amounted to 8.8%. These findings make it abundantly

evident that the rate of inhibition and treatment concentrations are directly correlated; the higher the concentration, the higher the percentage of inhibition.

Discussion

Researchers have discovered that zinc nanoparticles (ZnONPs) have antifungal effects because they successfully penetrate pathogenic microorganisms' cell membranes through the lipid bilayer **(Hulkoti and Taranath, 2014).** They have the ability to spread through food media **(Barsainya and Pratap Singh, 2018).** Zinc oxide (ZnO) showed obvious destruction of the cell walls and decomposition of the plasma of the internal organs of the tested fungi **(Farouk, 2016).** Zinc nanoparticles can kill germs or prevent their germination in addition to inhibiting the mycelial growth of pathogenic fungi. This is what happened in inhibiting the growth of the fungi *Botrytis cinerea* and *Penicillium expansum* when using ZnONPs (concentration of 3 mm/L) **(He et al., 2011). Lahuf et al. (2019)** found that concentrations of zinc nanoparticles (ZnONPs) (2.5, 5, 10, and 15 mg/ml) led to the inhibition of the fungus *R. solani* by (57, 71.03, 83.21, and 100%), respectively. **Bohan et al. (2017)** mentioned that the use of zinc oxide nanoparticles at concentrations (0.25, 0.5, 1.0 µg/ml) against the fungus *Fusarium* spp. at different periods (3, 7, 12, 15 days) led to inhibition of its growth, which amounted to (100, 75, 75%) after 3 days, (100, 75, 75%) after 7 days, (89, 56, 33%) after 12 days, and (25, 44, 100%) after 15 days. These results are consistent with **Yehia and Ahmed (2013)**, who indicated that ZnO nanoparticles may harm the fungus conidia. Zinc nanoparticles (ZnONPs) possess antifungal ability, and this was observed when they inhibited the fungus *Fusarium oxysporum* by 77% at a concentration of 12 mg/L. This was shown in electron microscopy through the presence of a clear deformation in the fungal hyphae of *F.*

oxysporum, which was treated with ZnONP **(Yehia and Ahmed, 2013). Wani and Shah (2012)** showed that ZnO nanoparticles had an antifungal effect on pathogenic fungi such as *Alternaria* spp., which saw a significant reduction in mycelial growth and spores treated with ZnO nanoparticles. According to **Celoto et al. (2008),** when added the hydroethanolic extract of Ruta graveolens to PDA culture media at a 20% concentration led to inhibits the mycelial growth of *Colletotrichum gloeosporioides* by 2.3%. **Cavalcanti, (2005)** stated that furanocoumarins, which are produced from *Ruta graveolens*, affect fungi in a number of ways, such as disruption of the plasma membrane, cytoplasmic granulation, inhibition of fungal enzymes and disruption of cell contents, which results in decreased mycelial growth and germination. **Duke et al. (2003)** revealed that rutin and chlorogenic acid, two phenolic compounds present in *R. graveolens* extract as flavonoids, had inhibitory activity against *Fusarium oxysporum* and *Penicillium expansum*. **Nehal et al. (2007)** stated that carnation showed the highest protective effect against disease incidence when applied as powder or extracts, and a high significant inhibitory effect on radial fungal growth was observed at different concentrations of carnation.

Conclusion

The study's findings demonstrated the effectiveness of *Ruta graveolens*, *Dianthus caryophyllus* extracts, and zinc nanoparticles against *F. incarnatum* and *R. solani* in vitro at different concentrations.

Table 2. Effect of *Ruta graveolens*, *Dianthus caryophyllus* extracts and their nanoparticles of ZnO in inhibiting *Fusarium incarnatum* in vitro

Treatments	**Concentrations**				
	1%	**2%**	**3%**	**4%**	**5%**
Ruta graveolens	52.2	53.8	61.6	65.5	73.3

	p	p	m	kl	hi
Dianthus caryophyllus	63.8 lm	70.5 ij	72.7 hi	79.4 def	81.2 de
ZnO NPs from *Ruta graveolens*	54.4 op	57.15 no	63.3 lm	68.2 jk	71.1 ij
ZnO NPs from *Dianthus caryophyllus*	52.7 p	57.7 n	62.2 m	65.5 kl	74.9 gh
Ruta graveolens +ZnO NPs	75.5 gh	76.6 fg	78.8 ef	80.0 de	82.2 cd
Dianthus caryophyllus +ZnO NPs	84.4 bc	86.6 b	91.1 a	92.2 a	93.3 a

Table 3. Effect of *Ruta graveolens*, *Dianthus caryophyllus* extracts and their nanoparticles of ZnO in inhibiting *Rhizoctonia solani* in vitro

Treatments	**Concentrations**				
	1%	**2%**	**3%**	**4%**	**5%**
Ruta graveolens	56.0 m	61.6 l	65.5 k	67.7 jk	71.1 i
Dianthus caryophyllus	19.9 p	26.6 n	26.0 n	59.4 l	67.1 jk
ZnO NPs from *Ruta graveolens*	15.5 q	23.3 o	27.7 n	69.4 ij	76.6 h

ZnO NPs from *Dianthus caryophyllus*	8.8 s	12.2 r	16.0 q	19.4 p	22.7 o
Ruta graveolens +ZnO NPs	87.7 cde	88.7 cd	90.0 bc	92.2 ab	93.3 a
Dianthus caryophyllus +ZnO NPs	82.2 g	83.3 fg	85.5 ef	86.6 de	90.0 bc

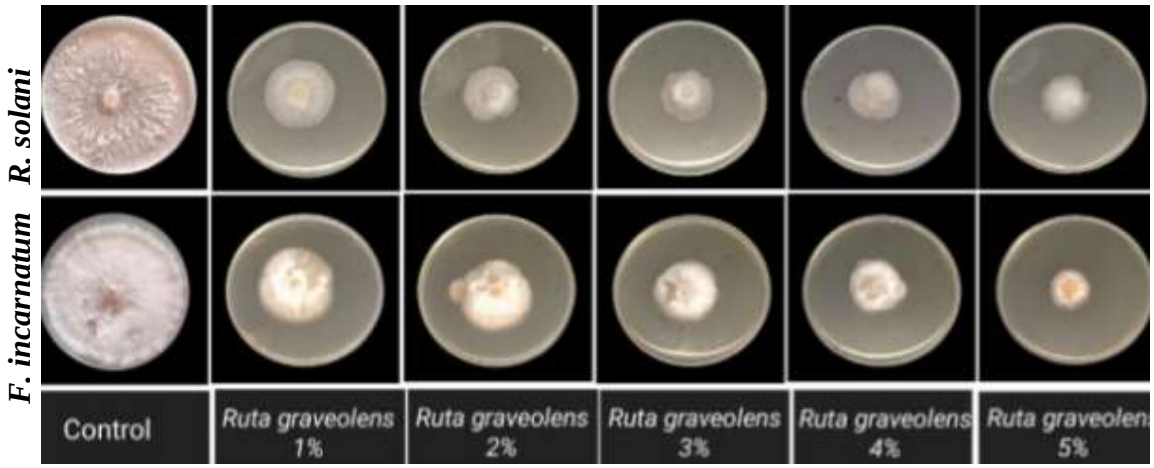


Figure 3. Effect of *Ruta graveolens* extract against *F. incarnatum* and *R. solani*

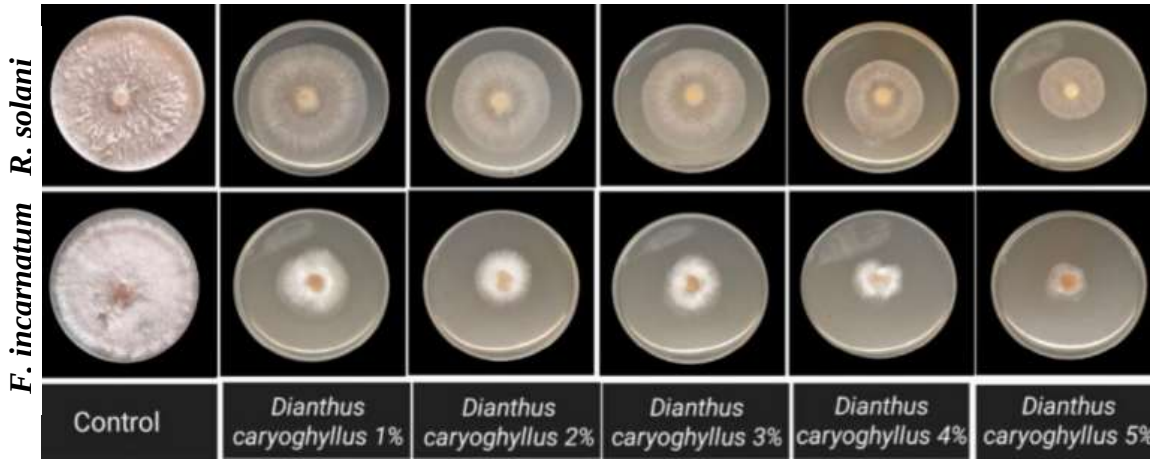


Figure 4. Effect of *Dianthus caryophyllus* extract against *F. incarnatum* and *R. solani*

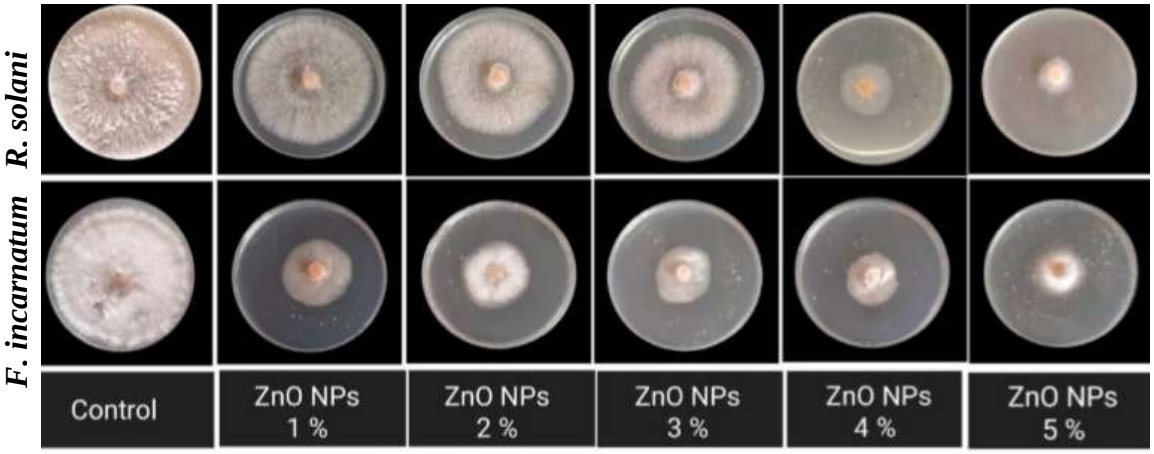


Figure 5. Effect of zinc nanoparticles from *Ruta graveolens* against *F. incarnatum* and *R. solani*

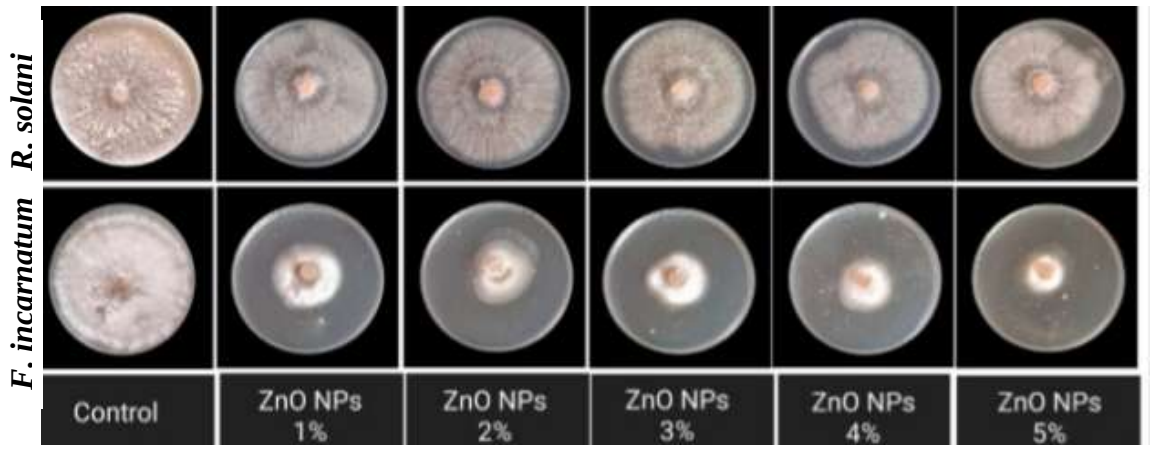


Figure 6. Effect of zinc nanoparticles from *Dianthus caryophyllus* against *F. incarnatum* and *R. solani*

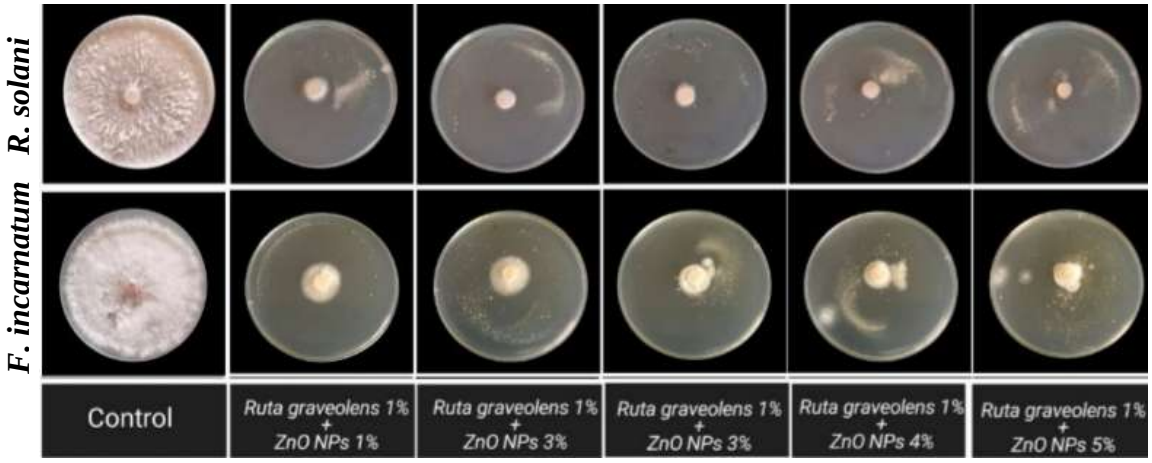


Figure 7. Effect of *Ruta graveolens* with zinc nanoparticles against *F. incarnatum* and *R. solani*

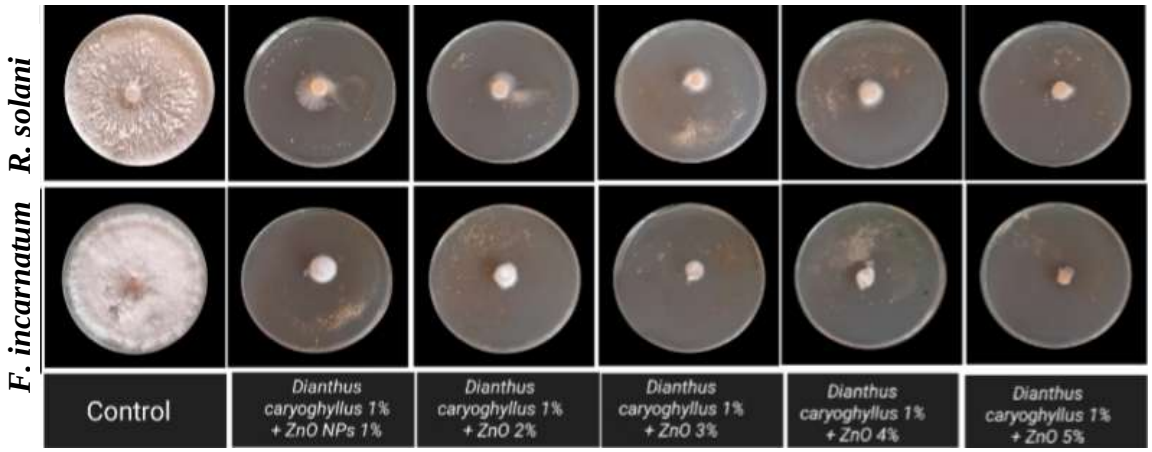


Figure 8. Effect of *Dianthus caryophyllus* with zinc nanoparticles against *F. incarnatum* and *R. solani*

References

Ahmed, S., Chaudhry, S. A., & Ikram, S. (2017). A review on biogenic synthesis of ZnO nanoparticles using plant extracts and microbes: a prospect towards green chemistry. Journal of Photochemistry and Photobiology B: Biology, 166, 272-284.

Al- Bawani FM, Eltayeb EA (2004). Antifungical compounds from induced Conium maculatum L. plants. Biochem Syst Ecol.32:1097–1108.

Alharbi, F. N., Abaker, Z. M., & Makawi, S. Z. A. (2023). Phytochemical Substances—Mediated Synthesis of Zinc Oxide Nanoparticles (ZnO NPS). Inorganics, 11(8), 328.

Al-Rawi, K. M. and Khalaf Allah A. M. 2000. Design and analysis of agricultural experiments. Second Edition. University of Al Mosul. Ministry of Education and Scientific Research. The Republic of Iraq.

Al-Snafi A. E, 2017. Chemical contents and medical importance of *Dianthus caryophyllus*- A review, IOSR Journal Of Pharmacy ,Volume 7, Issue 3 Version.1 , PP. 61-71 61.

Barsainya, M.; Pratap Singh, D. (2018). Green Synthesis of Zinc Oxide Nanoparticles by *Pseudomonas aeruginosa* and Their Broad-Spectrum Antimicrobial Effects. J. Pure Appl. Microbiol., 12, 2123–2134

Bohan A. J., Alhtheal E.D., Shaker Kh. S. (2017). "Synthesis Nano Zinc Oxide Materials and Their Activity on Fungus Growth" Engineering & Technology Journal, Vol. 13, Part B No. 0, pp. 27-84.

Cavalcanti LS, et al.(2005). Aspectos bioquímicos e moleculares da resistência induzida. In: Cavalcanti, LS et al. (Eds.). Indução de Resistência em Plantas a Patógenos e Insetos. Piracicaba: FEALQ, p.81–124.

Celoto MI B, Papa M FS, Sacramento LVS, Celoto FJ (2008). Atividade antifúngica de extratos de plantas a *Colletotrichum gloeosporioides*. Acta Sci Agron Maringá.30:1–5.

Duke SR, Baerson FE, Dayan AM, Rimando BE, Scheffler MR, Tellez DE, Wedge KK, Schrader DH, Akey FH, Arthur AJD, Lucca DM, Gibson HF, Harrison JK.Peterson DR, Gealy T, Tworkoski CL, Wilson JBM (2003). United States Department of Agriculture – Agricultural Research Service research on natural products for pest management. Pest Manag Sci. 59:708–717.

Elmer, W.H., White, J., 2016. The use of metallic oxide nanoparticles to enhance growth of tomatoes and eggplants in disease infested soil or soilless medium. Environmental Science: Nano 3 (5), 1072-1079. DOI:https://doi.org/10.1039/C6EN00146G.

Farouk, A.M. (2016). Improvement of Bean Resistance to Damping-Off and Root-Rot Diseases. Ph.D. Thesis, Cairo University, Cairo, Egypt.

He, L.; Liu, Y.; Mustapha, A.; Lin, M. (2011). Antifungal Activity of Zinc Oxide Nanoparticles against *Botrytis cinerea* and *Penicillium expansum*. Microbiol. Res., 166, 207–215. [CrossRef]

Hulkoti, N.I.; Taranath, T.C. (2014). Biosynthesis of Nanoparticles Using Microbes—A Review. Colloids Surf. B Biointerfaces, 121, 474–483.

Khan, S.A.; Shahid, S.; Lee, C.-S. (2020). Green Synthesis of Gold and Silver Nanoparticles Using Leaf Extract of ClerodendrumInerme; Characterization, Antimicrobial and Antioxidant Activities. Biomolecules, 10, 835.

Lahuf, A.A.; Kareem, A.A.; Al-Sweedi, T.M.; Alfarttoosi, H.A. (2019). Evaluation 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 Proceedings of the IOP Conference Series: Earth and Environmental Science, Banda Aceh, Indonesia, 21–22 August; IOP Publishing: Bristol, UK, 2019; Volume 365, p. 012033. [CrossRef]

Milesi SBM, Gontier E, Bourgaud F, Guckert A. (2001). *Ruta graveolens* L.: a promising species for the production of furanocoumarins. Plant Sci.161:189–199.

Nehal S. El-Mougy, Mokhtar M. Abdel-Kader.(2007). Antifungal effect of powdered spices and their extracts on growth and activity of some fungi

in relation to damping-off disease control, journal of plant protection research, Vol. 47, no. 3.

Salim H, Alsaady M, Al-zuhairi A, Kassoub F. 2023. Evaluation of the fungicidal activity of the aqueous extracts of some medicinal plants against *Fusarium* spp. Revis Bionatura;8 (2) 84. http://dx.doi.org/10.21931/RB/2023.08.02.84.

Salim H.A. and Simon S. 2015. Effect of carbendazim and solarized soil with *Pseudomonas fluorescens* spent mushroom compost against *Fusarium oxysporum* f.sp. *lycopersici* in Tomato, European academic research, vol. II, Issue 12,p15997-16010.

Salim H.A., Salman I.S., Jasim B.N. 2016..IPM Approach for the management of wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici* on tomato (*Lycopersicon esculentum*). Journal of Experimental Biology and Agricultural Sciences, 4: 742–747.

Sivasankarapillai, V. S., Krishnamoorthy, N., Eldesoky, G. E., Wabaidur, S. M., Islam, M. A., Dhanusuraman, R., & Ponnusamy, V. K. (2023). One-pot green synthesis of ZnO nanoparticles using Scoparia Dulcis plant extract for antimicrobial and antioxidant activities. Applied Nanoscience, 13(9), 6093-6103.

Suresh, D., Nethravathi, P. C., Rajanaika, H., Nagabhushana, H., & Sharma, S. C. (2015). Green synthesis of multifunctional zinc oxide (ZnO) nanoparticles using Cassia fistula plant extract and their photodegradative, antioxidant and antibacterial activities. Materials Science in Semiconductor Processing, 31, 446-454.

Wani A. H. and Shah M. A. (2012). A unique and profound effect of MgO and ZnO nanoparticles on some plant pathogenic fungi. Journal of Applied Pharmaceutical Science 02 (03): 40-44.

Yehia R. S. and Ahmed O. F.. (2013). *In vitro* study of the antifungal efficacy of zinc oxide nanoparticles against *Fusarium oxysporum* and *Penicilium expansum.* African Journal of Microbiology Research.Vol.7(19), pp.1917-1923,7May.