


Protective Role of Topsin M 70 Fungicide and Silicon Oxide Against White Mold Disease on Two Cultivars of Eggplant

Zahraa H. Abdulkhaleq¹, Eman K. Abdul-Karim^{1*} 

¹Department of Plant Protection, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq

ABSTRACT

This study was conducted at the Plant Protection Directorate / Technical Departments Complex in 2023 to identify the causal agent of white mold disease and evaluate the effectiveness of the fungicide Topsin M 70 and silicon oxide in controlling disease progression, infection rate, and severity. The results indicated that the *Sclerotinia sclerotiorum* (SS5) isolate exhibited the highest pathogenicity, with an infection rate of 100% and a severity of 82.5%. DNA electrophoresis analysis of the SS5 isolate revealed a single band with a molecular weight of 550 bp, showing 100% similarity to globally registered isolates in the NCBI gene bank. The nucleotide sequences were registered under accession number OR497817. The application of nano-silicon oxide (0.001 g/L), conventional silicon oxide (3 g/L), and the chemical fungicide Topsin M 70 (1 g/L) as foliar sprays on *Solanum melongena* cultivars Wasam and Barcelona exhibited protective effects. The chemical fungicide provided complete disease suppression, recording 0% infection rate and severity in both cultivars. Nano-silicon oxide resulted in an infection rate of 13.87% and severity of 5.44%, while conventional silicon oxide recorded 63.16% infection rate and 7.43% severity, compared to the control, which showed 50% infection rate and 25.69% severity. The area under the disease progression curve (AUDPC) was 130.95 and 143.21 in the control treatment, but it dropped to 0% with the application of the chemical fungicide.

Key words: Area under disease progress curve; Induced resistance; *Sclerotinia sclerotiorum*; Silicon oxide; Topsin M 70.



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INTRODUCTION

Eggplant (*Solanum melongena*) is one of the essential vegetable crops globally, belonging to the Solanaceae family. It is notable for being a significant nutritional source due to its content of various vitamins, in addition to essential elements such as potassium, iron, magnesium, manganese, phosphorus, copper, dietary fiber, and folic acid (Sharma & Kaushik, 2021; Rosa-Martínez et al., 2022). It is widely cultivated in tropical and subtropical regions. In Iraq, it is grown in open fields, greenhouses, and plastic tunnels. The cultivated area in Iraq reached approximately 54,469 dunams, with a production of 207.2 thousand tons in 2020 (Al-Juboory & Al-

Kubaisei, 2023). Eggplant farmers face a significant challenge in the form of infection by a wide range of plant pathogens, among which white mold disease, caused by the fungus *Sclerotinia sclerotiorum*, is one of the most aggressive and destructive. This pathogen has a broad host range, affecting up to 400 plant species (Al-Shujairi et al., 2022). Several materials have been utilized to combat the disease, including fungicides, which are known for their high efficacy in controlling pathogens, ease of application, and rapid results when used correctly to minimize environmental harm. Among the environmentally friendly and safe approaches are chemical compounds, as mineral nutrients

play a crucial role in plant growth and contribute to combating pathogens. Silicon particles, for instance, enhance plant growth, development, and reproduction, in addition to improving plant defense mechanisms against biotic and abiotic stress factors (Wang et al., 2022; Kovacs et al., 2022). Their efficacy increases in nano-sized forms, making them more effective against pathogens due to their distinct properties compared to their bulk counterparts. Their smaller size and higher surface area-to-volume ratio allow them to penetrate cell walls and plasma membranes, thereby enhancing their effectiveness (Rajeshkumar, 2019). The study aimed to evaluate the use of Topsin M 70 fungicide and silicon oxide in controlling white mold disease on eggplant plants.

MATERIALS AND METHODS

Samples of eggplant plants (stems) showing symptoms and signs of white mold disease were collected from greenhouses in Baghdad Governorate (Abu Ghraib and Yusufiyah) and Wasit Governorate (Al-Radassiyah) during January and February of 2023. The samples were collected using the diagonal sampling method and placed in polyethylene bags labeled with the collection location and date. The samples were then transported to the laboratory for isolation and identification of the causal pathogen. The isolation process was performed on each infected eggplant sample by cutting 0.5 cm sections near the infected area. These sections were sterilized with 70% ethanol for 2–3 minutes, then rinsed three times with sterilized distilled water and dried on sterile filter paper. Four sterilized sections were planted per petri dish containing sterilized Potato Dextrose Agar (PDA) medium, which had been autoclaved at 120°C and 1.5 kg/cm² pressure. The dishes were incubated at 25 ± 2°C for five days. The fungal isolates were identified based on colony characteristics, the nature of the fungal mycelium, and the structures formed by the fungus. The experiment was conducted in the lath house at the Plant Protection Directorate. Sterilized soil (autoclaved) was distributed into plastic pots, with each pot containing 2 kg of soil. Eggplant saplings, one month old, were transplanted at a rate of three saplings per pot,

with three pots assigned for each fungal isolate. The saplings were inoculated following the method described by Prova et al. (2018). A control treatment was included, consisting of non-inoculated plants. Disease severity was recorded seven days after inoculation. The DNA of the most pathogenic fungal isolate was extracted using a specialized commercial kit from Bioneer. The DNA of the pathogen was amplified using the primers ITS1 and ITS4 with the Polymerase Chain Reaction (PCR) technique, following confirmation of the genetic material's purity. For nucleotide sequencing, the PCR amplification products were sent to Macrogen, South Korea. The obtained nucleotide sequences were compared using the BLAST program on the NCBI website (<http://www.ncbi.nlm.nih.gov>) and subsequently deposited in the gene bank.

Evaluation of the Protective Role of Topsin M 70 and Silicon Oxide in Controlling the Pathogen in the Field: The experiment was conducted in a plastic greenhouse at the Plant Protection Directorate during 2023-2024. Necessary agricultural operations, including weed removal, plowing, leveling, furrow preparation, and the setup of a drip irrigation system, were performed. The greenhouse was divided into three blocks. Eggplant saplings of the "Barcelona" commercial cultivar and the "Wasam" cultivar were planted. Foliar treatments were applied three days before pathogen inoculation, including nano-silicon oxide (commercial formulation) at 0.001 g/L after ultrasonic homogenization for one minute, conventional silicon oxide at 3 g/L, and the fungicide Topsin M 70 at 1 g/L until runoff. Pathogen inoculation was performed using the most virulent fungal isolate, following the method of Prova et al. (2018). The treatments were as follows: nano-silicon oxide + pathogenic fungus, conventional silicon oxide + pathogenic fungus, Topsin M 70 + pathogenic fungus, and a control treatment consisting of plants inoculated with the pathogenic fungus only. The percentage and severity of infection were recorded seven days after fungal inoculation. Additionally, the area under the disease progress curve (AUDPC) was calculated over 50 days with

observations taken every seven days post-inoculation. The disease severity was assessed using a scoring system where 0 indicated no infection, and scores ranged from 1 (infection length 1–2 cm) to 7 (infection length greater than 12 cm). The area under the disease progress curve (AUDPC) was calculated using the formula

$$\text{AUDPC} = 1/2 \sum (2A_1 + 2A_2 \dots A_n)$$

This approach calculates the trapezoidal areas under the disease progression curve over 50 days, with observations recorded at seven-day intervals (Prova et al., 2018).

Evaluation of the Role of Treatments in Inducing Peroxidase Activity in Eggplant:

To assess the ability of different treatments to induce plant resistance, peroxidase enzyme activity was measured at 7, 14, and 21 days after foliar application of the following treatments. The treatments included: 1. Nano-silicon oxide + pathogenic fungus, 2. Conventional silicon oxide + pathogenic fungus, 3. Topsin M fungicide + pathogenic fungus, 4. Plant + pathogenic fungus (without treatment), 5. Nano-silicon oxide alone, 6. Conventional silicon oxide alone, 7. Topsin M fungicide alone, 8. Control (untreated plant). Enzymatic activity of peroxidase was determined according to Hammerschmidt *et al.* (1982), which consisted in the homogenization of 1 g of treated *Solanum melongena* leaves in 2 mL of sodium phosphate buffer. Filtered solution was then centrifuged (20 min, 6000

rpm, 4 °C) and the supernatant was recovered as source of enzyme and mixed 100 µL of the enzyme extract with 1.5 mL of a 0.5 M pyrogallol solution in a spectrophotometer cuvette. The reaction was started by the addition of 100 µL of 1% hydrogen peroxide. Ten readings of absorbance at 420 nm were obtained for 30 seconds using a spectrophotometer. Peroxidase activity was represented by rate of change in absorbance (Abs) per minute per gram of fresh weight (FW) using the following equation:

$$\text{Change in absorbance} = \text{Absorbance} / [\Delta\text{Time} \times \text{Fresh weight (g)}]$$

RESULTS AND DISCUSSION

Isolation and Identification of the Pathogenic Fungus from Infected Eggplant Plants:

The results of isolation and identification from eggplant plants infected with white mold disease revealed five isolates of *Sclerotinia sclerotiorum* on PDA medium (SS1, SS2, SS3, SS4, SS5). The fungal colonies were characterized by white growth, completely covering the petri dish within three days, and the formation of sclerotia after seven days of incubation (Figure 1). The sclerotia varied in size, number, and distribution patterns within the dishes depending on the isolate. These findings align with the characteristics of the pathogenic fungus described in previous studies (Melo et al., 2019; Ordonez-Valencia et al., 2015; Zanatta et al., 2019).

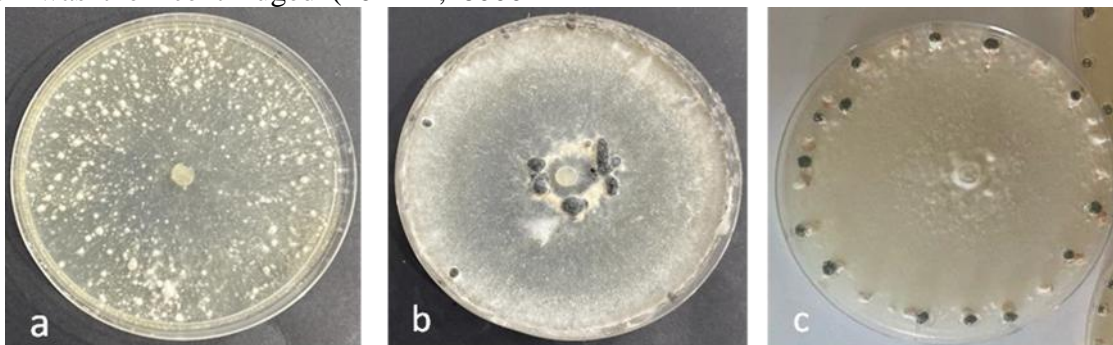


Figure 1. Morphological characteristics of *Sclerotinia sclerotiorum*: (a) fungal mycelium, and (b, c) fungal mycelial growth on PDA medium and sclerotial distribution

Pathogenicity Testing of Fungal Isolates in Pots:

The results in Table (1) indicate varying pathogenic abilities of the fungal isolates in pots under lath house conditions. The infection percentage ranged from 11.12% to 100% among the isolates. The SS5 isolate exhibited

the highest pathogenicity, with an infection percentage of 100% and a severity of 82.5%, compared to the SS4 isolate, which recorded 11.12% infection and 9.51% severity. In contrast, the control treatment showed 0% infection and severity. The results

demonstrated the ability of the fungal isolates to cause disease, despite variations in infection percentages. These findings are consistent with Al-Karaawi and Alwaily (2022), who confirmed the pathogenicity of *Sclerotinia*

sclerotiorum isolates on eggplant as well as other plant hosts (O’Sullivan et al., 2021; Smolinska & Kowalska, 2018). Based on these results, the SS5 isolate was selected for conducting the remaining experiments.

Table 1. Pathogenicity testing of fungal isolates on eggplant plants in pots.

Isolate code	Infection percentage (%)	Disease severity (%)
SS1	88.9	44.4
SS2	88.9	50.8
SS3	88.9	76.2
SS4	11.12	9.5
SS5	100.00	82.5
Control	0.00	0
L.S.D 0.05	2.145	27.96

*Each number represents the average of 3 replicates
Molecular Identification of the Most Pathogenic Fungal Isolate: The results of DNA amplification for the most pathogenic fungal isolate, SS5, using the diagnostic primer pair (ITS4 / ITS1), revealed the presence of a DNA band with a molecular weight of 550 bp on agarose gel (Figure 2). This finding aligns with the results of Al-Juboory and Al-Kubaisei (2023), who demonstrated the effectiveness of the ITS4 / ITS1 primer pair in identifying *S. sclerotiorum* isolates. The amplified ITS region is commonly used to detect genetic variations

among isolates, as this genetic region is conserved and provides species- or genus-specific sequences that distinguish it from other fungi (Abdul-Karim et al., 2023). The results of the nucleotide sequence analysis for the pathogenic fungal isolate showed 100% similarity with global isolates retrieved from the NCBI gene bank (Figure 3). The nucleotide sequences of the pathogenic fungal isolate were deposited in the gene bank under accession number OR497817.

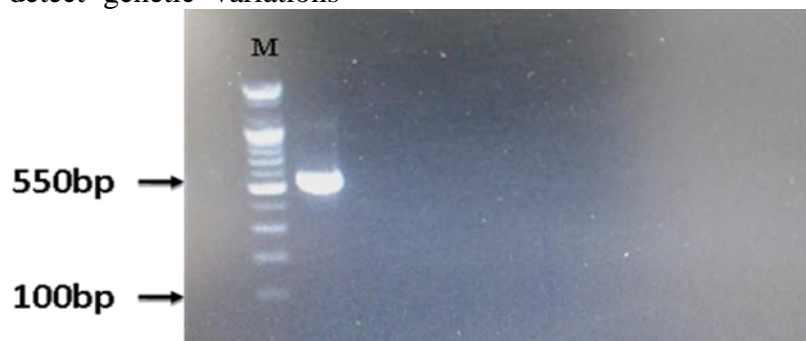


Figure 2. Electrophoresis of the pathogenic fungal isolate on agarose gel for amplified DNA fragments

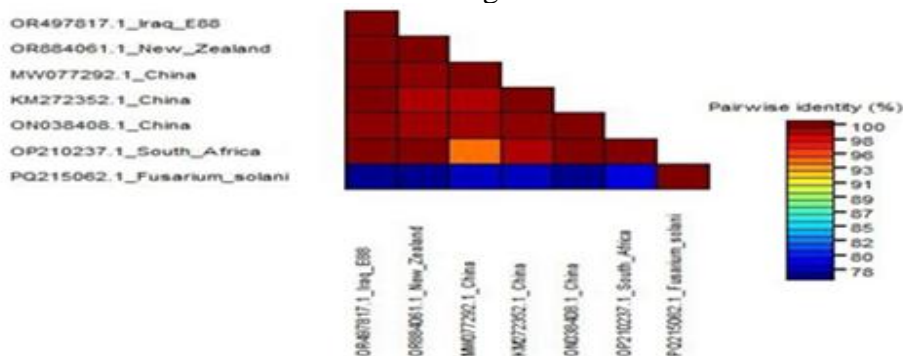


Figure 3. Genetic similarity values of the *S. sclerotiorum* isolate with its global counterparts

Evaluation of the Protective Role of Topsin M 70 and Silicon Oxide in Controlling the Pathogen in the Field:

The results in Table (2) demonstrated the effectiveness of the protective treatments in providing significant protection for eggplant plants against white mold disease. The Topsin M 70 treatment showed the best reduction in infection percentage and severity, achieving 0% for both cultivars. Nano-silicon oxide treatment resulted in an infection percentage and severity of 13.87% and 5.44% for the "Wasam" cultivar, respectively, and 16.62% and 7.62% for the "Barcelona" cultivar, respectively. In comparison, the control treatment recorded 50% and 25.69% for the "Wasam" cultivar and 50% and 27.94% for the "Barcelona" cultivar, respectively. The results indicated that the "Wasam" cultivar showed higher efficacy in reducing the infection percentage and severity, recording 9.64% and 16.24%, respectively,

compared to the "Barcelona" cultivar, which recorded 11.17% and 16.62%, respectively. This difference may be attributed to the inherent defenses or the rapid activation of defensive responses in the "Wasam" cultivar when exposed to external stress factors. The results demonstrated the effect of the applied treatments in reducing the area under the disease progress curve (AUDPC) for white mold disease caused by *S. sclerotiorum* on the "Wasam" and "Barcelona" eggplant cultivars, compared to the control treatment (no spray), which recorded the highest AUDPC values of 130.95 and 143.21, respectively. The chemical fungicide Topsin treatment resulted in the lowest AUDPC value of 0.0 for both cultivars. The nano-silicon oxide treatment recorded AUDPC values of 27.39 and 38.23, respectively, for the two cultivars, while the conventional silicon oxide treatment recorded values of 38.35 and 47.37, respectively.

Table 2. Effectiveness of nano and conventional silicon oxide and the chemical fungicide on the infection percentage and severity caused by the pathogenic fungus *S. sclerotiorum* in the field

Cultivars	Treatments	Infection percentage (%)	Severity percentage (%)
Wasam	Nano-silicon oxide	13.87	5.44
Wasam	Conventional silicon oxide	16.63	7.43
Wasam	Topsin M 70 fungicide	0.00	0.00
Wasam	Control	50.00	25.69
Barcelona	Nano-silicon oxide	16.62	7.62
Barcelona	Conventional silicon oxide	18.60	9.11
Barcelona	Topsin M 70 fungicide	0.00	0.00
Barcelona	Control	50.00	27.94
L.S.D 5%		0.31**	0.43**
Cultivars	Mean cultivars	Mean cultivars	
Wasam		9.64	16.24
Barcelona		11.17	16.62
L.S.D 5%		0.21	0.16**

These findings highlight the ability of the applied treatments to induce resistance in eggplant plants, effectively reducing the area under the disease progress curve (AUDPC) through various mechanisms. These include the mechanical enhancement of plant structural defenses and the activation of biochemical defenses. Nano-silicon plays a crucial role in reducing disease severity by depositing silica in plant epidermal cells and intercellular spaces, thereby increasing leaf thickness and improving the xylem and phloem structures. This forms a physical

barrier against pathogens (Al-Hilfie & Hussein, 2024). Hasan et al. (2020) reported that the use of nano-silicon particles reduced the infection percentage and severity of gray mold disease in faba bean caused by *Botrytis fabae*, treated plants exhibited increased cell wall thickness. Similarly, Zhang et al. (2023) demonstrated the effect of nano-silicon in enhancing resistance in turnip plants and reducing the disease index caused by *S. sclerotiorum*, with a 39–52% decrease compared to untreated control plants. The effectiveness of the Topsin fungicide is attributed to its mode of action,

which disrupts cellular processes and inhibits pathogen growth by interfering with key metabolic pathways. It affects cellular functions such as meiosis and intracellular transport, in addition to its selective toxicity to microorganisms (Wilson & Williamson,

2008). The use of Topsin M 70 at a concentration of 1 g/L effectively inhibited the growth of *Fusarium oxysporum*, the causative agent of wilt and root rot in beans, achieving a 100% inhibition rate (Salih & Al-Mansoury, 2021).

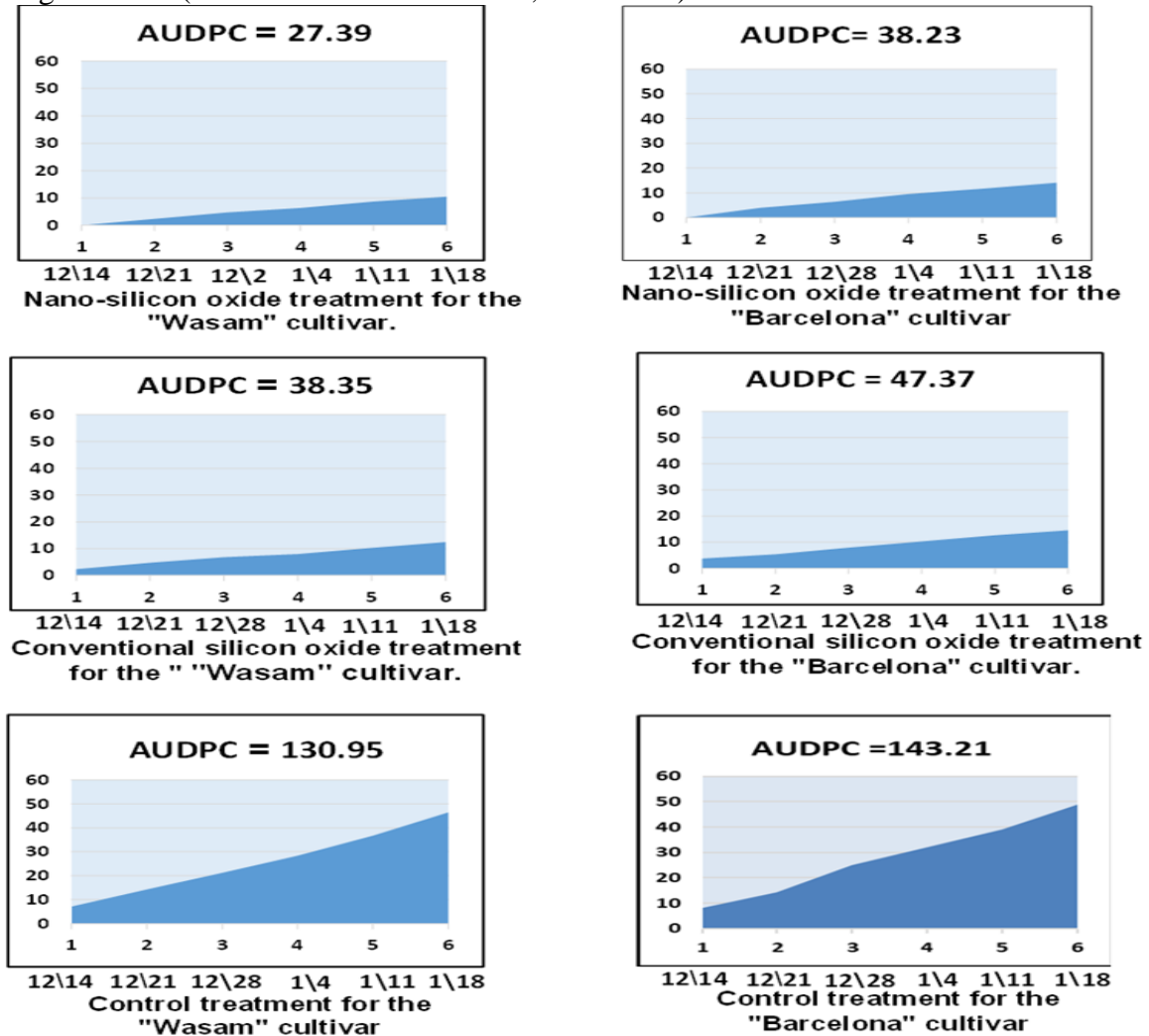


Figure 4. Area under the disease progress curve (AUDPC) for *S. sclerotiorum* on the eggplant cultivars "Wasam" and "Barcelona" under greenhouse conditions

Evaluation of the Role of Treatments in Inducing Peroxidase Activity in Eggplant:

The results revealed significant differences in the effect of treatments on peroxidase enzyme activity seven days after spraying, with the highest enzyme activity recorded after 14 days (Figure 5). The nano-silicon oxide + pathogenic fungus treatment and the conventional silicon oxide + pathogenic fungus treatment showed the highest activity, recording 26.43 and 25.90 U/mL-1 for the "Wasam" cultivar and 24.91 and 23.97 U/mL-1 for the "Barcelona" cultivar, respectively. In contrast, the chemical fungicide treatment

recorded values of 23.03 and 16.83 U/mL-1 for "Wasam" and 23.37 and 13.00 U/mL-1 for "Barcelona," respectively. The pathogen-only treatment (control) recorded lower values of 17.80 U/mL-1 for "Wasam" and 16.50 U/mL-1 for "Barcelona." These findings align with the study conducted by Wang et al. (2022), which reported a significant increase in peroxidase enzyme activity (ranging from 78.56% to 157.47%) in the leaves of tomato plants treated with nano-silicon oxide. Nano-silicon particles were shown to enhance antioxidant enzyme activity in tomato plants, boosting their ability to resist bacterial wilt caused by *Ralstonia*

solanacearum. Similarly, Abdelrhim et al. (2021) demonstrated that nano-silicon oxide particles induced the accumulation of defense-related compounds and mitigated oxidative stress by stimulating enzymatic defense mechanisms Guaiacol peroxidase, Catalase and Polyphenol oxidase and non-enzymatic defenses (phenols and flavonoids) to protect wheat seedlings from *Rhizoctonia solani* infection. The importance of the peroxidase enzyme in plants is significant, as it plays a key role in various metabolic pathways (Freitas et al., 2024; Pandey et al., 2017). Additionally, it contributes to plant defense mechanisms by stimulating the synthesis of lignin and suberin, increasing the polymerization of phenolic compounds into lignin-like substances, and depositing these materials in cell walls and papillae. This process impedes the growth and development of pathogens. Peroxidase also participates in ethylene production, which triggers the synthesis of phytoalexins, whose concentration increases when plants are exposed to pathogens (Almagro et al., 2009; Prasannath, 2017). The difference in potency of tested substances in activating peroxidase enzyme activity is due to their different modes of

action. Topsin M 70, as the consensus component of its active ingredient thiophanate-methyl (TM), will be mutated and internalized into a form of methyl benzimidazole carbamate when entering the plant. This compound exhibits fungicidal activity and interferes with the mitotic phase of fungal cells, preventing disease development (Bai et al., 2024; Wilson & Williamson, 2008). On the other hand, both nano- and bulk silicon have reported fungitoxic effects linked with plant defense induction. These responses include the activation of several defense-related enzymes such as peroxidase, phenylalanine ammonia-lyase, and polyphenol oxidase, as well as structural defenses such as lignin and suberin deposition (Hasan et al., 2020). The local cultivar "Wasam" showed greater responsiveness to the treatments, with a significantly higher peroxidase enzyme content compared to the commercial cultivar "Barcelona." This indicates that the "Wasam" cultivar possesses stronger defensive mechanisms, which contribute to inhibiting fungal mycelium growth and preventing its penetration into epidermal cells.

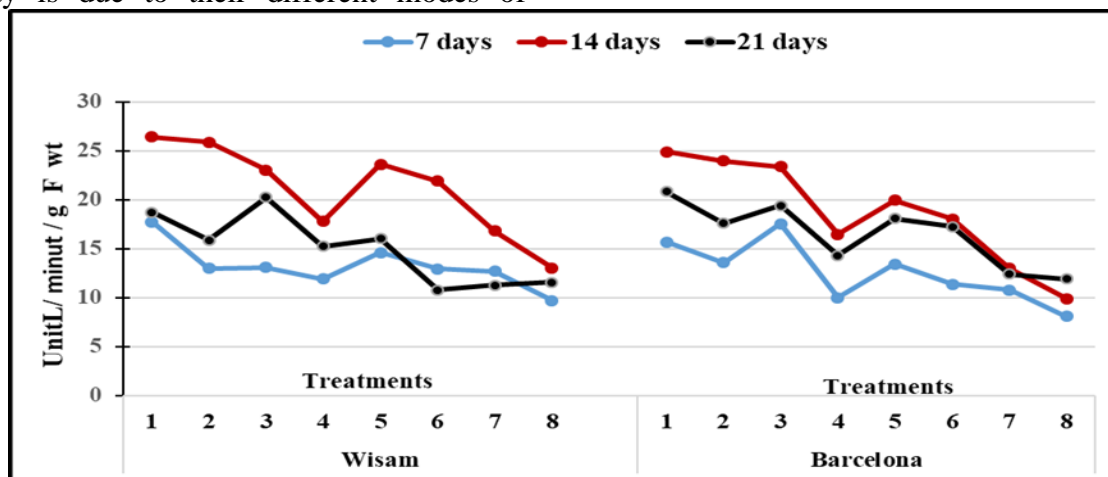


Figure 5. Efficiency of elicitation factors in the activity of the enzyme peroxidase in the plant. 1. nano-silicon oxide + pathogenic fungus, 2. conventional silicon oxide + pathogenic fungus, 3. Topsin M 70 + pathogenic fungus, 4. plant + pathogenic fungus, 5. nano-silicon oxide, 6. conventional silicon oxide, 7. Topsin M 70, and 8. control (plant only).

CONCLUSION

The study confirmed that *Sclerotinia sclerotiorum* isolate SS5 was the most pathogenic isolate associated with white mold disease on eggplant. Topsin M 70 at 1 g/L achieved complete protection against disease

development in both cultivars, while nano-silicon oxide reduced infection and disease severity more effectively than conventional silicon oxide. The treatments also enhanced peroxidase activity, especially in the Wasam

cultivar, indicating an induced defense response against the pathogen.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR/S DECLARATION

We confirm that all figures and tables in the manuscript are original to us. Additionally, any figures and images that do not belong to us have been incorporated with the required permissions for re-publication.

Ethical clearance and animal welfare: Not applicable./

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AUTHOR'S CONTRIBUTION STATEMENT

Zahraa H. Abdulkhaleq conducted the experimental work and prepared the manuscript. Eman K. Abdul-Karim supervised the study, contributed to the methodology, and reviewed the manuscript.

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الدور الوقائي للمبيد الفطري Topsin M 70 وأوكسيد السليكون ضد مرض العفن الأبيض على صنفين من الباذنجان

زهراء هشام عبدالخالق، إيمان خليل عبدالكريم
قسم وقاية النبات، كلية علوم الهندسة الزراعية، جامعة بغداد، بغداد، العراق

المستخلص

أجريت هذه الدراسة في دائرة وقاية المزروعات / مجمع الأقسام الفنية خلال 2023 لتشخيص المسبب المرضي لمرض العفن الأبيض وتقدير فعالية مبيد Topsin M 70 وأوكسيد السليكون في السيطرة على تطور المرض ونسبة وشدة الإصابة. أظهرت النتائج أن عزلة الفطر *Sclerotinia sclerotiorum* (SS5) كانت الأكثر إمرضية بنسبة إصابة 100% وشدة إصابة 82.5%. أظهرت نتائج الترحيل الكهربائي للحامض النووي DNA لعزلة SS5 وجود حزمة واحدة بوزن جزيئي 550 bp ونسبة تطابق 100% مع عزلات عالمية مسجلة في بنك الجينات NCBI، وسجلت التتابعات النيوكليوتيدية تحت رقم التسجيل. OR497817. أدى استخدام أوكسيد السليكون النانوي (0.001 غم/لتر) وأوكسيد السليكون العادي (3 غم/لتر) والمبيد الكيميائي Topsin M 70 (1 غم/لتر) رشاً على نباتات الباذنجان وسام وبرشلونة الى تأثير وقائي. سجل المبيد الكيميائي نسبة إصابة وشدة 0% للصنفين ، تلاه أوكسيد السليكون النانوي بنسبة إصابة 13.87% وشدة 5.44%، بينما سجل أوكسيد السليكون العادي 63.16% و7.43% مقارنة بالسيطرة التي بلغت 50% و25.69%. وكانت المساحة تحت منحنى تطور المرض 130.95 و143.21 في معاملة السيطرة، لكنها انخفضت إلى 0% باستخدام المبيد الكيميائي.

الكلمات المفتاحية: استحثاث مقاومة؛ الإنزيمات؛ المساحة تحت منحنى تطور المرض؛ *Sclerotinia sclerotiorum*.