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Utilizing certain disinfectants as alternatives to fungicides to manage *Macrophomina* root rot in sugar beet

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Abstract

This study aimed to determine the fungicidal effects of certain disinfectants, namely hydrogen peroxide (H_2O_2), n-alkyldimethylbenzylammonium chloride (n-ADBAC), and potassium permanganate ($KMnO_4$), on *M. phaseolina*, the causal pathogen of root rot in sugar beet (*Beta vulgaris* L.), *in vitro* and under greenhouse conditions. Mycelial growth of the pathogen was inhibited with different concentrations (0.00, 100, 200, 400, and 600 ppm) of the tested compounds. $KMnO_4$ at 600 ppm was the most effective in inhibiting the growth of the pathogen (77.41%) when compared to other treatments. In greenhouse tests, treating pathogen-infested soil with these disinfectants reduced the disease severity of *Macrophomina* root rot in sugar beet. The highest reduction in disease severity was achieved with 600 ppm $KMnO_4$. In addition, all treatments improved root agronomic characteristics, such as total soluble solids (TSS) and sugar percentage. These findings suggest that these disinfectants could serve as promising alternatives to traditional fungicides for managing *Macrophomina* root rot in sugar beet.

Keywords: *Beta vulgaris*, *Macrophomina phaseolina*, root rot, disinfectants, total soluble solids, sugar percent.

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1. Introduction

Sugar beet (*Beta vulgaris* L.) belongs to the Chenopodiaceae family and has a high sucrose concentration, which is used for sugar production (Zicari et al., 2019). Sugar extracted from sugarcane and sugar beet is used as a sweetener in domestic food and as an ingredient in the food industry for sweet-flavored substances. Sugar is mainly referred to as sucrose and, to some extent, as glucose and fructose (Duraisam et al. 2017). *Macrophomina phaseolina* is a soil-borne, necrotrophic pathogen present worldwide that affects more than 500 plant species (Abass et al., 2021; Marquez et al., 2021; Babu et al., 2007). *M. phaseolina* is a facultative saprophyte that survives in the soil through the formation of microsclerotia, which are pseudoparenchymatous tissue masses resistant to adverse environmental conditions (Shaner et al., 1999). Disinfectants are of great importance in eliminating microbes in various applications. Hydrogen peroxide (H_2O_2) is used for surface sterilization and is highly effective at inhibiting microbes. All H_2O_2 concentrations significantly reduced the linear growth of all the tested fungi. However, a 2% concentration completely inhibited the growth of *Rhizoctonia solani*, *Pythium sp.*, and *Fusarium solani* (Ali, 2018). H_2O_2 participates in many resistance mechanisms, including reinforcement of the plant cell wall, phytoalexin production, and enhancement of resistance to various stresses (Quan et al., 2008). It can also be used to control plant diseases, such as root rot and wilt disease in thyme (Ali, 2018) and alfalfa rust disease (Abdel-Monaim et al., 2012). Kyeong-Hwan et al. (2014) utilized hydrogen peroxide vapor in the agricultural field to inhibit the growth of pathogenic microorganisms. This was due to the fact that hydrogen peroxide could enter the microbial interior and react with lipid double bonds in the cell wall, affecting proteins, lipids, and polysaccharides, changing cell permeability, and ultimately causing cell lysis and death (McDonell and Russell, 2001). However, multiple studies have demonstrated the significance of H_2O_2 in promoting plant growth and increasing productivity (Khandaker et al., 2012). Orabi et al. (2015) stated that a lower level of treatment with H_2O_2 can have a significant positive effect on plant growth, growth regulators, antioxidant enzyme activity, fruit yield and quality of tomato. Quaternary ammonium compounds (QACs) are surfactants that penetrate the membranes of microorganisms, destroying proteins and nucleic acids, and leading to cell death (Gerba, 2015). Numerous studies have reported the effectiveness of QACs in controlling bacterial, fungal, and viral plant diseases (Bennett et al., 2011; Strobel, 2006; Tubajika, 2006). Among QACs, n-ADBAC is well-known for its strong antibacterial and antifungal properties (Oblak et al., 2013; Ohta et al., 2008). The textile industry has also utilized it as an antibacterial agent or insecticide (Kim and Sun, 2001). Studies have demonstrated that BAC, a potent bactericidal and fungicidal agent, reduces the size of organisms in multi-dose containers (Noecker and Miller, 2011). However, Izquierdo-García et al. (2021) and Nguyen et al. (2019) utilized a 1:100 dilution to completely prevent the survival of all *F. oxysporum* f. sp. *cubense* propagules over the length of all contact times, whether or not soil was present. QAC products have been reported to be effective against fungal plant pathogens (Bika et al., 2021; Baysal-Gurel et al., 2015). In a similar study, Tubajika (2006) showed that the colony diameter and mycelial dry weight of *Physalospora vaccinii* were reduced at 1,000 ppm ADBAC in *in vitro* growth tests. Potassium permanganate ($KMnO_4$) is a powerful oxidizing agent that can be used to

control plant diseases (Goutam and Bajpai, 2019). It acts as a disinfectant and fungicide, helping to eliminate pathogens such as fungi and bacteria. It is used for seed treatment, soil disinfection, and to control diseases such as damping-off, powdery mildew, and wilt in various crops (Sanchez-Saldana and Saenz, 2002). It can also be used as a fungicide to control a wide range of fungal infections (Goutam and Bajpai, 2019). Additionally, it can help reduce disease pressure and promote healthy root development in plants. This study aimed to determine the fungicidal effects of certain disinfectants, that is, hydrogen peroxide, n-alkyldimethylbenzylammonium chloride, and potassium permanganate, on *M. phaseolina*, the causal pathogen of root rot in sugar beet, *in vitro* and under greenhouse experiments. In addition, the impact of these treatments on yield parameters, such as total soluble solids (TSS) and sugar percentage, was investigated.

2. Materials and methods

2.1 Isolation and identification of the causal pathogen

Samples of naturally infected sugar beet plants were collected from various locations within the Assiut and El-Behera governorates in Egypt. Additionally, the collar portion of the infected plants was cut into 3-5 mm thick tissue sections, sterilized with 1% sodium hypochlorite solution for two minutes, rinsed thrice in sterilized distilled water, and dried on sterilized filter paper at room temperature. The developing hyphae were examined under a microscope at low magnification for typical hyphal growth of *M. phaseolina* and incubated at 25°C for 15 days. The purified fungi were identified based on their morphological and microscopic characteristics, as described by Sneh et al. (1991). Hyphal tips were transferred to PDA medium. Hyphal-tipped isolates identified as *M. phaseolina* were transferred to PDA slants and stored for further studies.

2.2 Pathogenicity assay

2.2.1 Inoculum production

Barley medium (75 g barley, 25 g clean sand, 2 g sucrose, 0.1 g yeast, and 100 ml distilled water) was used in the experiments. The barley medium was autoclaved for 20 min at 121°C on two separate days (Imran et al., 2021). *M. phaseolina* isolates were grown on potato dextrose agar (PDA) for 7 days. Each barley medium bottle was inoculated with two plugs (5 mm diameter) taken from the margin of a 1-week-old culture of the isolates grown on PDA medium in Petri dish. The bottles were then incubated at 25°C in the dark for two weeks.

2.2.2 Plant material and inoculation

Sand clay soil and plastic pots (30 cm Ø) were sterilized with a 5% formaldehyde solution and allowed to dry. Inoculum of each pathogen isolate was added to the soil in pots at 1% (w/w), one week before sowing (1 g inoculum/100 g soil), mixed well, and thoroughly irrigated. Three Pleno cultivar sugar beet seeds were sown in each pot. Three replicates were used for each isolate. Pots containing non-infested soil mixed with 1% barley grain medium were used as controls. The Agricultural Botany Department, Plant Pathology Branch, Faculty of Agriculture, Al-Azhar University (Assiut Branch), Assiut, Egypt, conducted the experiment in a greenhouse during the 2018/2019 growing season. The pots were irrigated and fertilized regularly in a greenhouse. Disease severity was recorded 120 days after sowing.

2.2.3 Assessment of root-rot severity

The severity of infection by root rot was assessed using the devised 0-7 scale by Engelkes and Windels (1996) as follows: 0 = No visible lesions, 1 = Arrested lesions at point of inoculation, 2 = Less than 5% shallow, dry rot canker, 3 = 5 to 24% deep, dry rot canker, 4 = 25 to 49% extensive rot, 5 = 50 to 89% rot extensive into interior root, 6 = 90 to less than 100%, most dead foliage, 7 = 100% dead plants.

2.2.3 Source of disinfectants

Hydrogen peroxide (H_2O_2) and potassium permanganate ($KMnO_4$) were obtained from El Nasr Pharmaceutical Chemicals Company, Abu Zaabal, Egypt. The n-alkyldimethylbenzylammonium chloride product was purchased from United Promotions, Inc. (Atlanta, USA).

2.4 Impacts of H_2O_2, n-ADBAC and $KMnO_4$ on *M. phaseolina* mycelial growth

The effectiveness of H_2O_2, n-ADBAC, and $KMnO_4$ at various concentrations (0.00, 100, 200, 400, and 600 ppm) on the mycelial growth of the pathogen was assessed *in vitro*. The concentrations were added to the PDA medium. 8-mm plugs of *M. phaseolina* culture grown on PDA plates were placed in the middle of PDA plates with the added compounds. Three replicate Petri dishes were used for each concentration. PDA Petri dishes without treatment served as controls. After a 7-day incubation period, the colony diameter of each plate was measured. The average length of the longest and shortest diameters was used to calculate the colony diameter. The following formula was used to determine the inhibition of mycelial growth: [(control radial growth – disinfectant-amended radial growth)/control radial growth] × 100.

2.5 Impact of H_2O_2, n-ADBAC and $KMnO_4$ soil treatments on managing root rot diseases under greenhouse conditions

The experiment was conducted during the 2019/2020 and 2020/2021 seasons at the Plant Pathology Branch of the Agricultural Botany Department at Al-Azhar University (Assiut Branch), Assiut, Egypt. *M. phaseolina* inocula were prepared and added to the soil, as mentioned before. Three Peleno sugar beet seeds were planted in sterile soil. One month after planting, each tested compound at a concentration of 600 ppm was added as a soil drenching. The treatments were applied twice at four-week intervals. The fungicide Double 56% {(Hymexazol 16% WP (W/W); Thiophanate Methyl 40% WP (W/W)} obtained from Shora Company for Agricultural Chemicals, Egypt, was applied at a concentration of 1 g/l water. The treatment with these compounds was repeated 90 days after the initial treatment. Control was achieved by planting sugar beet seeds in infected soil without treatment. Three pots were used as replicates for each treatment. As mentioned earlier, disease severity was calculated 120 days after planting. A hand refractometer was used to measure the T.S.S. percentage in the juice of fresh roots. Sugar content was measured at the sugar factory laboratory (Nobaryia Sugar Refining Company) using the standard polarimetric method described by Schneider et al. (2002).

2.6 Statistical analysis

MSTAT-C (1991) version 2.10 was used for all statistical analyses. The data were analyzed using one-way analysis. The results are presented as the mean and standard deviation (mean ± SD), and all measurements were performed in triplicate. The means were compared using Bartlett's test. The statistical

significance threshold was set at P = 0.05 (Gomez and Gomez, 1984).

3. Results

3.1 Isolation and identification of the causal pathogen

From diseased sugar beet plants (Figure 1A and B) gathered from various locations in the El-Behera and Assiut Governorates, 11 fungal isolates of *M. phaseolina* were obtained: seven and four isolates, respectively. The obtained isolates were identified as *M. phaseolina* by microscopic and cultural examinations. The isolates were identified based on the morphological and microscopic features of the mycelium (Figure 1C).

Figure 1: (A) Symptoms of *Macrophomina* root rot disease on sugar beet root, (B) cross-section of sugar beet root infected with *M. phaseolina,* and (C) mycelial growth of *M. phaseolina* on potato dextrose agar.

3.2 Pathogenicity test of *M. phaseolina* isolates on sugar beet plants

All *M. phaseolina* isolates infected the sugar beet cultivar and caused typical symptoms of root rot disease, as shown in Figure (2). The isolates tested for their ability to cause root rot in sugar beet plants varied greatly in terms of their disease severity. Isolate No. 8 (66.93%) was responsible for the greatest percentage of disease severity, followed by isolate No. 3 (58.43%) and No. 1 (52.56%). In comparison, isolates No. 9 and No. 10 were the least likely to cause infections (27.15 and 29.00%, respectively), with isolate No. 6 coming in following (34.44%). Based on the aforementioned findings, isolate No. 8 was used in subsequent tests.

3.3 Inhibitory effect of certain disinfectants on *M. phaseolina* mycelial growth *in vitro*

The effects of H_2O_2, n-ADBAC, and $KMnO_4$ on *M. phaseolina* mycelial growth were assessed *in vitro* using PDA medium (Table 1). Compared to the control, the addition of all tested compounds to the medium significantly inhibited the growth of *M. phaseolina*. Pathogen growth was most significantly inhibited by the addition of $KMnO_4$ at a 600 ppm concentration, which was followed by n-ADBAC. In contrast, H_2O_2 reduced mycelial growth the least. Furthermore, increasing the concentration of these compounds gradually increased the reduction in mycelial growth of the pathogen, with higher concentrations being more effective.

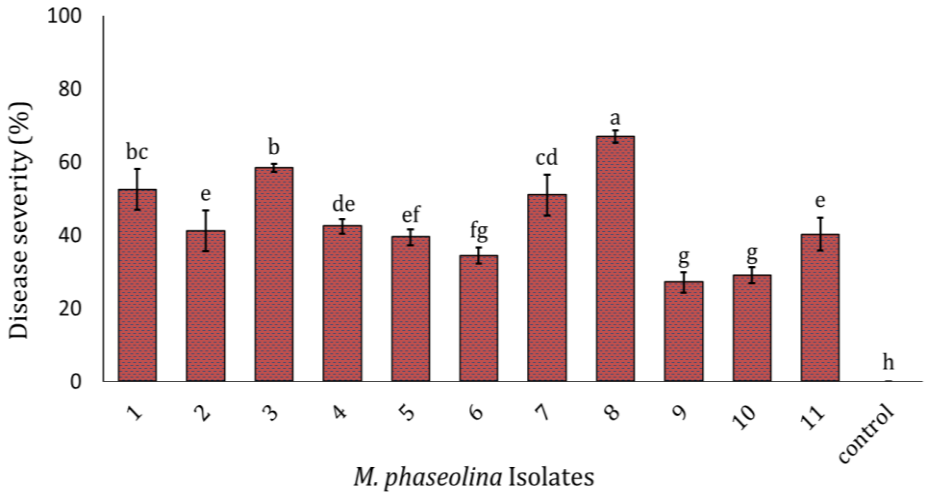

Figure 2: (A) Pathogenicity tests of *M. phaseolina* isolates on sugar beet plants under greenhouse conditions. The values are the mean ± standard deviation of three replicates. The same letters following column values are not significant according to Bartlett's test ($P \leq 0.05$).

Table 1: Effects of different H_2O_2, n-ADBAC, and $KMnO_4$ concentrations on *M. phaseolina* mycelial growth *in vitro*.

Concentration (ppm)	Growth inhibition (%)		
	H_2O_2	n-ADBAC	$KMnO_4$
0 (control)	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
100	21.11±1.11[b]	24.81±1.70[b]	31.48±1.70[b]
200	38.89±1.11[c]	44.07±0.64[c]	51.11±1.11[c]
400	54.81±0.65[d]	58.89±1.11[d]	60.74±0.64[d]
600	64.03±2.32[e]	68.52±3.21[e]	77.41±0.64[e]

The values are the three replicates' mean ± standard deviation. The same letters following column values are not significant, according to the Bartlett's test ($P \leq 0.05$).

3.4 Impact of disinfectants compounds and Double fungicide on sugar beet root rot caused by *M. phaseolina* in greenhouse experiments

The results in Table (2) show that all the disinfectant compounds tested and the double fungicide significantly reduced the severity of root rot in sugar beet plants caused by *M. phaseolina*. The findings of this study showed that compared to the control, the addition of $KMnO_4$ resulted in the least disease severity and the highest disease protection, with disease severity percentages of 17.33±2.11 and 16.71±2.11 in the 2019/2020 and 2020/2021 seasons, respectively. Generally, the fungicide Double 56% had the highest effect on controlling the disease. In contrast, H_2O_2 resulted in the least reduction in disease severity and disease protection, at 21.45±2.55 and 23.15±2.44% in both seasons. In addition, the data revealed significant differences among all treatments in the disease severity percentage in both seasons.

3.5 Impacts of disinfectants compounds and Double fungicide on TSS and sugar percentage in sugar beet roots

All treatments, including H_2O_2, n-ADBAC, and $KMnO_4$, increased TSS and sugar percentage in the 2019–2020 and 2020–2021 seasons compared to the untreated control

plants, as demonstrated by the results in Table 3. Data revealed that n-ADBAC recorded the highest percentages of TSS and sugar in sugar beet roots, which were 32.10±1.10 and 34.40±1.90 in both seasons, respectively. In contrast, Double fungicide resulted in the lowest values of TSS and sugar percentage, which were 23.70±1.45 and 22.50±1.60 in both seasons, respectively. The results indicate that there are no significant differences between H_2O_2 and n-ADBAC in their effects on TSS. In addition, there were no significant differences between n-ADBAC and $KMnO_4$ in their effects on sugar content.

Table 2: Effects of H_2O_2, n-ADBAC, $KMnO_4$ and Double fungicide on sugar beet root rot caused by *M. phaseolina* in greenhouse trials.

Treatments	Season 2019/2020		Season 2020/2021	
	Disease severity (%)	Disease reduction (%)	Disease severity (%)	Disease reduction (%)
H_2O_2	21.45±2.55^{b}	66.59	23.15±2.44^{b}	59.01
n-ADBAC	17.33±2.11^{bc}	73.01	20.32±1.88^{bc}	64.02
$KMnO_4$	14.07±1.99^{cd}	78.09	16.71±2.11^{cd}	70.41
Double 56%	10.18±2.22^{d}	84.15	12.45±2.12^{d}	77.96
Control	64.21±2.66^{a}	-	56.48±3.11^{a}	-

The values are the three replicates' mean ± standard deviation. The same letters following column values are not significant, according to the Bartlett's test ($P \leq 0.05$).

Table 3: Effects of $KMnO_4$, n-ADBAC, H_2O_2, and double fungicide on the sugar and TSS content of sugar beet roots.

Treatments	TSS (%)		Sugar (%)	
	Season 2019/2020	Season 2020/2021	Season 2019/2020	Season 2020/2021
H_2O_2	29.20±1.40^{a}	31.30±3.15^{a}	19.15±0.88^{a}	18.51±1.33^{b}
n-ADBAC	32.10±1.10^{a}	34.40±1.90^{a}	20.31±0.77^{a}	21.46±1.09^{a}
$KMnO_4$	25.40±1.30^{b}	23.10±1.60^{b}	20.07±0.88^{a}	20.71±1.11^{a}
Double 56%	23.70±1.45^{bc}	22.50±1.60^{b}	17.52±1.22^{b}	18.24±1.44^{b}
Control	22.00±1.80^{c}	23.60±1.30^{b}	15.61±1.11^{c}	15.36±1.11^{c}

The values are the three replicates' mean ± standard deviation. The same letters following column values are not significant, according to the Bartlett's test ($P \leq 0.05$)

4. Discussion

Sugar beet is cultivated for its high sucrose content, making it a major source of sugar production worldwide (Abou-Elwafa et al., 2020). Root rot diseases in sugar beet can cause significant yield loss, impacting both the quantity and quality of the harvest (Harveson, 2007). These diseases can lead to reduced root weight, lower sucrose content, and decreased juice purity, ultimately affecting the profitability of sugar beet production (El-Mansoub et al., 2020). In this study, the efficacy of H_2O_2, n-ADBAC, and $KMnO_4$ against root rot disease in sugar beet was investigated in vitro and in greenhouse experiments. The effects of H_2O_2, n-ADBAC, and $KMnO_4$ on *M. phaseolina* mycelial growth were assessed *in vitro*. In comparison to the control, each tested compound significantly slowed growth when added to the medium. In the current study, the data indicated that $KMnO_4$ had the strongest effect on the pathogen at a concentration of 600 ppm (77.41%), followed by n-ADBAC. These results are consistent with Goutam and Bajpai (2019), who showed that $KMnO_4$ inhibited the growth of fungi associated with mustard seeds such as *Aspergillus niger*, *Rhizopus nigricansis*, *A. flavus*, *A. fumigates*, and *A.*

luchuansis compared to the control. $KMnO_4$ is a powerful oxidizing agent that alters the cell walls of pathogenic microorganisms, interferes with their DNA structure, and exerts effective antimicrobial activity against protozoa, fungi, bacteria, and viruses (Sanchez-Saldana and Saenz, 2002). In a similar study, Tubajika (2006) showed that the colony diameter and mycelial dry weight of *Physalospora vaccinii* were reduced at 1,000 ppm ADBAC *in vitro* growth tests. Mild or no reduction in fungal growth and mycelial dry weight was observed at concentrations less than 100 ppm compared to the control. H_2O_2 exhibited the lowest inhibition of pathogen growth. Previous studies have also shown that it can trigger the plant's defense systems, such as increasing the activity of enzymes such as chitinase and peroxidase, leading to a significant increase in lignin and suberin. Furthermore, Copes (2009) noted that H_2O_2 is crucial for lignification and strengthening of cell walls where pathogens attack. QACs are cationic surfactants that penetrate the cell membranes of microorganisms (bacteria, fungi, and enveloped viruses), destroying proteins and nucleic acids, and causing cell lysis and death (Gerba, 2015; McDonell, 2007). The results of this study show that all the tested compounds and double fungicide significantly reduced the severity of root rot on sugar beet plants caused by *M. phaseolina*. The findings of this study showed that the addition of $KMnO_4$ resulted in the lowest disease severity and the highest disease protection compared to the control. These findings align with those of previous studies by Goutam and Bajpai (2019), who suggested the possible application of $KMnO_4$ as a disinfectant for mustard seeds to reduce seed fungal infection. Generally, the fungicide Double 56% had the highest effect in controlling the disease. Similar results with two QACs disinfectants showed 100% biocide activity against *F. oxysporum* f. sp. *cubense* TR4 microconidia in the absence of soil but at different exposure times of 5, 10, and 15 min. (Nel et al., 2007). In another study, the use of QACs showed high efficacy against spores of the cotton pathogen *F. oxysporum* f. sp. *vasifectum* (Bennett et al., 2011). Also, using H_2O_2 to successfully combat certain plant diseases, such as wilting tomato plant disease (Ojha and Chatterjee, 2012), chickpeas (Sarwa et al., 2005), root and stalk rot disease of cucumber caused by the pathogenic fungus *F. oxysporum* f. sp. *radices cucumerinum* (Yousefi et al., 2012), and blight in wheat caused by the fungal pathogen *F. graminearum* (Fe-Qi et al., 2012). In comparison to the control plants, the results indicated that all treatments (H_2O_2, n-ADBAC, and $KMnO_4$) improved sugar beet agronomic traits, including TSS and sugar percentage, in both seasons. The highest percentages of TSS and sugar in roots were found in n-ADBAC. These results were consistent with those of Leilah and Khan (2005), who found that the application of plant growth retardants, such as quaternary ammonium salts, affected the sugar percentage and root yield more than the control. However, the TSS and sugar percentage were the lowest in the double fungicide treatment. Mostafa (2021) observed that the application of 20 mM H_2O_2 treatment significantly increased the content of carotenoids, phenol, and total sugar. Yield, productivity, and fruit quality of mangoes under field conditions. Duman et al. (2013) observed that fruit thickness, titratable acidity (TA), and flesh color were considerably affected by all hydrogen peroxide applications.

5. Conclusion

Considering the previous results, it seems pertinent to indicate that the application of H_2O_2, n-ADBAC, and $KMnO_4$ was beneficial for reducing *Macrophomina* root rot in sugar

beet under greenhouse conditions. Moreover, these treatments increased the TSS and sugar percentage in the roots. Based on our results, we conclude that these treatments can be a good alternative to fungicides.

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