The Efficiency of sulfuric acid in suppressing the root rot of date palm offshoots (*Phoenix dactylfera* L.)caused by *Fusarium oxysporum*, (Schlech) Snyder &Hansen. enhanced growth, and photosynthesis pigments

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Abstract

The activity of different concentrations of sulfuric acid against the soil-borne pathogen *Fusarium oxysporum* as causative agents of root rot disease on offshoots of date palm(*Phoenix dactylifera* L.) in both culture medium and greenhouse experiment was evaluated. Sulfur plays an essential role in the defense of plants against biotic and abiotic stresses. The result of dual culture techniques were done using both control plates (*F. oxysporum* alone) and plates with different concentrations of sulfuric acid and *F. oxysporum* show a reduction of the mycelial radial growth colony of the pathogenic fungus *F. oxysporum* in PDA medium supplemented with different concentrations of sulfuric acid (0.1, 0.2, and 0.3 N) at about 75, 76.5, and 85%, respectively. The results of a greenhouse experiment reveal that the application of different concentrations of sulfuric acid (0.1, 0.2, and 0.3 N) to the soil pots inoculated with the inoculum fungus *F. oxysporum* reduced the disease severity by about 30.2, 26.26, and 12.5%, respectively, compared with *F. oxysporum* alone, which was 87%. It also increased the average of fresh and dry shoots and root systems and total chlorophyll and carotenoid content in comparison with the pathogen treatment alone.

Keywords: Date palm, Root rot disease, *F.oxysporum*, Sulfuric acid, Chlorophyll and Carotenoid.

Introduction

In Iraq, date palm trees are subject to a multitude of fungal infections that result in major decreases in the number of trees and their yield. Several soil-borne fungi have been identified as causative pathogens of date-palm root rot, wilt, and decline diseases, including *Fusarium oxysporum*, *F. solani*, *F. moniliforume, F. semitectatium, Thialoviopsis paradoxa*, and *Phomopsis phoenopsis* (Baraka et al. 2011). *F. oxysporum* is a soilborne pathogenic fungus that causes a variety of catastrophic diseases in over 100 plant species and poses a major danger to a number of economically important crops (Gordon & Martyn, 1997). It is classified as a species complex because distinct species might be pathogenic or non-pathogenic to different hosts. Dean et al. (2012) place it fifth among the top-10 fungal plant infections, and it has also become a human disease. If infected dead palm residues or offshoots with the pathogen's inoculum are used in compost, or if pathogen-contaminated soil is transferred from contaminated fields to uncontaminated fields, the disease can spread from contaminated regions to uncontaminated ones. Sulfur is an important component of plant nutrition, and demand varies with plant species. Sulfur fertilization has been shown to significantly increase sulfate ions in the nutrient solution, depending on many factors such as pH values, temperature, access to energy, corresponding sulfate concentration, strength, and the presence of other ions, as well as stress-related S-containing metabolites such as cysteine, GSH, and H2S (Salac et al., 2005). High sulfate concentrations may affect plant development and crop yield (Cerda et al., 1984). Field experiments have shown that inorganic sulfate salts applied to soil can significantly improve the disease resistance of crops. In addition, soil-applied sulfur had a significant repressive effect on *Pyrenopeziza brassicae* infection in oilseed rape, *Uncinula necator* infection in grapes, and *Rhizoctonia solani* infection in potato tubers (Bloem et al., 2004; Dubuis et al., 2005). Sulfur is indirectly responsible for the mobilization of phytotoxic chemicals such as aluminum and other trace elements (Komarnisky et al., 2003). The objectives of this work were an exploration of the effect of different sulfuric acid concentrations in the nutrient solutions and their respective effects against root rot disease caused by the fungal pathogen *F. oxysporum* on offshoots of date palms. We also research the host biochemical response in terms of photosynthesis pigment.

Materials and Methods

Preparation of different concentrations of sulphuric acid tested:

Concentration of H_2SO_4 = 96%, Density of H_2SO_4 = 1.840, Mass of H_2SO_4 present = 100 mL

Volume of H_2SO_4 / L = Actual mass/molar mass (Density)

Volume of H_2SO_4 / L = 100/ (1.840×1000) = 0.054 l

Concentration of H_2SO_4 = Volume of H_2SO_4 × Normality of H2SO4 × Equivalent weight

96% = 0.054 × N × 98.01

N = 18.14

To prepare 1000 mL of (0.1, 0.2, 0.3 N) solution,

$M_1V_1 = M_2V_2$

18.14 × V1 = 0.1 × 1000 mL . V1 = 5.5 mL / L

H_2SO_4	Water	Normality
5.5 ml	994.5 ml	0.1
11.0 ml	989.0 ml	0.2
16.5 ml	983.5 ml	0.3

To calibrate the acidic solution, distilled water was used with a Deluxe pH meter to determine the desired pH (4.0-5.0) then a pH pen was used to confirm that the desired pH = 4.0 .As a control, distilled water was used at pH 7.0.

Antagonisitic activity of sulfuric acid assay against *Fusarium oxysporum*

The experiment was performed to examine the antagonistic relationship between the pathogenic fungus *F. oxysporum* and different sulfuric acid concentrations. PDA medium with different sulfuric acid concentrations of 0.1, 0.2, and 0.3 N The medium was added to each Petri plate in an amount of around 20 mL. Five-mm disks were taken from the edge of the plates (5 days old) of the pathogenic fungus *F. oxysporum* colony (Representative fungus isolate used throughout this study was obtained from a previous study carried out in the Date palm research center, University of Basrah, by Jassim and Ati (2022). and placed in the Petri dish's center and incubated at 28 ^{o}C. For each treatment, five replicates were maintained. Petri dishes with a PDA medium devoid of any chemical compounds as a control The colony growth diameter of the fungus was measured in millimeters after incubation. Following the completion of the mycelial growth for the control treatments, the observations were computed. Following the formula, it was used to determine the rates of mycelial growth inhibition (GI%).

$$\textbf{Growth inhibition(\%)} = [dc-dt/dc]\times 100.$$

where dc is the average of mycelial growth in control and dt is the average of mycelial growth in treatment (Sing & Tripathi, 1999).

Effect of sulfuric acid concentrations on some growth parameters of date palm offshoots grown in soil infested with the *Fusarium oxysporum*.

A study was done in the greenhouse and laboratories of the Date Palm Research Centre, University of Basra, during the 2019–2020 growing season. The experiment was repeated twice. The *F. oxysporum* suspension preparation protocol has been described previously by Jein et al. (2012). In this study, 12-month-old date-palm plantlets (derived from the seeds of the Halawy cultivar) were sown in plastic pots (4 kilogram pots) loaded with autoclaved soil (1:1 peat moss:sand). The duplicate pot trials were set up and carried out according to a totally random design. Five different treatments were used in the experiment pots: controls (no sulphuric acid or fungal inoculation), plantlets only inoculated with *F. oxysporum*, plantlets treated with *F. oxysporum* and 0.1 N of sulphuric acid, plantlets treated with *F. oxysporum* and 0.2 N of sulphuric acid, and plantlets treated with F. oxysporum and 0.3 N of sulphuric acid. The sole treatment used in the control plantlets was CMC. *F. oxysporum* was inoculated at a concentration of 2×10^6 spores/ml. The solution of each concentration (0.1, 0.2, and 0.3 N) was added to distilled water and used at a rate of 3 cm/100 mL water/pot as soil drench according to their pH value of 4.0 and the control (7.0). Each replicate has three plantlets and was arranged in a Completely Randomized Design (CRD). All pots were placed in a greenhouse under appropriate conditions (28–30°C). After 28 days, root rot disease severity was calculated for some plant growth parameters of date palm offshoots, such as fresh and dry weights of shoots and roots, in addition to the effect on photosynthetic pigments.

The plants were scored for root rot disease severity using the root damage scale from 0 to 5 proposed by Abd-Elghany *et al*. (2021), following the formula: Where: 0 indicates no harm; 1 indicates little damage below 10%; 2 indicates moderate damage above 10%; 3 indicates medium-strength damage above 50%; 4 indicates extremely strong damage above 100%; and 5 indicates pure damage over 100%.

According to the procedure outlined Woltz and Arther index (Woltz & Arther, 1973) was used to determine disease severity. The disease severity was calculated according to the following equation:

$$\text{Disease severity (\%)} = \frac{\sum(\text{disease rate} \times \text{number of plant with this rate})}{\text{total number of plants} \times \text{maximum value of disease scale}} \times 100$$

Determination of photosynthetic pigment contents

The contents of photosynthetic pigments were recorded in mg g^{-1} FW. The total chlorophyll content was determined using 0.5 g of fresh sample leaves crushed in 15 mL of acetone (80%), according to the procedures suggested by Arnon (1949). The extraction was centrifuged at 5000 rpm for 5 min, and then the supernatant absorbance was measured at 663 and 645 nm using a spectrophotometer (CECL 2021, UK). The protocol of Lichtenthaler and Wellburn (1983) was used to measure the total content of carotenoids at an optical density of 470 nm using a spectrophotometer (CECL 2021, UK).

Results

Antagonistic activity of sulfuric acid assay against *F.oxysporum*

Our findings revealed that adding various concentrations of H_2SO_4 to the culture PDA medium was effective in reducing the diameter of the mycelial growth colony of *F. oxysporum* in the medium and that there was a significant difference between the various concentrations of H_2SO_4 in the culture medium for suppressing the growth colony of *F. oxysporum*. H_2SO_4 at 0.1, 0.2, and 0.3 N in the culture medium inhibited the mycelial growth colony of *F. oxysporum* about 75, 76.5, and 85% (these percentage equivalents 2.28, 2.12, and 1.38 cm of radial growth colony) respectively, when compared to the control(9 cm) (Table 1).

Table 1. Dual culture test for evaluating sulfuric acid concentrations against *Fusarium oxysporum*

Treatments	Dual culture test	
	Average of radial mycelial growth(cm)	% Growth inhibition
F. oxysporum + 0.1 N	2.28	75%
F. oxysporum + 0.2 N	2.12	76.5%
F. oxysporum + 0.3 N	1.38	85 %
F. oxysporum only	9.00	0.00
LSD 0.5%	0.06	-

The greenhouse experiment

The present study showed a significant difference in the pathogenic potential of the tested fungus when applied singly. The disease severity is shown to vary significantly depending on whether the fungus is used alone or in combination with different concentrations of sulfuric acid (Table

2). *F. oxysporum* infection led to the maximum disease severity on date palm offshoots, about 87%. As the severity caused by *F. oxysporum* decreased to 30.18%, 26.26%, and 12.44% at the application of different concentrations of 0.1, 0.2, and 0.3 N, respectively, the disease severity decreased with rising H_2SO_4 concentrations. In the meantime, the disease's severity was less severe when H_2SO_4 concentrations were raised compared to when *F. oxysporum* infection was the reason.

Table 2. Effect of different concentrations of sulfuric acid on disease severity caused by *F. oxysporum* and control(untreated)

	Disease severity of root rot disease		
Treatments	D	DSI	% Plant survival
Control(untreated)	0	-	100
F. oxysporum + 0.1 N	2	30.18	66.88
F. oxysporum + 0.2 N	2	26.26	78.66
F. oxysporum + 0.3 N	1	12.44	86.78
F. oxysporum only	4	87	12.84
LSD 0.5%		2.88	4.46

(Average scores for 15 plantlets for each treatment, where; D: disease rating scale and, DSI: disease severity index)

Effect on fresh, dry weights , and photosynthetic pigments

Results in Table (3) show significant differences between the averages of fresh and dry weights (shoots and roots) due to the tested treatments. Treatment with H_2S0_4 (0.1, 0.2, and 0.3 N) concentrations significantly increased the average weights of shoots and roots on date palm offshoots compared to *F. oxysporum* treatment. In most cases, differences were found between the dry and fresh weights of shoots and roots of plants grown in the presence of a different concentration of H_2SO_4 and between plants grown in the infested soil and those grown in uninfested soil (control). Moreover, significant differences between fresh weights and dry weights of roots and shoots were found to be due to the high concentration with an application of H_2SO_4 (0.3 N) as well. Total chlorophyll and carotenoid levels were significantly decreased under conditions of infection with *F. oxysporum*. The different concentrations of sulfuric acid significantly hamper the reduction in total chlorophyll and carotenoid pigment values (Table 3). When plantlets were inoculated with the pathogen *F. oxysporum*, the reduction percentages of chlorophyll and carotenoid compared to the control were 44% and 36%, respectively. Compared with *F. oxysporum* treatment alone, plantlets treated with different concentrations of sulfuric acid in the presence of *F. oxysporum* showed increases in total chlorophyll content by about 23%, 38%, and 49% , and total carotenoid content by about 31, 36, and 39%, respectively, at concentrations of 0.1, 0.2, and 0.3.

Table 3. Effect of different concentrations of sulphuric acid on some plant growth parameters and photosynthetic pigments in the presence of *F. oxysporum* and control.

Treatments	Shoots weight (g. plant)		Roots weight (g. plant)		photosynthetic pigments (mg. g FW)	
	fresh	dry	fresh	Dry	chlorophyll	Carotenoid
Control(untreated)	7.64	2.16	7.34	3.62	4.750	0.025
F. oxysporum + 0.1 N	6.82	2.08	6.73	2.48	3.442	0.023
F. oxysporum + 0.2 N	7.12	2,68	7.67	2.96	4.274	0.025
F. oxysporum + 0.3 N	8.38	3.36	8.96	3.64	5.167	0.026
F. oxysporum only	4.46	1.64	3.08	0.82	2.660	0.016
L.S.D≥ 0.5%	0.65	0.22	1.03	0.02	0.141	0.002

Discussion

In greenhouse experiments, the obtained data showed that all different concentrations of sulfuric acid (0.1, 0.2, and 0.3 N) in the presence of *F. oxysporum* decreased the disease severity of root rot in date palm offshoots and also increased all growth parameters such as fresh and dry shoots and root systems compared with *F. oxysporum* alone. In the present study, sulfur nutrition significantly influenced the disease severity for different host/pathogen combinations, and significant differences were found between the averages of each fresh and dry weight value (shoot and root) of date palm offshoots with elevated strength of H_2SO_4 applied. Furthermore, in lower acidic circumstances, such as pH values of 4 and 5, variations between the fresh and dry weights of shoots and roots were nearly obvious. Additionally, at various levels of H_2SO_4 between the inoculation plants, under disease stress, and/or the control plants, substantial changes in the fresh and dry weights of shoots and roots were discovered. Overall, when utilized correctly and sparingly, sulfur fertilizers can be advantageous for plant development. When utilizing these fertilizers, it's crucial to take into account any possible negative effects and environmental impacts. It is also advised to utilize soil testing and the directions on the fertilizer box to determine the right quantity of sulfur fertilizer to use. According to Wang et al. (2003), southern blight on maize caused by *Bipolaris maydis* and stern rot on oilseed rape caused by *Sclerotinia sclerotiorum* both saw a decline in disease severity between 8 and 20 days following fungal inoculation. According to previous studies by Dubuis et al. (2005), oilseed rape's sulfur nutritional status was associated with resistance to *Leptosphaeria maculans*' blackleg, *Botrytis cinerea's* grey mold blight, and *Phytophthora brassicae*. Sulfur fertilization greatly decreased the disease index for light leaf spot caused by *Pyrenopeziza brassicae* and black scurf caused by *Rhizactonia solani* in field experiments with oilseed rape and potatoes, respectively (Schnug et al., 1995; Klikocka et al., 2005). Specifically, infection rates and disease severity in potato tubers caused by *R. solani* are reduced by about 41% and 29%, respectively (Klikocka et al., 2005). The direct toxicity of sulfur, the toxicity of its reduction product hydrogen sulfide (H2S) outside of the fungal hyphae, or the reduction of sulfur to H2S after entering the fungal cell are all factors in sulfur's effectiveness (Boerner, 1997). The generation of lethal quantities of H2S is caused by lipophilic sulfur that may pass straight into the cell wall of the fungus and disrupt redox processes in the pathogen's metabolism (Boemer, 1997). One of the elements, sulfur (S), contains the sulfur amino acids cysteine and methionine in addition to several other substances, such as glutathione or ferro dioxin (Kowalska, 2005). Klikocka et al. (2005) found that soil treated with sulfur led to a rise in a range of fungal infections on several crops in greenhouse and field settings. According to Haneklaus et al. (2009), sulfur metabolites such as cysteine, glutathione, gaseous S emissions, phytoalexins, glucosinolates, and elemental S depositions are crucial for plant defense against fungi. The fungus *Phaeomoniella chlamydospora*, which causes grapevine trunk (esca) disease, was suppressed by cysteine in a concentration-dependent manner in both spore germination and mycelial growth (Roblin et al. 2018). Methionine (Met), which plays a crucial role in cellular metabolism, including protein synthesis, processes of transmethylation through S-adenosyl-L-methionine (SAM) (Rahikainen et al. 2018), as well as many defensive responses to biotic stressors, is the other significant sulfuric acid in plants. For instance, treatment with Metionine significantly decreased the severity of the disease brought on by *Sclerospora graminicola* infection in a sensitive cultivar of pearl millet (*Pennisetum glaucum)* (Sarosh et al. 2005). Metionine treatment induces the generation of hydrogen peroxide (H_2O_2), a key element in plant defense signaling, and upregulates the expression of different defense-related genes in grapevine (*Vitis vinifera*) (Boubakri et al. 2013). *Plasmopara viticola* development in grapevine plants was likewise inhibited by metionine treatment. Additionally, it was discovered that Met had direct antifungal action but was less potent than Cysteine both *in vitro* and *in vivo* (Boubakri et al.

2013). Recent research has shown that transgenic Arabidopsis plants that express a modified form of thionin (Mthionin) exhibit decreased *Fusarium graminearum* development by preventing the germination of fungal spores and the proliferation of hyphal tissue in plants (Hao et al. 2020). Without having a major impact on the host microbiome, sulfur-rich (cysteine) peptides can particularly inhibit infection by a certain pathogen in a specific host or hosts. In greenhouse and field trials, sulfur fertilization decreased the disease severity for different host/pathogen associations by 5–50% and 17–35%, respectively (Haneklaus et al., 2006). This conclusion illustrates the potential of SIR that has not yet been fully realized, and only a breakthrough in gradually advancing systemic induced resistance (SIR) in industrial sectors will make it possible to integrate SIR into existing plant protection strategies. Other sulfur metabolites, such as phytoalexins, sulfur-rich proteins (thionins), and localized cellular deposition of elemental sulfur, may also contribute to plants' resilience to stress and pests (Cooper and Williams, 2004; Hell and Kruse, 2007). The current study showed that growing date palm offshoots under acidic soil conditions (pH = 4-5) helped reduce root infections brought on by *Fusarium oxysporum*. Based on research with three different sulfuric acid concentrations (0.1, 0.2, and 0.3N), 3 mL/100 mL water from each strength was administered as a soil drench in potted soil contaminated with *F. oxysporum* and observed to see if disease symptoms occurred in comparison to control plants. Secondary metabolites that include sulfur are crucial for plant disease resistance. The relationship between root disease and sulphuric acid concentrations was examined, and it was discovered that the high sulphuric acid strength (0.3 N) had caused the rapid development in the date palm offshoots under study and exhibited decreased disease severity. As a result, agricultural techniques for limiting plant diseases that use the preceding approach of acidification may be taken into consideration. Caracuel et al. (2003) discovered that pH (6) was ideal for *F. oxysporum* to thrive and become aggressive. In this context, Garrett et al. (2006) found that soil responses (pH) and ambient variables have a significant impact on how severe plant diseases are. According to Zhao et al., (2008), sulfur's antioxidative protective properties play a significant role in the physiology of plants and aid in their defense against pests and environmental challenges. Infection with Fusarium species causes photo-suppression and a decrease in the amount of chlorophyll, which negatively affects the photosynthetic reaction. A decrease in the absorption of minerals required for the synthesis of chlorophyll, such as magnesium, would indirectly lower the chlorophyll concentration in sick plants and have an impact on the photosynthetic response (Sheng et al. 2008). The effectiveness of photosynthesis is also dependent on the function of 1,5-

bisphosphate carboxylase ribulose (Rubisco)-containing carbon assimilation enzymes, which can be harmed by pathogen infection (Dehgahi et al. 2015). Reduced levels of various proteins in the thylakoid membrane and RuBPCC, notably soluble proteins of the leaves, are a possible cause of the reduction in photosynthetic processes caused by pathogen assault (specifically photosystem II). Photosynthesis' nocturnal fluctuations can show crucial physiological processes occurring in plants. It may also be used to examine how environmental factors or plant activity affect photosynthesis (Li et al., 2013; Hu et al., 2014). According to Odiyi and Eniola (2015), soil application treatments (pH 5, 6, and 7) significantly increased photosynthetic rates and had the greatest effects on plant height, leaf area, fresh weight, relative growth rate, leaf chlorophyll content, and harvest index. The effectiveness of plant physiological activities is subsequently increased by the addition of acids to the soil, which also speeds up the mineralization of nutrients and increases the amount of accessible nutrient pool in the soil (Liu et al., 2005).

Conclusions

Sulfur plays an essential role in the defense of plants against biotic and abiotic stresses. Sulfur provides antioxidant and physiological protection against a variety of abiotic stressors, playing a crucial role in the basic metabolism of plants. In conclusion, the results of this study show that different concentrations of sulfuric acid (0.1, 0.2, and 0.3 N) reduced the radial mycelial growth colony in dual culture, also *in vitro* provided protection of date palm offshoots against infection by *F. oxysporum*, reduced the disease severity, increased the fresh and dry shoots and roots systems, and enhanced the total chlorophyll and carotenoid pigments compared with *F. oxysporum* treatment only.

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فعالية حامض الكبريتيك في تثبيط مرض تعفن الجذور في فسائل نخيل التمر المتسبب عن الفطر *Fusarium oxysporum* (Schlech. Snyder &Hansen) وتحسين مؤشرات النمو وصبغات التمثيل الضوئي

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الخلاصة

تم تقييم فعالية تراكيز مختلفة من حامض الكبريتيك ضد مسبب مرض تعفن الجذور في فسائل نخيل التمر و المتسبب عن الفطر *Fusarium oxysporum* مختبريا في الاوساط الزرعيه والبيت البلاستيكي. يلعب الكبريت دورا حيويا في عملية التمثيل الغذائي للنباتات حيث يمنح النبات وظائف فسيولوجيه واقيه ومضادة للاكسدة ضد العديد من الاجهادات اللاحيائية كما يلعب دورا اساسيا في الدفاع عن النباتات ضد الاجهادات الحيوية المختلفة. أظهرت نتائج أختبار الزراعة المزدوجة باستخدام تراكيز مختلفة من حامض الكبريتيك ضد الفطر الممرض في الوسط الزرعي(البطاطا والدكستروز والاكر) تثبيط النمو القطري لمستعمرة الفطر الممرض عند التراكيز 0.1 و0.2 و0.3 N حيث بلغت النسبة المئويه للتثبيط 75 و76 و85% على التوالي. كما بينت نتائج تجربة البيت البلاستيكي أن تراكيز حامض الكبريتيك المضافة الى تربة الاصص الملوثة بلقاح الفطر الممرض قد قللت من شدة الاصابة بالمرض بنحو 30.2 و26.3 و12.5 % على التوالي مقارنة بمعاملة الفطر الممرض لوحده والتي بلغت شدة الاصابة بالمرض 87%. كما ادت المعاملة بالتراكيز المختلفة لحامض الكبريتيك الى زيادة في مؤشرات النمو المدروسة حيث زادت من معدل الاوزان الطرية والجافة لكل من المجموع الخضري والجذري كما زادت من محتوى الكلوروفيل والكاروتين الكلي مقارنة بمعاملة الفطر الممرض لوحده.

الكلمات المفتاحية: فسائل نخيل التمر، مرض تعفن الجذور، حامض الكبريتيك، *Fusarium oxysporum*، الكلوروفيل، الكاروتين.