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pH VARIATION INHIBITS THE MYCELIAL GROWTH OF *FUSARIUM* SPP. AND *COCHLIOBOLUS SATIVUS*

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A B S T R A C T

Soluble silicon (Si) helps field crops, whether directly or indirectly, counteract fungal diseases, but *in vitro* inhibition of Fusarium head blight (FHB) pathogens and *Cochliobolus sativus* causing spot blotch (SB) and common root rot (CRR) has not been reported. The influence of Si on the mycelial growth of 16 FHB isolates of four agents (*F. culmorum*, *F. verticillioides*, *F. solani* and *F. equiseti*) and 54 *C. sativus* cultures (32 SB isolates + 22 CRR isolates) was investigated in four experiments. The growth of 70 isolates was not significantly inhibited (P>0.05) on Si added potato dextrose agar (PDA) dishes in the first experiment. However, when the pH of Si added PDA dishes was raised to 8.79, 9.57, 10.19 and 10.59, there were differences (P<0.05) in fungal growth of 16 FHB and 54 *C. sativus* isolates compared to Si added PDA dishes (pH was equivalent to 6.56) in the second experiment. NaOH added PDA medium was tested for 6 fungal isolates of the five analyzed species in the third experiment and it was found to have similar results to NaOH added Si-PDA. More importantly, differences in growth rates were observed for the 6 isolates in the fourth experiment when discs were transferred from NaOH added Si-PDA to PDA without NaOH and soluble silicon, indicating that NaOH added Si-PDA was fungicidal rather than fungistatic. Our results provide conclusive evidence that inhibition of fungal growth was principally due to the alkaline pH effect of NaOH added to Si PDA dishes. No fungicidal effects of Si were observed, suggesting that reduction of FHB, SB and CRR diseases can not be achieved through direct silicon application. Thus, our data highlight that other physical, biochemical, and/or molecular defense mechanisms could be behind the possible decreases in severity of FHB, SB and CRR diseases in cereal plants treated with low concentrations of Si. As far as we know, this is the first work exploring the fungicidal effect of Si on mycelial growth of causative agents of FHB, SB and CRR diseases *in vitro*.

Keywords: cereal phytopathogenic species, colony diameter, fungal inhibition, media pH, mycelial growth.

INTRODUCTION

Globally, small-grain cereals can be totally deteriorated by pathogenic deuteromycetes *Fusarium* fungi causing Fusarium head blight (FHB) and ascomycetous *Cochliobolus sativus* species responsible for spot blotch (SB) and common root rot (CRR), account for ultimate of the major devastating diseases of cereal cultivation (Nutter *et al.,* 1985; Parry *et al.,* 1995; Mathre *et al.,* 2003). FHB results from the development of a complex of at minimal 17 causative agents undergo under *Fusarium* genus, principally by *F. graminearum* and *F. culmorum*. Additionally, other species are minimum repeatedly added agents such as *F. equiseti*, *F. poae* and *F. cerealis*, and, to a lower range, *F. solani*, *F. verticillioides* and *F. oxysporum* (Bottalico and Perrone, 2002).

Outbreaks of soil-borne FHB species occurring in seasons with frequent rainfall and high humidity can reduce yield and contaminate wheat and barley grains with dangerous mycotoxins (McMullen *et al.,* 2012). *C. sativus*, a widespread air-borne fungus of barley, is an economically predominant species because it can create remarkable decrease in productivity and quality of the harvest (Mathre, 1997). Also, *C. sativus* acting as a soil-borne fungus is accountable for great economical damages in dry-land barley cultivation (Fernandez *et al.,* 2009).

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Successful control of FHB, SB and CRR cannot be fulfilled via the employ of singular management policy owing to the fact that each possesses its own restrictions. Using several management designs comprising genetic resistance, agricultural, biological and chemical is a strong methodology for FHB, SB and CRR control (Gyawali, 2010; Aada, 2013; Dahl and Wilson, 2018; Sakr, 2020a). In fact, the application of bioactive silicon (Si) has shown potential to enhance crop production, mitigation of mineral toxicity and nutrient inequality, supplying of organizational hardness and tolerance to fungal, bacterial and viral pathogens, and insect pests (Ma and Yamaji, 2006; Sakr, 2016a,b, 2017). To date, To date, the underlying *mechanisms* that govern fungal *disease* protection by silicon treatments are not yet fully understood. However, silicon can assist plants in alleviating disease severity via prohibiting pathogen penetration (1) via structural strengthening, (2) by preventing pathogen infection and establishment through stimulating systemic acquired resistance, (3) *throughout* antifungal component production, as well as (4) through enhancing plant resistance by stimulating multiple signaling pathways and defense-related gene expression (Debona *et al.,* 2017; Sakr, 2018). Concerning FHB disease, Yobo *et al.* (2019) reported that granular and foliar potassium silicate had a possible capacity to reduce FHB as compared to the fungus-inoculated management application in wheat plants. Recently, Sakr, (2021b,c) found that FHB disease reduction was observed on wheat and barley with the treatment of 1.50 g/kg of soil and 1.7 mM soluble silicon. However, no study has been undertaken to characterize the influence of soluble silicon on reducing the incidence of SB and CRR outbreaks on barley.

The fungicidal effect's hypothesis suggests that silicon might operate as the initial defending impediment in silicon enriched plants, and may prevent fungus infestation and successive colonization by suppressing fungal development on the plant surface. Generally, there is a considerable framework of published papers highlighting that reduction of fungal infection severity through foliar silicon treatments is the outcome of a immediate impact on the fungus rather than one mitigated by the host (Reviewed in Sakr, 2016b; Debona *et al.,* 2017). Till now, some *in vitro* studies have been investigated this hypothesis in several *phytopathogen*s; however, the contradictory results showed the presence or absence of antifungal activity following silicon treatment (Bi *et al.,* 2006; Bekker *et al.,* 2009; Li *et al.,* 2009; Liu *et al.,* 2010; Shen *et al.,* 2010; Fayadh and Aledani, 2011; Khan *et al.,* 2013; Ge *et al.,* 2017). As far as we know, there are no reports about the fungicidal effectiveness of silicon on mycelial growth of causative agents of FHB, SB and CRR diseases. With a view to better characterizing the role of silicon in FHB, SB and CRR control, *in vitro* experiments were therefore initiated to analyze the direct impact of soluble silicon on the mycelial growth of 70 fungal isolates of five cereal phytopathogenic species (i.e., *F. culmorum*, *F. verticillioides*, *F. solani*, *F. equiseti* and *C. sativus*).

MATERIALS AND METHODS

Fungal isolates: Sixteen fungal isolates belonging to four FHB species, i.e., *F. culmorum*, *F. solani*, *F. verticillioides* (synonym *F. moniliforme*) and *F. equiseti*, were isolated from spikes showing typical disease symptoms sampled over the 2015 growing season in different sites of Ghab Plain, one of the major Syrian wheat cultivation regions. Fifty four *C. sativus* isolates were isolated in 2015 from both naturally infected barley leaves displaying spot blotch (SB) symptoms (32 isolates) and barley sub-crown internodes showing common root rot (CRR) symptoms (22 isolates) in several localities of Syria. Fungi were multiplied individually in 9 cm diameter Petri dishes including potato-dextrose agar (PDA, HiMedia, HiMedia Laboratories) for 10 days at 22 ± 1 ºC in an incubator (JSPC, JS Research Inc.) under darkness to permit mycelial development. Lately, the 16 FHB fungi were molecularly treated by random amplified polymorphic DNA (Sakr, 2021a). The 70 monosporic cultures were preserved in sterile distilled water at 4°C and by freezing at -16°C till demeaned (Sakr, 2019; 2020b).

Culture media preparation: *Si added PDA :* In order determine the impact of soluble silicon on the 70 fungi, Si-PDA dishes including 0.00, 1.67, 3.33, 5.00, or 6.67 mM soluble silicon (SiO_2, Kieselsaure, Carl Roth GmbH + Co. KG) were prepared by adding sterile SiO_2 powder to hot (50 to 60°C) PDA and then flooding the mix into Petri dishes. The mean pH value of Si-PDA dishes containing all tested above

SiO_2 concentrations was equivalent to 6.56.

Sodium hydroxide added Si-PDA: Amendment with soluble silicon did not increase the pH of the PDA medium. To define whether the raise of pH affect the multiplication of the fungi, the pH of the soluble silicon added medium containing 1.67, 3.33, 5.00, or 6.67 mM soluble silicon was adjusted to 8.79, 9.57, 10.19 and 10.59 with 4 M NaOH (Avonchem, SK 116PJ) (the pH of 4 M NaOH was equivalent to 14) before sterilization. The pH of the NaOH added Si-PDA was equivalent to the pH (8.79, 9.57, 10.19 and 10.59, respectively) of the PDA medium added with four potassium silicate concentrations (1.67, 3.33, 5.00 and 6.67 mM (K_2SiO_3), respectively) as described by Shen *et al.* (2010). NaOH added Si-PDA was prepared by adding sterile SiO_2 powder and sterile sodium hydroxide powder to PDA and then flooding the mix into Petri dishes. The mean pH value of PDA control dishes was equivalent to 6.56.

Sodium *hydroxide* added PDA medium*:* To determine whether NaOH added PDA influenced the development of the six fungal isolates (F1 (*F. culmorum*), F7 (*F. solani*), F15 (*F. verticillioides*), F43 (*F. equiseti*), C.S. 14, a SB isolate and C.S. 41, a CRR isolate (*C. sativus*)) of the five tested species, PDA dishes containing 1.67, 3.33, 5.00, or 6.67 mM NaOH were equipped by supplementing sterilized sodium *hydroxide* solution to PDA and then flooding the mix into Petri dishes. The pH of the NaOH added PDA was equal to the pH of NaOH added Si-PDA (8.79, 9.57, 10.19 and 10.59, respectively). The mean pH value of PDA control dishes was equivalent to 6.56.

For the three experiments, the pH of each solution was quantified with a pH meter (Hanna Ins., HI 8521). The pH of the added PDA medium could not be quantified immediately for the reason that it was solid; however, the measures of the soluble silicon, NaOH added Si-PDA and NaOH-PDA added potato dextrose broth (PDB) were conducted prior the adding of agar.

In vitro experiments*:* Fungicidal efficacy was measured as the development range methodology adapted by Li *et al.* (2009). A 7 mm disk was separated from the edge of *vigorous developing 10 day old culture plate* of each culture and set at the middle of each application or control dish. The dishes were then laid at random way in an incubator at 22 ± 1 ºC under continuous *darkness*. Colony diameter was obtained by *measuring two vertical axes* at 24 h intervals until 72 h. Each treatment was repeated five times and each experiment was repeated twice.

Determining whether NaOH added Si-PDA is fungicidal or fungistatic*:* To determine whether NaOH added Si-PDA is fungicidal or fungistatic, growth rates were observed for six isolates (F1, F7, F15, F43, C.S. 14 and C.S. 41) of the five tested species when discs were transferred from 72 h-old-NaOH added Si-PDA to PDA without NaOH and soluble silicon. The dishes with disks were then randomly placed in an incubator at 22 ± 1 ºC in the dark until 72 h. Each treatment was replicated five times and each experiment was repeated twice.

STATISTICAL ANALYSES

All statistical data were fulfilled using DSAASTAT add-in version 2011. Data were tested by one-way analysis of variance (ANOVA). For each isolate, treatment averages within an incubation time were compared using the Fisher's LSD test. Differences at $P<0.05$ were estimated to be significant.

RESULTS

Effect of soluble silicon on colony growth in vitro: The mean growing of 70 fungal isolates of five species (*F. culmorum*, *F. verticillioides*, *F. solani*, *F. equiseti* and *C. sativus*) causing FHB, SB and CRR diseases was not inhibited on silicon added dishes (Table 1 and Figure 1). Colony diameters of all species were not significantly ($P>0.05$) smaller than the control at all soluble silicon concentrations. These results imply that amendment of soluble silicon at all tested concentrations did not raise the pH of medium (equivalent to 6.56). Thus, the pH value of 6.56 was a crucial factor in no inhibiting the colony growth of all tested isolates.

Effect of NaOH added Si-PDA on colony growth in vitro: NaOH added Si-PDA markedly inhibited ($P<0.05$) mean mycelial growth for 70 fungal isolates of *F. culmorum*, *F. verticillioides*, *F. solani*, *F. equiseti* and *C. sativus*, with greater inhibitory effects at higher concentrations (Table 2). Colony diameters of all species were significantly smaller than the control (pH was equivalent to 6.56) measured on medium at pH values of 9.57, 10.19 and 10.59. It was probable that it was the higher pH of the added medium which suppressed colony growing of all fungi caused FHB, SB and CRR diseases.

Table 1. Mean colony diameters of 5 isolates of *F. culmorum*, 6 isolates of *F. solani*, 4 isolates of *F. verticillioides*, one isolate of *F. equiseti*, 32 isolates for *Cochliobolus sativus* causing spot blotch and 22 isolates for *C. sativus* causing common root rot grown on potato-dextrose agar medium added with soluble silicon. The pH of the culture medium was not adjusted

Fungal species	Incubation period (h)	Colony diameter (mm)				
		Not-treated	A1[b]	A2	A3	A4
F. culmorum	24	7.5±0.9a[c]	7.3±0.8 a	7.9±0.5 a	7.6±0.3 a	7.7±0.4 a
	48	20.3±3.7a	21.0±4.6a	21.5±4.0a	21.9±4.5a	21.0±4.5a
	72	39.5±10.4a	39.1±10.4a	39.6±10.0a	40.4±11.0a	39.6±10.4a
F. solani	24	9.8±1.7a	9.4±1.4a	9.7±1.3a	9.5±1.3a	9.6±1.2a
	48	33.0±7.6a	33.7±7.3a	33.5±8.2a	33.8±8.3a	33.6±8.4a
	72	56.3±9.0a	56.7±8.8a	55.9±8.8a	56.2±8.9a	56.7±8.7a
F. verticillioides	24	9.3±1.2a	9.7±1.2a	9.9±1.0a	9.7±1.1a	9.9±0.9a
	48	28.9±6.8a	29.1±6.6a	29.5±7.6a	29.6±7.0a	29.4±7.4a
	72	53.5±13.0a	53.8±13.6a	53.2±12.5a	53.9±13.0a	54.4±13.0a
F. equiesti	24	7.0±1.2a	6.8±0.8a	6.8±0.8a	6.4±1.1a	7.0±0.7a
	48	30.4±0.9a	30.6±1.1a	30.2±1.1a	29.8±0.8a	29.4±1.3a
	72	48.4±1.1a	48.4±0.5a	49.2±1.1a	48.6±0.5a	48.4±0.9a
Cochliobolus sativus causing spot blotch	24	12.0±4.7a	12.1±4.6a	12.1±4.7a	12.1±4.8a	12.0±4.6a
	48	32.2±9.6a	32.6±9.9a	32.3±9.9a	32.3±9.9a	32.3±9.8a
	72	50.2±11.9a	50.2±11.7a	50.3±11.9a	50.2±11.9a	50.1±11.7a
C. sativus causing common rot root	24	10.4±4.3a	10.5±4.3a	10.6±4.1a	10.5±4.4a	10.6±4.4a
	48	27.5±6.7a	27.8±6.5a	27.5±6.7a	27.9±6.8a	27.6±6.7a
	72	48.2±11.7a	48.4±11.8a	48.1±11.5a	48.2±11.7a	48.0±11.5a

[b] A1, A2, A3 and A4 represent PDA dishes added with 1.67, 3.33, 5.00 and 6.67 mM soluble silicon, respectively.
[c] Findings are the averages ± standard deviation (SD). Averages ± SD within a line followed by the identical letter are not significantly (P<0.05) different based on Fisher's LSD test.

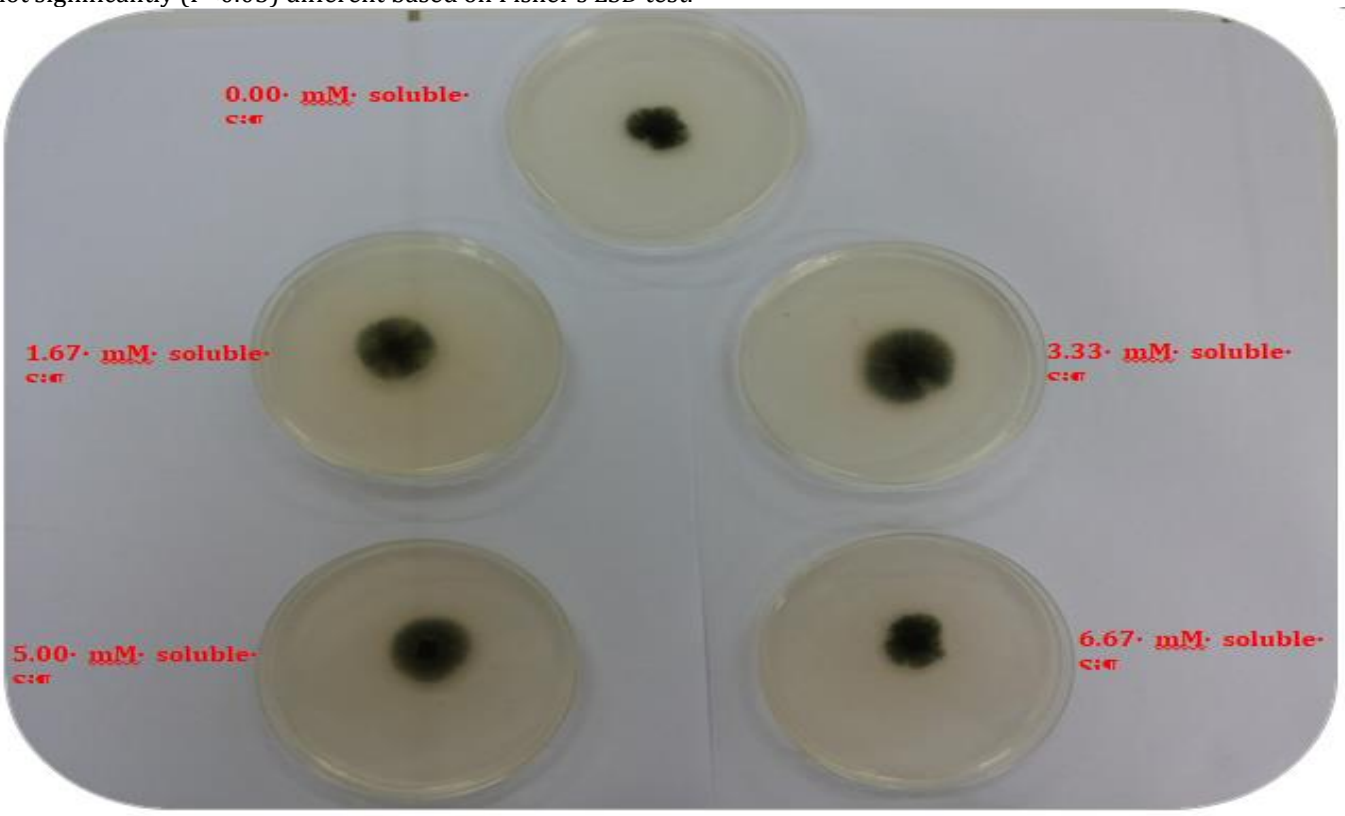

Figure 1. Mycelial growth of fungal colonies for isolate C.S. 50 (*Cochliobolus sativus* causing common root rot on barley) on potato-dextrose agar medium added with 0.00, 1.67, 3.33, 5.00 and 6.67 mM soluble silicon at 72 hours after infection.

Table 2. Mean colony diameters of 5 isolates of *F. culmorum*, 6 isolates of *F. solani*, 4 isolates of *F. verticillioides*, one isolate of *F. equiseti*, 32 isolates for *Cochliobolus sativus* causing spot blotch and 22 isolates for *C. sativus* causing common root rot grown on potato-dextrose agar (PDA) medium added with soluble silicon. The pH of the soluble silicon added medium was adjusted with NaOH that it was equivalent to the pH (8.79, 9.57, 10.19 and 10.59, respectively) of the PDA medium added with four potassium silicate concentrations (1.67, 3.33, 5.00 and 6.67 mM (K_2SiO_3), respectively) as described by Shen *et al.* (2010)

Fungal species	Incubation period (h)	Colony diameter (mm)				
		Not-treated	A1[b]	A2	A3	A4
F. culmorum	24	7.5±1.2a[c]	5.2±1.1b	4.5±0.7bc	3.8±0.7cd	2.7±1.0d
	48	20.4±3.8a	18.0±3.5ab	16.4±2.1bc	14.2±2.2bc	13.3±2.8c
	72	39.7±10.4a	31.9±11.5ab	26.5±8.2b	23.3±6.9b	20.6±6.1b
F. solani	24	9.4±1.0a	7.3±1.5b	6.2±0.8bc	5.3±1.6c	4.8±1.2c
	48	33.7±7.4a	27.7±7.8ab	25.4±7.4ab	23.0±7.2b	20.5±7.2b
	72	56.3±7.9a	47.0±7.7b	40.9±7.9bc	33.1±6.7cd	30.7±6.6d
F. verticillioides	24	9.4±1.7a	7.8±1.1b	6.7±0.6bc	5.9±0.3c	5.6±0.7c
	48	29.5±6.7a	26.1±5.5ab	21.2±7.6abc	17.9±3.6bc	16.6±5.1c
	72	54.1±13.0a	47.5±14.4ab	37.1±10.5ab	33.8±11.9b	33.0±9.6b
F. equiesti	24	7.8±1.1a	4.8±0.4bc	5.2±1.3b	4.6±0.9bc	3.8±0.4c
	48	31.8±1.1a	20.4±0.8b	20.1±0.7b	18.6±0.5c	15.5±0.7d
	72	49.2±2.2a	44.0±0.5b	32.8±0.7c	30.4±0.3d	28.5±0.5e
Cochliobolus sativus causing spot blotch	24	12.2±4.6a	10.1±4.1b	8.3±3.4c	6.8±3.0cd	5.6±2.4d
	48	32.4±9.6a	28.0±8.5b	24.2±7.7b	20.1±6.7c	17.0±6.2c
	72	50.4±11.9a	44.3±10.5b	38.4±9.6c	32.4±8.5d	26.9±8.4e
C. sativus causing common rot ro	24	10.5±4.3a	8.7±3.5ab	7.3±2.7bc	5.9±2.8cd	4.5±1.5d
	48	27.4±6.7a	23.9±6.8ab	21.1±5.9b	16.8±5.7c	12.7±4.7d
	72	48.1±11.3a	41.8±9.7b	37.1±9.2b	30.3±8.9c	23.6±7.4d

[y] A1, A2, A3 and A4 represent PDA dishes added with 1.67, 3.33, 5.00 and 6.67 mM soluble silicon, respectively. The pH of the soluble silicon added medium was adjusted with NaOH that it was equal to the pH (8.79, 9.57, 10.19 and 10.59, respectively). The mean pH value of PDA control dishes was equivalent to 6.56.

[c] Findings are the averages ± standard deviation (SD). Averages ± SD within a line followed by the identical letter are not significantly (P<0.05) different based on Fisher's LSD test.

Effect of NaOH added PDA on colony growth in vitro NaOH added PDA significantly suppressed (P<0.05) mycelial growth for 6 fungal isolates of the five tested species, with greater inhibitory effects at higher concentrations (Table 3). Colony diameters of all isolates were significantly smaller than the control (pH was equivalent to 6.56) measured on medium at pH values of 9.57, 10.19 and 10.59.

Determining whether NaOH added Si-PDA is fungicidal or fungistatic*:* Significant differences in growth rates (P<0.05) were observed for the six isolates of the five tested species when they were transferred from 72 h-old-NaOH added Si-PDA to PDA without NaOH and soluble silicon (Table 4).

Table 3. Colony diameters of 4 isolates of four Fusarium head blight agents and 2 isolates of *Cochliobolus sativus* causing spot blotch and common root rot grown on potato-dextrose agar (PDA) medium added with sodium *hydroxide*

Isolates (identification)	Incubation period (h)	Colony diameter (mm)				
		Not-treated	A1[b]	A2	A3	A4
F1 (*F. culmorum*)	24	8.2±2.5a[c]	4.0±1.9b	4.8±1.9b	2.8±1.1bc	2.2±0.4c
	48	23.6±1.5a	18.8±3.6b	16.6±1.7bc	14.8±2.0cd	13.0±1.6d
	72	54.2±2.9a	49.5±3.8b	39.4±2.6c	31.8±2.1d	31.6±3.5d
F7 (*F. solani*)	24	8.0± 0.7a	5.4±1.5b	4.2±0.8b	3.0±0.4c	3.0±0.7c
	48	24.6±2.1a	21.6±2.2b	17.2±1.5c	17.6±2.3c	16.4±1.1c
	72	46.0±1.6a	38.4±3.3b	31.8±4.2c	25.6±1.8d	20.0±1.6e
F15 (*F. verticillioides*)	24	8.8±1.3a	7.8±0.8a	6.8±1.1b	6.2±1.1c	5.8±1.1c
	48	24.2±1.3a	21.3±1.3b	14.2±1.6c	13.6±1.8c	10.7±1.0d
	72	41.4±1.5a	30.5±0.7b	27.7±1.1c	22.3±1.2d	22.3±1.5d
F43 (*F. equiesti*)	24	8.2±1.5a	5.2±0.4bc	5.0±1.0b	5.0±1.7bc	3.6±0.5c
	48	32.2±1.5a	20.6±1.0b	20.5±1.1b	19.0±1.2c	15.7±1.0d
	72	48.8±1.6a	43.6±0.6b	33.0±0.6c	30.0±0.6d	28.6±0.6e
C.S. 14 (spot blotch)	24	9.6±1.9a	6.6±3.0b	6.4±2.5bc	4.6±0.5bc	3.6±1.1c
	48	29.0±2.4a	22.2±1.8b	20.0±1.9c	19.2±1.1c	18.0±3.2c
	72	49.4±3.8a	47.9±1.2a	41.6±1.6b	33.8±2.4c	33.2±2.3c
C.S. 41 (common root rot)	24	10.8±1.6a	9.2±1.5a	8.0±0.7b	7.8±1.1b	4.2±0.9c
	48	29.6±1.7a	27.0±3.0ab	25.4±3.6b	18.6±1.8c	15.8±2.2d
	72	49.8±1.3a	48.2±0.8b	41.8±2.7c	35.2±1.1d	30.6±1.9e

[b] A1, A2, A3 and A4 represent PDA dishes added with 1.67, 3.33, 5.00 and 6.67 mM NaOH, respectively. The pH of the NaOH added PDA was equal to the pH of NaOH added Si-PDA (8.79, 9.57, 10.19 and 10.59, respectively). The average pH value of PDA control dishes was equal to 6.56.

[c] Findings are the average of five replications ± standard deviation (SD). Averages ± SD within a line followed by the identical letter are not significantly (P<0.05) different based on Fisher's LSD test.

Table 4. Comparisons among reductions in colony diameters measured after 72 h of 4 isolates of four Fusarium head blight agents and 2 isolates of *Cochliobolus sativus* causing spot blotch and common root rot grown on potato-dextrose agar (PDA) medium when they were transferred from 72 h-old-NaOH added Si-PDA

Isolates (identification)	Colony diameter (mm)							
	A1[b]	PDA[c]	A2	PDA	A3	PDA	A4	PDA
F1 (*F. culmorum*)	49.3±3.9a[z]	39.0±0.7b	39.3±2.7a	31.4±0.9b	31.8±2.1a	24.0±1.0b	30.5±3.7a	22.8±1.8b
F7 (*F. solani*)	38.4±3.3a	32.3±0.9b	31.6±1.3a	23.8±1.3b	26.0±1.4a	20.4±0.5b	20.0±1.5a	14.1±2.7b
F15 (*F. verticillioides*)	30.5±0.7a	25.6±1.7b	26.9±1.3a	20.8±1.3b	21.5±1.0a	15.4±0.9b	22.3±1.2a	15.0±1.0b
F43 (*F. equiesti*)	43.6±0.6a	34.6±1.1b	33.0±0.6a	22.4±0.9b	30.3±0.3a	22.0±1.0b	27.8±1.6a	16.4±1.4b
C.S. 14 (spot blotch)	48.7±1.9a	40.2±0.4b	41.0±1.5a	34.8±1.1b	33.4±2.1a	26.8±1.9b	32.9±2.7a	23.4±1.1b
C.S. 41 (common root rot)	48.6±0.5a	37.2±1.5b	41.6±2.3a	35.2±1.9b	35.0±1.2a	28.0±1.0b	29.4±0.9a	21.8±1.1b

[b] Colony diameter of fungal isolates measured after 72 h on PDA dishes added with 1.67 (S1), 3.33 (S2), 5.00 (S3) and 6.67 (S4) mM soluble silicon, respectively. The pH of the soluble silicon added medium was adjusted with NaOH that it was equivalent to the pH (8.79, 9.57, 10.19 and 10.59, respectively).

[c] Colony diameter of fungal isolates measured after 72 h when they were transferred from 72 h-old-NaOH added Si-PDA on PDA dishes without NaOH and soluble silicon.

[d] Finings are the average of five replications ± standard deviation (SD). Averages ± SD within a line followed by the identical letter are not significantly (P<0.05) different based on Fisher's LSD test.

DISCUSSION

Important fungal diseases of wheat and barley, i.e., FHB, powdery mildew, spot blotch, blast and leaf blast, have been efficiently controlled following soluble silicon supplying (Reviewed in Sakr, (2016b); Debona *et al.* (2017)), Yobo *et al.,* 2019; Sakr, 2021b,c) because these small grain cereals are classified as high silicon absorbers and accumulators (Ma and Yamaji, 2006). An *in vitro* checking proceeding which supplies fast and *in situ* trust findings is a remarkable phase in checking effective control measures of FHB, SB and CRR species. However, such *in vitro* procedures are not applicable yet on the growth of *F. culmorum*, *F. verticillioides*, *F. solani*, *F. equiseti* and *C. sativus*. Thus, the findings in this study are the primary data in detecting the potential antifungal effect of soluble silicon in a large set of fungal cultures recovered from several Syrian cereal growing area and exhibiting several morphological, pathogenic and genetic characteristics (Sakr, 2020a, 2021a, unpublished data).

In the current research, we do not utilize potassium silicate or sodium silicate as the source of silicate commonly used in studies investigating the impact of silicon on the growing of fungal isolates *in vitro* (Bi *et al.,* 2006; Bekker *et al.,* 2009; Li *et al.,* 2009; Liu *et al.,* 2010; Shen *et al.,* 2010; Fayadh and Aledani, 2011; Khan *et al.,* 2013; Ge *et al.,* 2017). However, the soluble silicon concentrations and the values of pH in NaOH added Si-PDA applied here are similar to those of potassium silicate reported by Shen *et al.* (2010). The compound in our experiment can be comparable to the sodium or potassium silicate media in terms of containing soluble silicon and sodium and its similar adjusted alkalinity. Therefore, we can compare our results generated in media containing NaOH added Si-PDA with those obtained with sodium silicate added PDA. NaOH added Si-PDA had an inhibitory effect on the growth of all tested FHB, SB and CRR isolates of species *F. culmorum*, *F. verticillioides*, *F. solani*, *F. equiseti* and *C. sativus*. These results agree with those reported by Bi *et al.* (2006) for *Alternaria alternata*, *F. semitectum* and *Trichothecium roseum* on sodium silicate added medium, Bekker *et al.* (2009) for eleven phytopathogenic fungi (*F. solani, F. oxysporum, Colletotrichum coccodes, Mucor pusillus, Drechslera* spp, *Phytophthora cinnamomi, Pythium* F-group, *Alternaria solani, Verticillium theobromae, Curvularia lunata*, *Stemphylium herbarum* and *Sclerotinia sclerotiorum*) on medium added with potassium silicate, Li *et al.* (2009) for *F. sulphureum* on sodium silicate added medium, *Liu et al. (2009) for Penicillium digitatum on* sodium silicate added medium*,* Shen *et al.* (2010) for *F. oxysporum* f. sp. *fragariae*, *F. oxysporum*, *Pestalotiopsis clavispora* and *Rhizoctonia solani* on PDA medium added with potassium silicate, Fayadh and Aledani, (2011) for *R. solani* on sodium silicate added PDA medium, Khan *et al.* (2013) for *Macrophomina phaseolina* on sodium silicate added medium, and Ge *et al.* (2017) for *T. roseum* on medium added with sodium silicate. These published findings and our data indicate the sensitivity of several species within deuteromycetes *Fusarium* genus to *in vitro* potassium silicate, sodium silicate or NaOH added Si-PDA application. Also, this report highlighted, for the first time, the inhibition of colony growth of ascomycetous *C. sativus* species causing SB and CRR diseases. Furthermore, it is valuable to record that potassium silicate, sodium silicate or NaOH added Si-PDA inhibit mycelial growth not only for soil-borne fungi but also for air-borne pathogens. More importantly, differences in growth rates were observed for some isolates of the five tested species when discs were transferred from NaOH added Si-PDA to PDA without NaOH and soluble silicon. This indicated that NaOH added Si-PDA was fungicidal rather than fungistatic against *F. culmorum*, *F. verticillioides*, *F. solani*, *F. equiseti* and *C. sativus.*

Although the suppressive effect that NaOH added Si-PDA had on fungal growth is clear (the pH values were equivalent to 8.79, 9.57, 10.19 and 10.59, respectively), the growing of 70 fungi of five species was not suppressed when the PDA culture medium was added only with soluble silicon concentrations similar to sodium silicate concentrations (the mean pH value was equivalent to 6.56). Thus, findings from this report indicated that the inhibition of fungal growing is principally due to the alkaline pH effect of NaOH to added Si PDA dishes. During the current investigation, NaOH added PDA medium was tested for some fungal isolates of the five analyzed species and it was found to have similar results to NaOH added Si-PDA. Our data agree with Shen *et al.* (2010) position; they argue that silicon does not seem to directly affect pathogens, being no fungicidal. The decrease of colony growing of four pathogens on PDA medium added with potassium silicate was due to a pH impact (Shen *et al.,* 2010). In fact, the *most* optimal *mycelial growth* for most fungi happens at pH 5 to 6.5; however, alkaline conditions can inhibit fungal growing (Ingold, 1973). In dissimilarity to

our results and those obtained by Shen *et al.* (2010), Bekker *et al.* (2009); Fayadh and Aledani, (2011) found that the restrained impact of potassium silicate and sodium silicate, respectively, on fungal growth *in vitro* was mostly due to a fungicidal effect. The differences in these data may be attributable to that the potassium silicate and sodium silicate concentrations used by Bekker *et al.* (2009); Fayadh and Aledani, (2011) were 50 to 60 times and 30 to 74 times, respectively, higher than that in our experiment and the research conducted by Shen *et al.* (2010). It should be indicated that the concentrations tested by Bekker *et al.* (2009) and Fayadh and Aledani, (2011) are inapplicable for field application due to the high pH of the resulting potassium silicate/sodium silicate solutions could cause phytotoxicity. In fact, potassium silicate/sodium silicate concentrations commonly do not exceed 1.67 mM in field studies investigating the effeteness of silicon for decreasing plant disease incidence (Laane, 2018; Zhou *et al.,* 2018). It is valuable to record that the range of NaOH amended Si-PDA concentrations tested in our study is advisable for field treatment. However, there is still a need to further analyses need to be done to assess the effect of higher silicate concentrations on plant development.

Based on the information generated in this study, we conclude that although sodium silicate acts as a promising effective controlling measure against fungal diseases, our results revealed that the NaOH added Si-PDA at low concentrations (1.67 to 6.67 mM) did exhibit antifungal activity against the causative agents of FHB, SB and CRR diseases because of the alkaline pH effect of NaOH added to Si PDA dishes. No fungicidal effects of soluble silicon were observed, suggesting that reduction of FHB, SB and CRR disease severity can not be achieved through direct silicon application. Thus, our data highlight that other physical, biochemical, and/or molecular defense mechanisms could be behind the possible decreases in severity of FHB, SB and CRR diseases in cereal plants treated with low concentrations of Si. In our experimental station, studies aimed to investigate the role of silicon to reduce FHB (*F. culmorum*, *F. verticillioides*, *F. solani* and *F. equiseti*) incidence and severity on wheat and barley plants are in progress under controlled (Sakr, 2021b,c) and field conditions, and encouraged results were obtained. Further research should be conducted to better understand the bioactive role of silicon on biological functions involving cell turgor pressure of hyphae and spores, mycelium symmetry and asymmetry, hyphal swelling, curling, cupped shape, reproductive growth including asexual and sexual spore germination, and the cellular architecture alterations in FHB, SB and CRR fungi. In future, the use of silicon along with fertilizers and other biological control agents to reduce the severity of these important fungal diseases should be investigated.

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