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Effectiveness of silicon and silicate salts for controlling black root rot and induced pathogenesis-related protein of strawberry plants



Farid Abd-El-Kareem, Ibrahim E. Elshahawy and Mahfouz M. M. Abd-Elgawad*

Abstract

Background: Black root rot is a complex disease caused by one or more of fungal pathogens especially *Rhizoctonia solani* and *Fusarium solani*. It is a serious, yield-limiting disease of strawberry plants. A considerable incidence of black root rot has been observed in recent years in Egypt.

Materials/methods: Evaluation of silicon as well as potassium, sodium, and calcium silicates against black root rot and induced pathogenesis-related (PR) protein under field conditions was investigated herein. Four concentrations, i.e., 0, 2, 4, and 6 g/l of silicon, potassium, sodium, and calcium silicates, were tested to study their effect on growth of strawberry black root fungi (*F. solani* and *R. solani*) under laboratory and field conditions.

Results: Complete inhibition of fungal linear growth was observed with 4 and 6 g/l for *R. solani* and *F. solani*, respectively, in the laboratory. Under field conditions, all tested concentrations significantly reduced the disease incidence and severity. The highest reduction of such disease incidence and severity was obtained with potassium silicate and calcium silicate applied separately as soil treatment combined with foliar sprays which reduced the disease incidence and severity by 92.7 and 91.9, and 91.7 and 91.1%, respectively. The highest yield increase, i.e., 77.1 and 72.8%, was obtained with potassium silicate and calcium silicate, respectively, applied as soil treatment + foliar spray. Results indicated that 8 to 12 new protein patterns (bands) appeared in the tested treatments.

Conclusions: No-standalone management measure is perfectly effective against the black root-rot complex. Thus, an integrated management including tactful cultural practices and applying foliar and soil inputs, e.g., silicon salts reported herein, can effectively control the disease and enhance strawberry yield.

Keywords: Black root rot, Protein patterns, Silicon, Silicate salts, Strawberry

Background

The current increase in strawberry (*Fragaria × ananassa* Duchesne) acreage in Egypt is frequently associated with high infestation with soilborne fungi. The lack of their control can result in a significant reduction in strawberry yield and fruit quality. Black root rot, a complex disease caused by one or more of fungal pathogens, e.g., *Fusarium oxysporum* (Juber et al. 2014), *Macrophomina phaseolina* (Hutton et al. 2013), and *Rhizoctonia* spp. (Fang et al. 2013), ranks high among other diseases. A

considerable incidence of this root rot disease has been observed in recent years in Egypt. Our field observations indicated that it is frequently caused by *Rhizoctonia solani* and *Fusarium solani* in Egypt. The disease is characterized by feeder rootlet killing, deterioration, and blackening of the main root system and a decline in vigor and productivity of the plant stand causing damage to the host and considerable reduction in the yield (Botha et al. 2001; Fang et al. 2013; Ceja-Torres et al. 2014).

Silicon (Si) has been reported to play a vital role to effectively mitigate various environmental stresses and enhance plant resistance against both fungal and bacterial pathogens (Forbes and Watson 1992; Wang et al. 2017).

* Correspondence: mahfouzian2000@yahoo.com
Plant Pathology Department, National Research Centre, Dokki, Giza 12622, Egypt

Its amount within plant tissues of different species considerably varies due to differences related to root uptake capacity. Generally, Si uptake occurs via plant roots as silicic acid $[\text{Si}(\text{OH})_4]$, an uncharged molecule, and passes through the plasma membrane by two Si transporters, Lsi1 and Lsi2, which function as influx transporters and efflux transporters, respectively (Wang et al. 2017). Silicon has an important potential in the plant growth and development (Datnoff et al. 2001). Potassium silicate is the main source of soluble potassium and silicon. In general, plants require silica to resist against biotic and abiotic stress (Ma 2004). Soil amendment by such a solution resulted in enhancing plant growth, yield, and disease and insect resistance and reduced the harmful mineral toxicities (Bélanger et al. 1995). So, fungal diseases such as powdery mildew and root rot were suppressed while the amount of chemical fungicides released into the environment was decreased by application of silicic acid (Bélanger et al. 1995; Ma 2004).

Thus, the development of safe alternatives to environmentally unfriendly fungicides would be useful to avoid environmental pollution and health hazards (Kumar 2007; Khalifa et al. 2013). Since Si play a vital role in leaf development, consequently exposing more leaves to light, it can increase the efficiency of plant canopy photosynthesis. On the other hand, plants exposed to different environmental stresses including disease infections exhibit changes in membrane leakage that lead to loss of membrane integrity (Blokhina et al. 2003).

The aim of this study was to evaluate the effect of silicon, as well as potassium, sodium, and calcium silicates, against strawberry black root rot under laboratory and field conditions in Egypt. The effect of these treatments on protein patterns of strawberry plants was examined.

Materials and methods

Pathogens and plant material

Pathogenic isolates of *F. solani* and *R. solani*, the causal agents of black root disease of strawberry plants, were kindly provided by Plant Pathology Department, National Research Centre, Giza, Egypt. Strawberry seedlings (cv. Festival) were obtained from Vegetable Crops Research Dept., Agricultural Research Centre, Giza, Egypt. At the end of November 2017, strawberry seedlings were transplanted into a field with loamy clay, well-drained soil, and final harvest was at the end of April, 2018.

Laboratory testing

Three concentrations, i.e., 2, 4, and 6 (ml/l) of silicon, potassium, sodium, and calcium silicates, in addition to the untreated control (only water), were tested to study their effect on linear growth of strawberry black root rot fungi (*F. solani* and *R. solani*) in the laboratory. These concentrations were added individually to sterilized potato dextrose agar (PDA) before solidification and then poured in

sterile 9-cm-diameter Petri-plates. After solidification, the plates were inoculated with 6-mm fungal disc in the center of the plate and incubated at $25 \pm 1^\circ\text{C}$. Five plates per concentration for each fungus were used as replicates; five plates served as control/ fungus. Linear growth was recorded, and reduction in linear growth was estimated.

Field testing

A field experiment was carried out at Tukh district, El-Qalioubia governorate, Egypt. Chosen field was naturally highly infested with strawberry black root rot fungi from previous season. The experiment was conducted under natural infection by both *F. solani* and *R. solani* in plots (4×8 m), each comprised of 8 rows (32 holes/ row and a seedling was sown in each hole) in a randomized complete block design with three replicates (plots) for each treatment. Strawberry seedlings were planted in loamy clay well-drained soil to a depth of 10 cm. In addition, irrigation, fertilization, and other nutrients were added as recommended (El-Shemy et al. 2013). Silicon, potassium, sodium and calcium silicates were tested at concentration of 6 g/l to study their effect on strawberry black rot, plant weight, and yield under field conditions.

Application

Silicon, potassium, sodium, and calcium silicates, each at concentration of 6 g/l, were applied as soil treatments as well as soil + foliar spray every 15 days for 4 months. Soil in intimate contact with strawberry roots received 40 ml of each treatment but soil and plant received 80 ml of each treatment for soil + foliar spray as fifty-fifty.

Assessment of disease incidence

The percentage of disease incidence was calculated 100 days after transplanting as follows:

$$\text{Disease incidence\%} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Disease severity (DS) was recorded at the end of the experiment, 5 months after transplanting, according to the following scale (Morocco 2006): 0: plant well developed, no disease symptoms; 1: no visible symptoms on plant foliage, 25% of roots discolored; 2: plant slightly stunted, black necrosis on petiole bases, 26–50% of roots discolored; 3: plant stunted, black necrosis on petiole bases, yellowing and death of outer leaves, 51–75% of roots discolored; 4: plant severely stunted, outer leaves collapsed, younger leaves bluish green and wilting, > 75% of roots discolored; and 5: roots almost completely discolored and plant almost dead.

$$\text{Disease severity\%} = \frac{\sum (\text{Disease grade} \times \text{Number of plants in each grade})}{\text{Total number of plants} \times \text{Highest disease grade}} \times 100$$

Effect on plant weight and yield of strawberry plants under field conditions

Fresh and dry weights per plant were determined at harvest. Accumulated strawberry fruit yield (Ton/fed-dan) for each treatment was determined.

Protein pattern analysis

Isolation of total plant proteins

Sixty days after transplanting, the whole plant tissues were rapidly frozen with liquid nitrogen to make the plant more fragile. The frozen plant tissues were ground before shaking in the buffer (Fido et al. 2004). Each dried sample was mixed with 1 ml of water-soluble protein extraction buffer in Eppendorf tube and left in refrigerator overnight, then vortexed for 15 s and centrifuged at 5000 rpm on 4 °C for 15 min. The supernatants containing water-soluble proteins were transferred to new Eppendorf tubes and kept at deep-freezer until electrophoretic analysis.

Molecular weight estimation by electrophoresis

To determine the relative molecular weight of extracted proteins, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on a stacking and separating gel according to the method of Laemmli (1970) using Mini-gel electrophoresis (BioRad, USA). The molecular weight of the isolated proteins was estimated in comparison to standard molecular weight

markers (standard protein markers, 11–245 kDa; Sigma, USA). The protein bands were visualized by staining with Coomassie Brilliant Blue G-250 (Sigma, USA) after documentation (Darwesh et al. 2015).

Statistical analysis

Tukey test for multiple comparisons among means was utilized (Neler et al. 1985).

Results

Laboratory tests

Results in Table 1 indicate that all tested treatment concentrations significantly reduced the linear growth of both fungi. Complete inhibition of linear growth was obtained with 4 and 6 g/l for *R. solani* and *F. solani*, respectively. Concentration at 2 g/l showed moderate effect.

Field experiment

Results in Table 2 reveal that all tested concentrations significantly reduced the disease incidence and severity. The highest reduction of disease incidence and severity was obtained with potassium silicate and calcium silicate applied as soil treatment + foliar sprays which reduced the disease incidence and severity by 92.7 and 91.9, and 91.7 and 91.1%, respectively, followed by sodium silicate applied as soil treatment + foliar spray which reduced the disease incidence and severity by 81.2 and 82.4%. Other treatments showed moderate effect.

Table 1 Effect of silicon as well as potassium, sodium, and calcium silicates on linear growth of strawberry black root rot fungi under laboratory conditions

Treatment	Concentration (g/l)	<i>Fusarium solani</i>		<i>Rhizoctonia solani</i>	
		Linear growth	Reduction %	Linear growth	Reduction %
Silicon	2.0	44.5 b*	50.6	38.0	57.8
	4.0	21.0 d	76.7	00.0 d	100.0
	6.0	00.0 e	100.0	00.0 d	100.0
Potassium silicate	2.0	48.0 b	46.7	40.2 b	55.6
	4.0	25.0 c	72.2	00.0 d	100.0
	6.0	00.0 e	100.0	00.0 d	100.0
Sodium silicate	2.0	50.0 b	44.4	35.0 c	61.1
	4.0	18.0 d	80.0	00.0 d	100.0
	6.0	00.0 e	100.0	00.0 d	100.0
Calcium silicate	2.0	33.0 c	63.3	44.0 b	51.1
	4.0	18.0 d	80.0	00.0 d	100.0
	6.0	00.0 e	100.0	00.0 d	100.0
Actamyl (Fungicide)	3.0	00.0 e	100.0	00.0 d	100.0
Control	0.0	90.0 a	0.0	90.0 a	0.0

*Figures with the same letter in a column are not significantly ($P \leq 0.05$) different

Table 2 Effect of silicon as well as potassium, sodium and calcium silicates on root rot disease incidence and severity of strawberry plants under field conditions

Treatment	Application	Black root rot disease			
		Disease incidence	Reduction%	Disease severity	Reduction%
Silicon	Soil	12.7 b*	69.0	10.2 b	72.4
	Soil + foliar	6.7 d	83.7	6.0 c	83.8
Potassium silicate	Soil	9.3 c	77.3	8.5 bc	77.0
	Soil + foliar	3.0 f	92.7	3.0 e	91.9
Sodium silicate	Soil	12.3 b	70.0	10.2 b	72.4
	Soil + foliar	7.7 d	81.2	6.5 c	82.4
Calcium silicate	Soil	9.7 c	76.3	8.5 bc	77.0
	Soil + foliar	3.4 f	91.7	3.3 e	91.1
Actamyl 3 g/L (Fungicide)	Soil	5.3 e	87.1	5.7 d	84.6
	Soil + foliar	4.7 e	88.5	5.2 d	85.9
Control		41.0 a	0.0	37.0 a	0.0

* Figures with the same letter in a column are not significantly ($P \leq 0.05$) different

Effect on weight of strawberry plants under field conditions

Results in Table 3 reveal a significant increase in the fresh and dry weight of strawberry plants by all tested concentrations. The highest increase was obtained with potassium silicate and calcium silicate applied as soil treatment + foliar spray which increased the fresh and dry weight of strawberry plants by 82.2 and 166.7, and 81.8 and 158.3%, respectively. Other treatments showed intermediate effect.

Effect on strawberry yield

All tested concentrations significantly increased strawberry fruit yield (Table 4). The highest increase was obtained with potassium silicate and calcium silicate applied as soil treatment + foliar sprays which increased fruit yield by 77.1 and 72.8%, respectively. Potassium silicate and calcium silicate applied as soil treatment as well as sodium silicate and silicon as soil + foliar spray increased fruit

yield more than 35.7% with no significant difference compared to the fungicide (Actamyl).

Effect on protein patterns

Results in Table 5 and Fig. 1 indicate that 12, 10, 9, and 8 new protein bands appeared in the treatments of calcium silicate, sodium silicate, potassium silicate, and silicon, respectively.

Discussion

Black root rot is a disease complex of strawberry caused by *Rhizoctonia fragariae*, *Ceratobasidium* [teleomorph] sp., *Coniothyrium fuckelii*, *Diaplella coniothyrium* [teleomorph]= *Leptosphaeria coniothyrium*, *Hainesia lythri*, *Discohainesia oenotherae* [teleomorph], *Idriella lunata*, *Pyrenochaeta* sp., *Pythium* spp., *Pythium ultimum* (Anonymous 2018). It is a serious, yield-limiting

Table 3 Effect of silicon as well as potassium, sodium and calcium silicates on weight of strawberry plants under field conditions

Treatment	Application	Weight (g)/plant			
		Fresh	Increase %	Dry	Increase %
Silicon	Soil	160.0 c*	45.5	17.0 d	41.7
	Soil+ foliar	180.1 b	63.7	22.0 b	83.3
Potassium silicate	Soil	182.0 b	65.5	22.1 b	84.2
	Soil+ foliar	200.4 a	82.2	32.0 a	166.7
Sodium silicate	Soil	157.0 c	42.7	19.2 c	60.0
	Soil+ foliar	180.0 b	63.6	22.0 b	83.3
Calcium silicate	Soil	177.0 b	60.9	19.5 c	62.5
	Soil+ foliar	200.0 a	81.8	31.0 a	158.3
Actamyl 3 g / L (Fungicide)	Soil	150.0 c	36.4	18.2 d	51.7
	Soil+ foliar	150.0 c	36.4	18.2 d	51.7
Control		110.0 d	0.0	12.0 e	0.0

*Figures with the same letter in a column are not significantly ($P \leq 0.05$) different

Table 4 Effect of silicon as well as potassium, sodium, and calcium silicates on strawberry fruit yield under field conditions

Treatment	Application	Yield (tons/feddan)	Increase %
Silicon	Soil	8.2 d*	17.1
	Soil+ foliar	9.8 b	40.0
Potassium silicate	Soil	10.0 b	42.9
	Soil+ foliar	12.4 a	77.1
Sodium silicate	Soil	8.0 d	14.3
	Soil+ foliar	9.5 cb	35.7
Calcium silicate	Soil	10.2 b	45.7
	Soil+ foliar	12.1 a	72.8
Actamyl 3 g/L (Fungicide)	Soil	10.0 b	42.9
	Soil+ foliar	10.0 b	42.9
Control		7.0 d	0.0

*Figures with the same letter are not significantly ($P \leq 0.05$) different

disease that reduces annual production by about 30% in the Western Cape Province of South Africa, recognized as the most important root disease of strawberries in this area (Botha et al. 2001; Juber et al. 2014; Fang et al. 2013). In Egypt, the disease is frequently caused by *F. solani* and *R. solani* but exact yield loss is unknown. We tested herein four concentrations, i.e., 0, 2, 4, and 6 g/l of silicon,

Table 5 Molecular weights for protein patterns of strawberry plants as affected with silicon and silicate salts analyzed using gelaxiralyzer 2010 program

Control	Potassium silicate	Calcium silicate	Sodium silicate	Silicon
159	191	173	174	182
119	170	156	156	165
105	151	136	136	145
87	132	130	127	121
70	116	117	113	106
60	106	111	99	100
55	98	98	94	88
48	80	93	83	79
44	65	81	67	71
39	54	75	63	67
35	50	67	52	63
33	45	64	48	58
32	42	58	45	50
30	38	49	41	43
	35	46	38	39
	32	41	36	37
		37	34	35
		36	33	33
		34	31	32
		32	30	30

potassium, sodium, and calcium silicates, to study their effect on linear growth of strawberry black root fungi (*F. solani* and *R. solani*) under laboratory conditions. Results indicated that all tested concentrations significantly reduced the linear growth of both fungi. Complete inhibition of linear growth was obtained with 4 and 6 g/l for *R. solani* and *F. solani*, respectively. All concentrations tested herein significantly reduced the disease incidence and severity under field conditions with their differences. Consequently, the highest yield increase was obtained with potassium and calcium silicates applied as soil treatment + foliar spray.

As numerous pathogens could be entangled in the black root-rot complex, no single control measure is totally effective. Therefore, cultural practices should be strictly followed as preventive measures before applying the aforementioned treatments as a last resort. Many Egyptian growers have already put some of these practices in effect especially avoiding heavy, wet soils and improve drainage in marginal soils by tactful irrigation system and tillage as well as planting on raised beds. Moreover, the source of seedlings must be examined to ensure healthy white-rooted plants. Also, it is essential to incorporate organic matter such as buffalo/poultry fertilizer as well as straw from a rotational grain crop before bed construction. On the other hand, some Egyptian growers neglect crop rotation although strawberry should be rotated for at least 2 years before replanting. Preplant fumigation of the soil is frequently helpful, but some unauthorized fumigants, sometimes used by Egyptian growers, such as methyl bromide should be avoided.

Bekker et al. (2006) reported the effect of soluble potassium silicate (20.7% SiO₂) on *Phytophthora cinnamomi*, *Sclerotinia sclerotiorum*, *Pythium* sp., *Mucor pusillus*, *Drechslera* sp., *Fusarium oxysporum*, *F. solani*, *Alternaria solani*, *Colletotrichum coccodes*, *Verticillium theobromae*, *Curvularia lunata*, and *Stemphylium herbarum*. Inhibition of mycelial growth was dose-related with 100% inhibition at 80 ml (pH 11.7) and 40 ml (pH 11.5) soluble potassium silicate per liter of agar, for all fungi tested with the exception of *Drechslera* sp. and *F. oxysporum* at 40 ml in one experiment. Only *Sclerotinia sclerotiorum* and *Phytophthora cinnamomi* were completely inhibited at all soluble potassium silicate concentrations between 5 and 80 ml/l agar, while all the other fungi were only partially inhibited at potassium silicate concentrations of 5, 10, and 20 ml/l agar. Likewise, percentage inhibition was positively correlated with dosage. Bekker et al. (2009) demonstrated in vitro inhibition of mycelial growth of phytopathogenic fungi grown on potassium silicate-amended media.

Nada et al. (2014) stated that potassium silicate was more effective than calcium and sodium silicates in reducing damping-off incidence and in improving plant growth parameters of coriander. Also, Kanto et al. (2006)

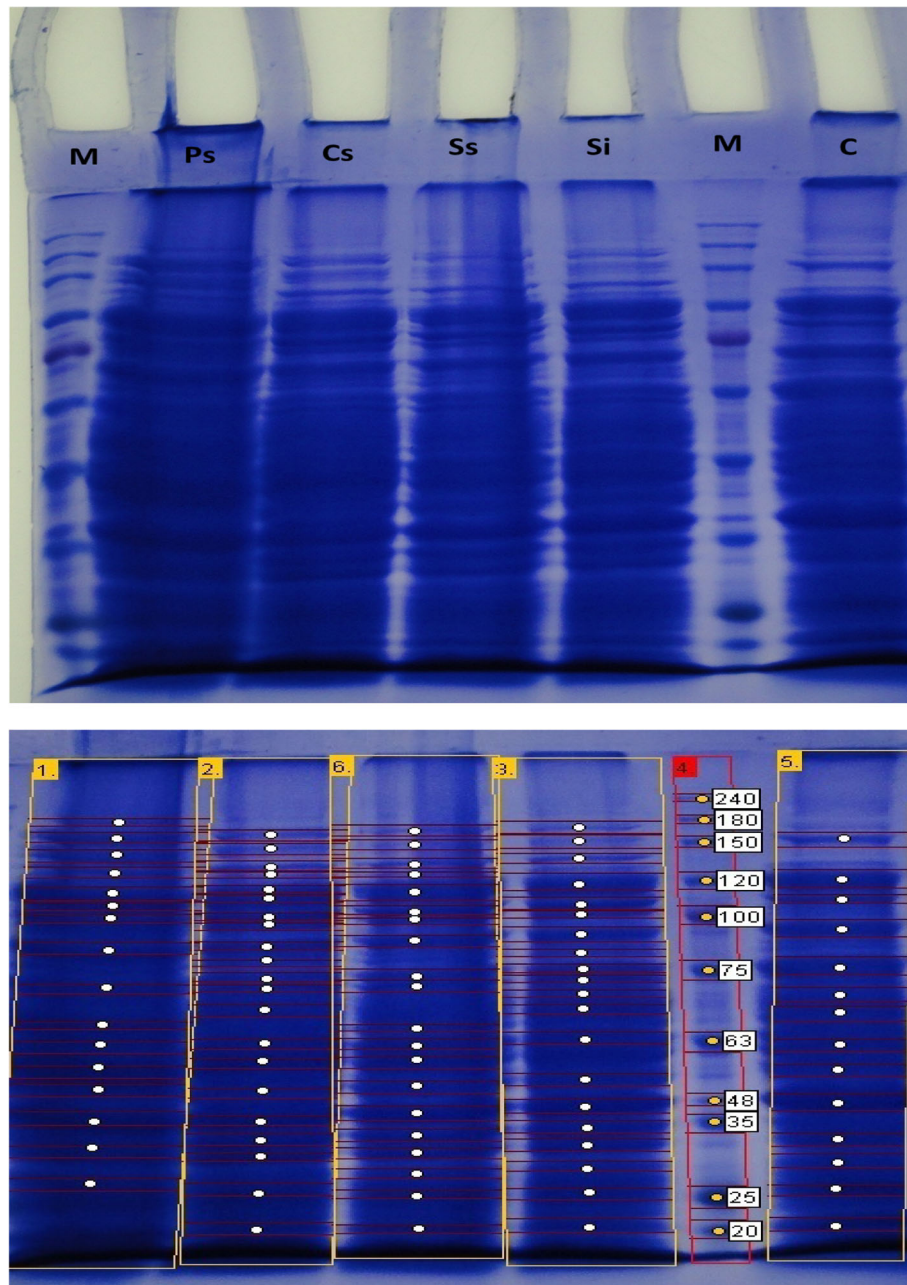


Fig. 1 Protein patterns of strawberry plants as affected with silicon and silicate salts. Upper patterns: Ps = potassium silicate, Cs = calcium silicate, Ss = sodium silicate, Si = silicon, C = Control, M = marker; Lower patterns: 1 = potassium silicate, 2 = calcium silicate, 3 = sodium silicate, 4 = marker, 5 = Control, 6 = silicon

reported that both potassium and calcium silicates suppressed Fusarium wilt of cucumber for 3 years more than sodium silicate. They also found that liquid potassium silicate as soil drench to control the powdery mildew of strawberry in the soil acted more efficiently as preventive rather than curative treatment; strawberry leaf treated by silicate was harder than control leaf. Moreover, Jayawardana et al. (2014) reported that root and foliar application of soluble potassium silicate caused decrease in disease

incidence and increase in plant growth and fruit quality parameters. Liang et al. (2005) noticed that silicon can prevent pathogen penetration into host tissues. Eventually, it is likely that reduction in disease incidence in plants treated with silicon sources under field conditions is not probably due to the fungistatic effects of silicon, but silicon could act as physical barrier against pathogen penetration or silicon can be used as inducer for defense response in plant (Shen et al. 2010).

On the other hand, Cherif and Bélanger (1992) found that treating cucumber roots with soluble silicon resulted in an increase in the activities of peroxidase and polyphenoloxidase and that silicon can stimulate accumulation of polymerized phenolic compounds. These results agreed with ours reported herein but for different host and plant pathogen. There are numerous explanations of the silicon role to suppress the rot disease. The emerging role of silicon as a biologically active element capable of improving the natural defense system of the plant; Si-treated plants exhibited increased activity of peroxidases, chitinases, polyphenol oxidases, and flavonoid phytoalexins, which play an important role in the resistance of the plant to fungal pathogens (Fawe et al. 1998). Furthermore, the higher production of glycosylated phenolics, antimicrobial products such as diterpenoid phytoalexins and a proline-rich protein in the silicon-treated plants, indicated that these products can have a role in the protection effects of Si against plant diseases (Rodrigues et al. 2003, 2010). The bioactivity of Si as a regulator of plant defense mechanisms may be explained through the biochemical properties, e.g., Si binds with hydroxyl groups of proteins which are involved in signal transduction. Also, Si may interfere with cationic cofactors of the enzymes which influence pathogenesis-related (PR) events.

Nevertheless, plant reactions to the attacking plant pathogens are very complex and involve the activation of set of genes, encoding different proteins. These proteins can induce biochemical and physiological changes in plants, such as physical strengthening of the cell wall through lignification, suberization, and callose deposition, by producing phenolic compounds, phytoalexins and PR proteins which subsequently prevent various pathogen invasions (Li et al. 2001; Ebrahim et al. 2011).

Results in the present study indicated that 12, 10, 9, and 8 new protein patterns (bands) appeared in the treatments of calcium silicate, sodium silicate, potassium silicate, and silicon, respectively. In this respect, Ryals et al. (1996) reported that production and accumulation of PR proteins in plants in response to invading pathogen and/or stress conditions is very important. Phytoalexins are mainly produced by healthy cells adjacent to localized damaged and necrotic cells, but PR proteins accumulate locally in the infected and surrounding tissues and also in remote uninfected tissues. Production of PR proteins in the uninfected parts of plants can prevent the affected plants from further infection.

Conclusions

The black root rot becomes a major problem in many strawberry fields in Egypt. To avoid environmental pollution and health hazards in managing the disease, an integrated approach is necessary which includes tactful cultural practices and applying foliar and soil inputs, e.g.,

silicon salts reported herein. Such an approach can effectively control the disease and enhance strawberry yield.

Abbreviations

PR: Pathogenesis-related; Si: Silicon; SDS-PAGE: Sodium dodecyl sulphate polyacrylamide gel electrophoresis

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Authors' contributions

All authors participated in the development and implementation of the research plan and subsequently written it. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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