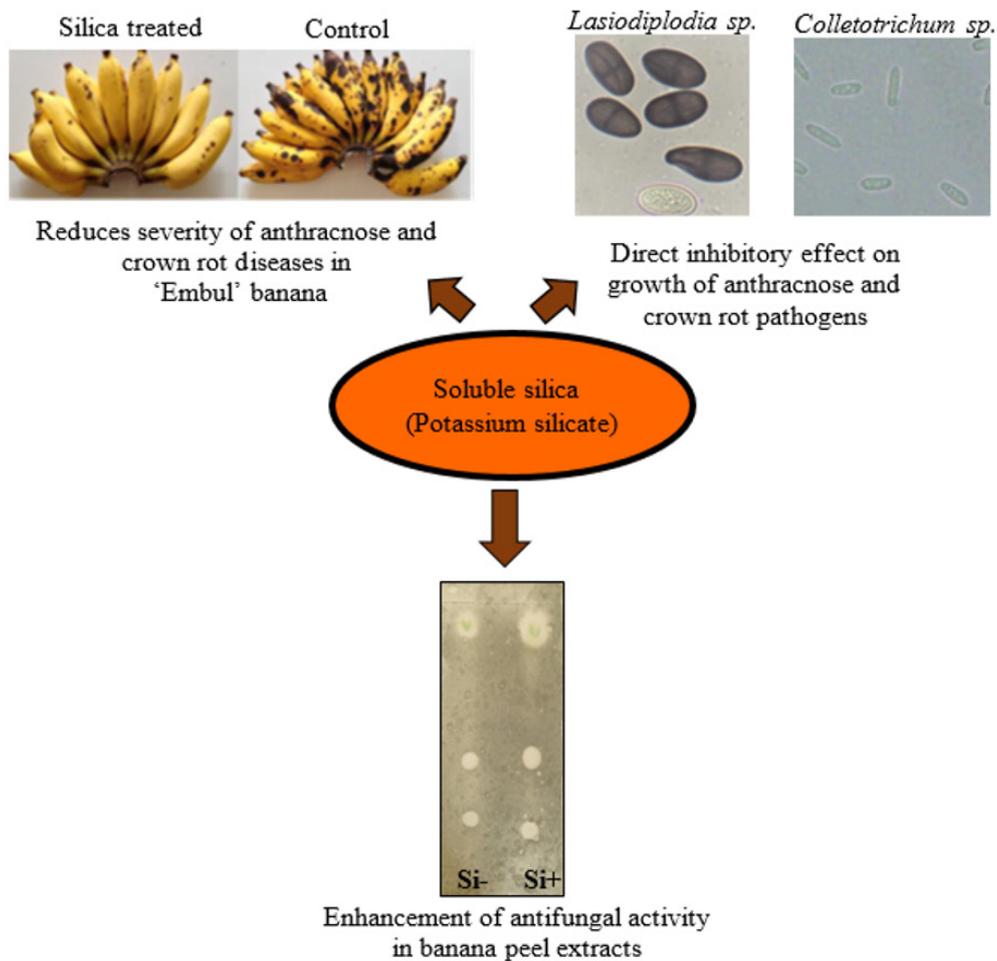# Direct inhibitory effect and enhanced antifungal activity of postharvest soluble silica treatment against anthracnose and crown rot pathogens in banana

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Highlights

- Dip-treatment with soluble silica reduced the severity of anthracnose and crown rot disease in banana.
- Soluble silica imposed a direct inhibitory effect on the fungal pathogens *Colletotrichum* sp. and *Lasiodiplodia* sp.
- Silica-treated banana exhibited enhanced pre-formed antifungal activity in the fruit peel.

SHORT COMMUNICATION

Direct inhibitory effect and enhanced antifungal activity of postharvest soluble silica treatment against anthracnose and crown rot pathogens in banana

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Abstract: Soluble silica (Si) treatment has been reported to control postharvest fungal diseases and extend shelf life of bananas. In this study, the effects of soluble silica treatment on antifungal activity of fruit peel and direct inhibitory effect against anthracnose and crown rot (CR) pathogens in banana were investigated. Mature banana cv. 'Embul' fruits, treated with potassium silicate (1500 µg mL^{-1} K$_2$SiO$_3$ solution for 20 min) or with water (control) were artificially inoculated with anthracnose pathogen (*Colletotrichum* sp.) and CR pathogen (*Lasiodiplodia* sp.), and assessed for anthracnose lesion diameters and CR Index. Bioassays with *Cladosporium* and *Colletotrichum* sp. were performed to assess antifungal activity in ethyl acetate extracts of Si treated (Si+) and control (Si-) fruit peel tissues. Total phenolic (TP) contents of Si+ and Si- peel extracts were determined by Folin Ciocalteu assay. To test direct inhibitory effect of Si on growth, the two pathogens were grown in Potato Dextrose Broth (PDB) media amended with silica (0, 500, 1000, 1500 and 2000 µg mL^{-1}) and harvested for mycelial dry weights and spore counts. A significant delay in symptom development and reduction of severity of both diseases was observed in Si+ fruits. In bioassay plates, three antifungal zones (R_f= 0.14, 0.48, 0.79) appeared in both Si+ and Si- extracts, indicating them to be pre-formed antifungal compounds (phytoanticipins). Si+ extracts showed higher antifungal activity and TP levels. Reduction of mycelial growth and spore production was observed with increased Si levels in PDB. Si-enhanced resistance to fungal rots in bananas can be partly attributed to enhanced phytoanticipin levels, including phenolics, and direct inhibitory effect on pathogens.

Keywords: Anthracnose; banana; crown rot; soluble silica.

INTRODUCTION

Postharvest fungal diseases are a major cause for losses during banana handling chain. Use of systemic fungicides, such as benomyl and thiabendazole (TBZ), has been practicing by farmers as a conventional way of controlling postharvest fungal diseases; anthracnose and crown rot (Khan *et al*., 2001). Nevertheless, indiscriminate use of fungicides has resulted in the emergence of fungicide-resistant strains (Kablan *et al*., 2012) and health problems. Finding alternatives to synthetic fungicides is thereby important to overcome their negative effects and to satisfy the food security.

Use of soluble silica (Si) has been practicing as an alternative method to control pre- and postharvest diseases in many crops. Silica is considered as a Generally-Regarded-As-Safe (GRAS) compound and also a natural defense inducer compound. Si accumulates in plants and utilizes various beneficial effects for crop species, such as cucurbits (Ratnayake *et al*., 2016) and legumes (Guérin *et al*., 2014). Studies show that Si could lighten plant disease through (1) blocking penetration via structural fortification, (2) hindering of pathogen settlement through stimulating systemic acquired resistance, (3) antimicrobial compound production, and (4) enhanced plant resistance by simulating the defense-related gene expression (Wang *et al*., 2017).

Soluble silica has been used to control decay and maintain postharvest quality of several fruit and vegetable commodities. This disease control was closely associated with Si-enhanced disease resistance in fruit tissues (Hassan *et al*., 2021).

Literature related to Si effects of postharvest quality of banana is limited. We recently reported that the dip-treatment with soluble silica (potassium silicate; K$_2$SiO$_3$) extends postharvest life of 'Embul' banana (Nikagolla *et al*., 2019). The objective of this study was to further investigate the possible mechanism/s of silica-induced resistance to anthracnose and crown rot, the major postharvest fungal diseases in banana.

MATERIALS AND METHODS

Plant material

Mature banana (*Musa acuminata* AAB) cv. 'Embul' fruits were and prepared for experiments as described by Nikagolla *et al*. (2019).

Isolation and identification of causative fungal pathogens of anthracnose and crown rot diseases

Isolation and sub-culturing of fungal pathogens were carried out using protocols described by Agrios (2005).

Morphology of fungal colonies was observed under light microscope (Olympus CX21FS1, UK) for identification.

Effect of soluble silica on anthracnose and crown rot development on artificially inoculated banana

Effects of Si treatment on severity of disease caused by challenge-inoculated anthracnose and CR pathogens were tested on banana fruit. Banana 'fingers' and 'hands' were used for the testing of anthracnose and crown rot, respectively. Bananas were dip-treated with previously identified effective soluble silica level (Si+); 1500 µg mL^{-1} of K$_2$SiO$_3$ for 20 min and its respective control treatment (Si-); distilled water for 20 min (Nikagolla *et al.*, 2019).

Anthracnose and Crown rot development

A conidial suspension (1×10^8 conidia mL^{-1}) was prepared using the anthracnose pathogen cultures grown on PDA. Twenty-four hours after the silicon treatment, 20 µL drops of the suspension were placed at two points along the fruit surface. Both Si+ and Si- fruits were incubated under ambient temperature (27±3 °C) and 95-100% relative humidity. Anthracnose lesion diameters (cm) were measured daily.

Mycelial segments of (1 cm×2 cm) from 14 d old cultures of CR pathogen were inoculated on crown scar of banana hands. Fourteen days after inoculation, rot development was measured and expressed as Crown Rot Index (CRI).

Testing the direct inhibitory effect of soluble silica on growth of anthracnose and crown rot pathogens *in-vitro*

Effect of soluble silica on *in-vitro* growth of anthracnose pathogen and CR pathogen was tested as described by De Costa and Gunawardhana (2012). Potato Dextrose Broth (PDB) having final concentrations of 0, 500, 1000, 1500 and 2000 µg Si mL^{-1} (final pH 6.5) were prepared and mycelial discs (9 mm diameter) of anthracnose and CR pathogens, were introduced separately to flasks containing 50 mL of Si amended PDB media. The flasks were incubated at 27±3 °C for seven days as static cultures, and the mycelial masses were collected to quantify the dry weight. Mean spore production under different silica concentrations was quantified as described by De Costa and Gunawardhana (2012).

Effect of Si treatment on antifungal activity in banana fruit peel

Thin layer chromatography (TLC) coupled with bioassays using *Cladosporium cladosporioides* and *Colletotrichum* sp. (anthracnose pathogen) were performed to assay antifungal activity. Si+ and Si- fruits were stored under ambient conditions for 24 h before extraction of antifungal compounds. Frozen peels were extracted with ethyl acetate. Aliquots of 50 µL were spotted in and separated by TLC using chloroform (CHCl$_3$): methanol (CH$_3$OH) (95:5 v/v) as the developing solvent system. Bioassays were conducted (Adikaram and Bandara, 1998) to locate antifungal compounds on TLC. Conidial suspensions (1×10^6 conidia mL^{-1}) of *C. cladosporioides*

and *Colletotrichum* sp. sprayed on separate TLC plates and incubated in a moist chamber at room temperature (27±3 °C). After five days of incubation, TLC plates sprayed with *Colletotrichum* sp. were placed in a closed box containing a saturated iodine solution to locate antifungal zones.

Total phenolic compound contents (TPC) in banana peel

Two days after silicon treatment, Si+ and Si- peel tissues were oven dried at 40 °C for complete dryness. The dried powder was used to visualize the bands (grayish blue) with phenolic compounds on TLC plates after chromatographic separation in chloroform:methanol (CHCl3:CH3OH) (95:5 v/v) and spraying with Folin Ciocalteu (FC) reagent. TPCs were quantified using FC assay coupled with spectrophotometry (Singleton *et al.*, 1999). TPC was determined using a calibration curve plotted with gallic acid as a standard expressed as mg. GAE/gdw.

Experimental design and data analysis

Challenge inoculation study on anthracnose disease development was carried out for 15 replicate single fruits. CR disease development experiments were carried out for 8 replicate hands of banana having 12-18 fingers in each. Each experiment including antifungal activity assays was repeated three times, and pooled data were subjected to statistical analysis. For broth culture experiments, three replicate flasks per Si concentration were employed. All experiments were arranged in Complete Randomized Design. Two sample T test and Mann-Whitney test were done to compare anthracnose lesion diameters and Crown rot development, respectively. Mean mycelial weight and spore count data were subjected to analysis by One-way ANOVA. The data were analyzed using Minitab 15 statistical software.

RESULTS

Isolation and identification of causative fungal pathogens of crown rot and anthracnose diseases

Pathogenic fungus isolated from anthracnose symptomatic tissues produced pinkish white colonies on PDA. Mycelium was septate, and hyaline and conidia were aseptate, hyaline and frequently ellipsoid. Based on the colony morphology and microscopic characteristics, the causative fungus was identified as *Colletotrichum* sp. The CR pathogen isolated on PDA produced colonies initially white in colour, but changed to smoke-grey to black with time on the upper and lower surfaces. Conidia were hyaline sub-ovoid to ellipsoidal, unicellular and with time became light to dark-brown color thick walled, equally bi-celled and oblong. Based on those characteristics, the pathogen was identified as *Lasiodiplodia* sp.

Effect of soluble silica on anthracnose and crown rot development on artificially inoculated banana

Anthracnose and crown rot development

Si+ banana 'hands' showed significantly lower crown
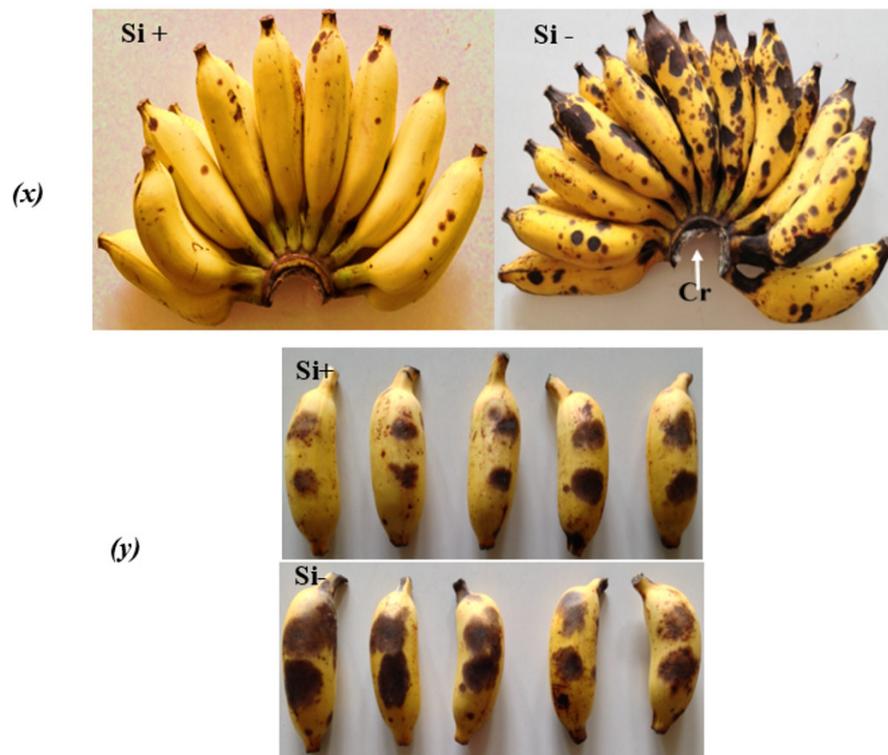
Figure 1: (*x*) Crown rot development (Cr) on fruits (Si+) and (Si-) at 14 d after challenged inoculation with *Lasiodiplodia* sp. (*y*) Development of anthracnose symptoms in (Si+) and (Si-) banana 12 d after challenge inoculation *Colletotrichum* sp.

rot index (3.0) compared to that in Si- fruits (5.0) by 14 d after inoculation with the pathogen *Lasiodiplodia* sp. (Figure 1x). Anthracnose lesions started to appear seven days and three days after incubation in Si+ and Si-fruits, respectively. A significantly ($p < 0.05$) lower disease development was observed (Figure 1y) at 12 d of incubation of Si+ (0.5 ± 0.04 cm) compared to that in Si-fruits (1.4 ± 0.07 cm).

Direct inhibitory effect of potassium silicate on growth of *Colletotrichum* sp. and *Lasiodiplodia* sp. *in-vitro*

Spore production of *Colletotrichum* sp. was negatively affected by increased Si concentrations in broth cultures (Figure 2x). Although *Colletotrichum* sp. showed intense spore production, *Lasiodiplodia* sp. did not produce any reproductive structures during the seven-day incubation period. Higher fungal growth of both pathogen species was observed in PDB media without Silica treatment.

A complete inhibition of mycelial growth of *Colletotrichum* sp. was observed with 2000 µg mL^{-1} Si, and that of *Lasiodiplodia* sp. was observed in 1500 and µg mL^{-1} Si level and above (Figure 2y).

Effect of Si treatment on antifungal activity in banana fruit peel

The bioassay with *C. cladosporioides* revealed the presence of three zones of antifungal activity in banana peel extracts at R_f values 0.14, 0.48 and 0.79 both in Si+ and Si- samples. The areas of inhibition at all three R_f values in Si+ extracts were bigger than those of Si- indicating enhanced antifungal activity upon Si treatment (Table 1). However, in bioassay with *Colletotrichum* sp. only one faint antifungal zone at R_f 0.79 was visible.

Phenolic content in banana fruit peel

TLC plates sprayed with FC reagent showed two zones in faint blue color at R_f 0.14 and 0.79, indicating the presence of phenolic compounds. Si+ peel extracts had significantly higher TPC (3.614 ± 0.170) compared to the Si- extracts (3.014 ± 0.059).

DISCUSSION

This study reveals that dip treatment of potassium silicate effectively reduces anthracnose disease severity in banana during storage under tropical ambient conditions. Soluble silica treatment had been effective in controlling a number of *Colletotrichum* sp. in several commodities including Capsicum (Jayawardana *et al.*, 2016).

It was previously reported that soluble silica delays ripening of banana by reducing the rate of ethylene production (Nikagolla *et al.*, 2019). The delay in ripening may have contributed to slow development of anthracnose and crown rot since there is a direct relationship between postharvest rot developments along ripening in banana. According to literature, silicates efficiently control postharvest rots during storage, delay the onset of infection, and slow down the infection process. Overall, the reduction of severity of rot has been more significant with elevated silicate concentrations.

(x)

(y)
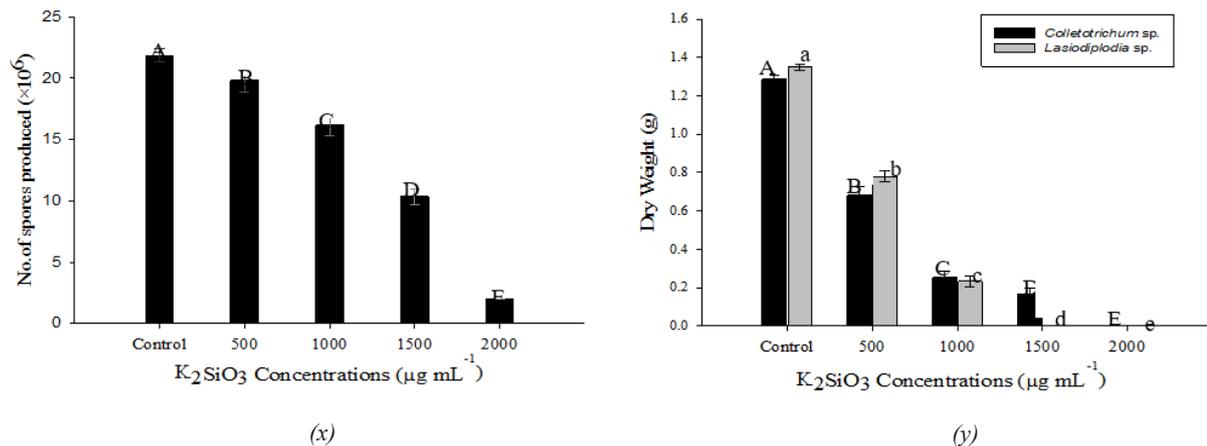
Figure 2: *(x)* Mean number of spores of *Colletotrichum* sp. after seven days of incubation in PDB amended with different concentrations of K_2SiO_3. *(y)* Mean mycelial dry weights of *Colletotrichum* sp. and *Lasiodiplodia* sp. after seven days of incubation in PDB media amended with different concentrations of K_2SiO_3. Different letters on bars indicate significant difference at $p < 0.05$. Vertical line in each bar indicates standard error of mean.

Table 1: Areas of inhibition in antifungal assay with *Cladosporium cladosporioides* of Silica treated (Si+) and control (Si-) extracts.

Treatment	Area of inhibition (mm²)		
	$R_f = 0.14$	$R_f = 0.48$	$R_f = 0.79$
Si+	290 ± 0.10	328 ± 0.10	720 ± 0.20
Si-	272 ± 0.20	302 ± 0.30	556 ± 0.40

$n = 2$ replicate TLC plates.

Reduction of mycelial growth of anthracnose and crown rot pathogens in silica-amended media indicates a direct inhibitory effect of Si on them. This may also have partly contributed to reduced rot severity in silica treated fruits. Similar inhibitory effects of Si on mycelial growth have been reported on several agriculturally important pathogens including *Alternaria solani*, *Fusarium oxysporum* and *L. theobromae* (Bekker *et al*., 2009). Electron microscopic studies revealed that sodium silicate treatment has caused changes, such as mycelial sparsity, asymmetry, curling, and twisting in *F. sulphureum*, where the extent of hyphal damage increased with higher silica concentrations (Li *et al*., 2009). These evidences suggest the potential use of soluble silica as an alternative to conventional fungicides.

Many crop plants treated with Si showed enhanced activity of defense-related enzymes or compounds *viz* peroxidase, polyphenol oxidase, chitinase, ß-1,3-glucanase, phenylalanine ammonia lyase (PAL) and phenolics (Ratnayake *et al*., 2016). The bioassays on TLC indicated that, silicon dip treatment could not induce production of new antifungal compounds, but enhanced the activity of preformed antifungal compounds (phytoanticipins) in banana peel. Increased levels of phenolic compounds may have contributed partly for enhanced antifungal activity. According to Behiry *et al.* (2019), phenolic compounds, such as ferulic acid and caffeic acid, present in banana peel may have some role in this antifungal activity against postharvest pathogens.

As previously reported, postharvest silica treatment increases the total Si levels in the banana fruit peel and also the fruit firmness (Nikagolla *et al*., 2019). Application of Si also creates a mechanical barrier to pathogens by forming a cuticle-Si double layer in epidermal tissues. The accumulated Si also may have provided a barrier thus reducing the severity of anthracnose disease in banana.

CONCLUSION

Silica dip treatment reduces the anthracnose and crown rot severity of banana stored under tropical ambient conditions. Overall, this study suggests Si-enhanced chemical mechanisms that may contribute to reduction of disease severity in banana fruits as (i) direct fungi-toxic effect on soluble silicon on pathogenic fungi *Colletotrichum* sp. and *Lasiodiplodia* sp. (ii) enhancement of phytoanticipin levels in fruit peel. Histological studies are being continued to gain a better idea on Si-enhanced structural defense responses in banana.

ACKNOWLEDGEMENT

DECLARATION OF CONFLICT OF INTEREST

We declare that we do not have any conflict of interest.

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