### **RESEARCH ARTICLE**

# Potassium silicate treatment enhances natural disease resistance in *Capsicum annuum* L. and reduces anthracnose disease development

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Abstract: The study investigated the possibility of enhancing natural disease resistance (NDR) of chili (Capsicum annuum L.), cvs. HYW and CA8, against anthracnose disease, using potassium silicate (Kasil®) as a soil drench or postharvest spray treatment. Anthracnose disease in chili, in this study, was shown to be caused by Colletotrichum acutatum. Potassium silicate (Kasil®), applied as a postharvest spray to the fruit of either cultivar, at 100, 200 and 400 mg/l, reduced anthracnose development significantly (p = 0.05) when the fruits were challenge-inoculated 3 days after treatment. Treatment was slightly more effective at 200 mg/l. There was 100% of reduction of anthracnose in treated fruits of cv. CA-8 throughout an  $8^{th}$  day period of storage following fruit inoculation. In the cv. HYW, the same treatment showed 100% disease reduction only initially, 4 days after inoculation. The treatment was also applied as a pre-harvest soil drench commencing from flower initiation, at 200 mg/l, weekly for 4 weeks. The treatment reduced anthracnose disease significantly (p = 0.05) in harvested fruits, challenge-inoculated with C. acutatum. Spore germination assay revealed that potassium silicate had no antifungal effect to C. acutatum. Potassium silicate (Kasil®)-treated fruits, inoculated with C. acutatum after harvest, showed greater accumulation of phytoalexins and increased level of  $\beta$ -1, 3-glucanase in fruit tissue.

*Keywords:* Anthracnose, Enhanced resistance, Potassium silicate (Kasil<sup>®</sup>), *Colletotrichum acutatum*.

## INTRODUCTION

Anthracnose disease, caused by *Colletotrichum* species, is a threat in chili pepper (*Capsicum annuum* L.) production in tropical and sub-tropical countries (Oanh *et al.*, 2004). The alternative methods to fungicidal application to control the anthracnose, has become a must due to various reasons, include; environmental pollution, health concern of the consumers and also development of fungicideresistant pathogen populations etc. The enhancement of plant disease resistance plays a vital role in minimizing the postharvest losses and adjusting crop production to meet global population, increases. It also satisfies the requirements, such as, no toxicity to pathogens, plants or animals, not affecting plant growth, development and yield, broad spectrum of defense and long lasting (Kessman *et al.*, 1994; Tally *et al.*, 1999; Kuc, 2001). There were several studies in the past proved that the enhanced natural disease resistance could be achieved with silicon (Si) treatment, in a wide range of tropical and sub-tropical fruits and vegetables (Weerahewa and Somapala, 2016; Huang *et al.*, 2011; French- Monar *et al.*, 2010). This has been achieved through different role of silicon, include; reduced mineral toxicity, increased photosynthetic activity, superior nutrient imbalance, and enhanced drought and frost tolerance (Ma, 2004).

Si application enhancing the disease resistance by two different methods against infections; (i) either by Si deposition on plant tissues and leads in preventing the pathogen penetration and/or (ii) by synthesis of antipathogenic compounds. Therefore, the main objective of this research was to characterize the induced resistance responses of chili against an economically important disease, the anthracnose by the pre-harvest soil amendment of potassium silicate (Kasil®).

### MATERIALS AND METHODS

#### **Collection of samples**

Fresh and healthy chili pepper samples cvs. Hungarian Yellow Wax (HYW) and CA8 were obtained from retail markets at Colombo (Western Province), Sri Lanka. Chili fruits showing symptoms typical to anthracnose disease were also collected separately in polythene bags. Healthy and diseased fruits were taken to the laboratory experiments were carried out at the Department of Plant Sciences, Faculty of Science, University of Colombo, Sri Lanka.

#### **Isolation of fungi**

Segments of diseased tissues from chili pepper, collected from local markets, were surface sterilized in 2% sodium hypochlorite for 3 min and after washing in sterile distilled water, the segments were transferred under sterile conditions onto Potato Dextrose Agar (PDA) medium. Culture plates were incubated at  $27^{\circ} - 30^{\circ}$ C. The colony morphology of the cultures and the shapes of the conidia were recorded.



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## Postharvest application and optimization of Potassium silicate (Kasil<sup>®</sup>)

The effect of postharvest application of Potassium silicate (Kasil<sup>®</sup> 2236(K32), weight ratio SiO<sub>2</sub>: K<sub>2</sub>O = 2.23, weight % SiO<sub>2</sub> = 24.8) and development of anthracnose disease in chili pepper *c*vs. HYW and CA-8 was tested. Solutions were prepared by dissolving the required amount of Potassium silicate (Kasil<sup>®</sup>) in sterile distilled water to obtain final concentrations of 0 (control), 100, 200 and 400 mg/l. Tween 20 (50µl /l) was added to all solutions as a wetting agent.

Eighty (80) healthy chili pepper fruits, consisting 40 samples from each cultivar ( $n = 40 \ge 2$ ), were used for this experiment. Surface sterilized samples were arranged in eight moist chambers ( $n = 10 \times 4 \times 2$ ), sprayed with the Potassium silicate (Kasil®) solution, prepared in above concentrations, using atomizer pump (KnfNeuberger D-79112, Freiburg) and were incubated at the room temperature (28± 2 °C). After 72 hours of incubation, the samples were wiped with sterile cotton wool, soaked with SDW in order to remove any chemicals remaining on the surface of the fruits and were challenge inoculated with 20  $\mu$ l conidia suspension (1 x 10<sup>6</sup> spores/ml) of C. acutatum. Four to five inoculations were made along the long axis, depending on the length of the fruit. The diameter of lesions was measured daily in two places of the inoculated site (at right angles to each other) and the lesion area was calculated. All treatments were arranged in a CRD. Data were analyzed by ANOVA using general linear model (GLM) procedure and means were separated by Duncan's Multiplel Range Test using SAS computer software (Release 6.12), at p<0.05 significant level.

## Testing antifungal effect of potassium silicate (Kasil<sup>®</sup>) on spore germination of *C. acutatum*

Suspensions of potassium silicate (Kasil®) solutions were prepared by dissolving the required amount of the chemical in sterile distilled water, to obtain the final concentrations of 0, 20, 100 and 200 mg/l and with Tween 20 (50µl /l) as a wetting agent. Slide germination test with the conidia suspension of C. acutatum was carried out using the above solutions. 10 µl of conidia suspension was mixed with 10  $\mu$ l of chemical elicitor solution on a glass slide. Control contained 10 µl of suspension of conidia and 10 µl of SDW. The slides were incubated in moist chambers at room temperature ( $28\pm2^{\circ}c$ ). After 24 h, a drop of lacto phenol in cotton blue was added to terminate further germination. The total number of conidia and the number of germinated conidia with appressoria were counted in 3 randomly selected microscopic fields (x 400). A total of 18 microscopic fields were observed per concentration. Percent germination and appressoria formation were determined for each concentration.

## Assessment of enhanced defense responses of fruit samples, treated with potassium silicate (Kasil<sup>®</sup>)

## TLC bioassay

Potassium silicate (Kasil®) treated and control (SDW)

fruits, inoculated with conidial suspension of C.acutatum, were used in this experiment. Extraction was done in ethyl acetate (10g tissue / 50 ml ethyl acetate), from 50g of peel tissues, collected from beneath the inoculated sites under vacuum with constant magnetic stirring for 1 h and was filtered through Whatman No. 1 filter paper. Fresh ethyl acetate was added to the residue and extraction was repeated for two more times. The extracts were pooled and evaporated to dryness (Adikaram and Ratnayaka Bandara, 1998) in a rotary evaporator at 40 0C (Staurt RE 300). The crude residue was collected in 500 µl of ethyl acetate. A 100 µl aliquot of this extract was spotted on glass plate coated with silica gel (13 % CaSo<sub>4</sub>, GF<sub>254</sub>, BDH). The plate was then developed in chloroform: methanol (95:5 v/v) and air dried over night to remove remaining solvent. The developed TLC plate was sprayed with the conidia suspension of C. cladosporioides, using an atomizer pump (Knf, Neuberger, D-79112) and the TLC plate was incubated in a moist chamber at 20 °C. Presence of antifungal compound was identified by the lack of aerial mycelium in the relevant zone (Klarman and Stanford, 1968).

## Defense-related enzymes $-\beta$ -1, 3-glucanase

Small segments of fruit peel of chili pepper (1 x 1cm area and 2mm depth) surrounding the inoculated sites were excised and were extracted using the method of Dann and Devarall (2000). From these segments, 1g of sample was homogenized at 11,000 rpm in a pre-cooled centrifuged tube with approximately 1% (w/w) PVPP (Polyvinylpolypyrolidone) and 5 ml, 50mM potassium acetate buffer pH 5, containing 1mM EDTA and 5mM reduced glutathione that was added immediately prior to homogenization. Extracts were centrifuged and supernatants were stored at -17  $^{\circ}$ C until used as crude extract in assay of enzyme activities.

 $\beta$ -1, 3-glucanase activity was assayed based on the method described by Dann and Devarall (2000). The assay relies on the release of a soluble and measurable dye when the substrate, azurine-crosslinked Pachyman (AZCL-Pachmyman), is hydrolysed by endo-  $\beta$ -1, 3-glucanase. The substrate was developed and formulated as a tablet by Megazyme Australia P/L. Potassium acetate buffer (1.6 ml, 10mM, pH 5) and 0.4 ml of a crude extract was added to a centrifuge tube and allowed to equilibrate to 30 °C for 3 minutes. One substrate (Pachyman) tablet was suspended 4 ml of double-deionized water and vortexed (Whirlimixer<sup>TM</sup>, Fisher brand) to maintain a homogenous suspension. The reaction was initiated by the addition of 0.4 ml of the substrate suspension and was stopped after 10 minutes by addition of 2.8 ml of 20% (w/v) Tris. The tube was vortexed, at room temperature (28  $^{\circ}C \pm 2$ ) for 5 minutes and centrifuged at approximately 9000 G for 3 min. Absorbance was measured at 610 nm against a blank containing substrate but no enzyme extract, using a spectrophotometer (Cam Spec M302). The Enzyme activity was expressed as change in optical density. The experiment was repeated twice with different sets of peel samples. Data were analyzed by ANOVA using general linear model (GLM) procedure and means were separated by Duncan's Multiple Range Test using SAS computer

software (Release 6.12), at p < 0.05 significant level.

## Pre-harvest treatment of potassium silicate (Kasil®) in chili pepper cvs. HYW and CA-8

#### Field preparations

Five weeks old chili pepper seedlings, cvs. HYW and CA-8, were obtained from the Agriculture department, Colombo-03, Sri Lanka. Forty-eight plants from each cultivar were transplanted to 5 L capacity black polythene bags, containing compost 48 x 2 = 96). Plants were grown in rows 1 m apart, with 0.5 m spacing between plants within rows, giving a plant population of 2 plants m<sup>-2</sup>. Plants were supported vertically by 3m long stick. Chemical fertilizers were applied to the plants as recommended by Department of Agriculture [Urea: Muriate, of potash: Rp9k phosphate, 110: 150:190 (g)]. Three doses of fertilizers were applied to the plants at 30, 45 and 60 days after planting. No pesticides or insecticides were applied to the plants, during the study. Diseased or dead plant parts were immediately removed from the field and destroyed.

#### Experimental design

The pre-harvest soil drench experiment followed as Randomized Complete Block Design (RCBD) with 2 blocks and each block contained total of 48 replicate plants (n = 12x2x2). Among those, 24 replicates for the Potassium silicate (Kasil<sup>®</sup>) treatment and the remaining plants were (24) treated with sterile distilled water as control. The concentration of 200 mg/l of potassium silicate (Kasil<sup>®</sup>) (the optimum concentration) was applied as soil drench at weekly intervals, for 4 weeks, commencing from the initiation of flowering.

## Challenge inoculation of fruits, harvested from Potassium silicate (Kasil<sup>®</sup>) treated plants

The challenge inoculation of the fruit samples, harvested from the Potassium silicate (Kasil<sup>®</sup>) treated plants, were done as described.

Data were analyzed by One-way ANOVA to identify the effect of silicon treatment on anthracnose lesion area and disease incident at  $\alpha$ =0.05 using SAS statistical package (SAS Corporation, release 6.12) and means were separated by Duncan Multiple Range Test.

#### Measurement of fruit weight, length and girth

Individual fruit weight was measured using a digital balance

(Setra EL - 410S, Setra Systems Inc., USA) for 8 replicate fruits. Length of the fruit from stem end to blossom end was measured using a measuring tape. Fruit girth was determined by measuring individual fruit with a tape at the widest midpoint of each fruit. For these measurements, 8 replicates per treatment were used.

For statistical analysis of physiochemical parameters, both the treated and control samples obtained from 2 blocks were pooled separately and One- way ANOVA was performed to obtained significant difference between the Potassium silicate (Kasil<sup>®</sup>) treatment and control using SAS statistical package (SAS Corporation, Release 6.12).

## TLC bioassay for assessment of antifungal activity

For *Cladosporium* bioassay, peel extracts were obtained from treated and control fruits and 50g of sample from each treatment was extracted.  $50\mu l$  of peel extracts from treated and control fruits. Two to three days later the plates were observed for inhibition zones.

## Defense-related enzymes $-\beta - 1$ , 3-glucanase

The  $\beta$ -1, 3-glucanase activity assay was carried out as described. from the peel extracts of the chili peppers, from treated and control plants.

#### Statistical analysis

The data were statistically analyzed by using also analyzed using analysis of variance ( $P \ 0.05$ ), with DMRT and LSD values used for multiple range tests with SPSS.

## RESULTS

#### **Collection of diseased Sample**

The diseased chili pepper samples, collected from wholesale and retail markets, were with characteristic symptoms of anthracnose, such as circular or angular sunken lesions with concentric rings of acervuli on which orange conidial masses also were observed as described by Hadden and Black (1989) (Figure 1).

#### **Isolation of fungi**

Initial identification of the isolates was carried out based on the morphological descriptions of the spores and colony of *Colletotrichum* species outlined by Mordue (1971) and Sutton (1992). Distinct morphological types on PDA were observed 7 days after sub culturing. Colonies on PDA, the conidial that were effused, white becoming orange then turning greenish gray as the cultures aged on PDA. Colony



Figure 1: Chili pepper samples with anthracnose symptoms, collected from the local markets of the study area.

reverse was brownish orange to black. Bright orange spore masses were produced outward from the center of the colony. Older cultures developed black acervuli around the center of the colony. No setae were observed. Mycelia were branched, septate and hyaline. In addition, *C. acutatum* was identified from the conidial characteristics; hyaline, unicellular, and cylindrical with obtuse apices and tapering bases. The monoconidial culture, obtained from the single spore isolation also confirmed the similar morphological characteristics of *C. acutaum* and was used in the rest of the experiments throughout the study unless otherwise stated.

# Pathogenicity of *C. acutatum*, isolated from diseased pepper samples

The isolate of *C. acutatum* produced typically necrotic, sunken anthracnose symptoms on both unwounded and wounded chili of both cultivars. The initial symptom was observed on wound inoculated fruits 3 days after inoculation (DAI) in Cv. HYW and severe sunken symptoms were observed 5 DAI (Figure 2).

However, the anthracnose development was commenced 3 and 5 DAI in cv. CA-8 in the wounded and unwounded



Figure 2: Pathogenicity of C. acutatum on chili peppers (cvs. CA-8 and HYW) 5, 7 and 9 DAI.

DAI	5	5	6	;	7		8	
Concentration of silicon (mg/l).	LA(cm <sup>2</sup> )	% DR						
0	0.79*		1.92ª		4.62 a		5.74 ª	
50	0.33 <sup>b</sup>	55	0.75 <sup>b</sup>	59	3.22 ª	30	4.18 a	27
200	0.30 <sup>b</sup>	62	0.67 <sup>b</sup>	63	2.19 <sup>b</sup>	53	3.78 ª	34
400	0.37 <sup>b</sup>	54	1.12 в	39	3. 37 <sup>ab</sup>	27	4.14 ª	26
1000	0.39 b	51	1.43 <sup>ab</sup>	26	3.86 ab	16	4.62 ª	22

\*Means of each column, followed by the same letters for Lesion Area development in each treatment, are not significantly

different according to Duncan's multiple ranger test (p<0.05). LA-Lesion Area (cm<sup>2</sup>), % DR - % Disease Reduction

**Table 2:** Effect of postharvest treatment of potassium silicate (Kasil®) on the anthracnose development in chili pepper cv.CA-8.

DAI	7	,	8		9		10	
Concentration of potassium silicate (Kasil®)mg/l)	LA(cm <sup>2</sup> )	% DR						
0	0.64*		1.02ª		2.86 ª		4.22 ª	
50	0.26 <sup>b</sup>	59	0.52 <sup>b</sup>	49	1.84ª	36	3.98 ª	28
200	0.24 <sup>b</sup>	62	0.48 <sup>b</sup>	53	1.66 <sup>b</sup>	42	3.58 ª	30
400	0.27 <sup>b</sup>	58	0.72 <sup>ab</sup>	30	2.09 <sup>ab</sup>	27	3.64 ª	18
1000	0.31 <sup>b</sup>	52	0.75 <sup>ab</sup>	26	2.66 <sup>ab</sup>	20	3.82ª	20

\*Means of each column, followed by the same letters, for lesion area development in each treatment, are not significantly different according to Duncan's multiple ranger test (p<0.05). LA-Lesion Area (cm<sup>2</sup>), % DR - % Disease Reduction

fruit samples, respectively. and the lesion diameter was measured in both cultivars at 24 hours intervals.

## Potassium silicate (Kasil<sup>®</sup>) treatment and anthracnose disease assessment

Reduction in lesion area was observed in all 3 treatments (100, 200 and 400 mg/l) compared to the untreated fruits of both cultivars. Also, the percentage disease reduction (%DR) was higher in all the treatments, compared to control fruits of either cultivars (Table 1 & 2). However, the treatment with the concentration of 200mg/l potassium silicate (Kasil<sup>®</sup>) showed better reduction in disease severity and % DR among all three treatment. As shown in table 2, cv. CA-8 showed the greater disease reduction and more than 50% protection up to 8 DAI at 200mg/l.

As shown in table 2, cv. CA-8 showed the greatest disease reduction and more than 50% protection was observed until 8 DAI at 200mg/l.

## Determination of antifungal activity of potassium silicate (Kasil<sup>®</sup>)

The results of spore germination test indicated that there was no significant reduction in conidia germination observed up to 200 mg/l of the concentrations of potassium

silicate (Kasil<sup>®</sup>) treatments (Figure. 3), whereas reduction in spore germination was observed at higher concentrations (400 and 1000 mg/l) of potassium silicate (Kasil<sup>®</sup>).

## Challenge inoculation and assessment of disease development of fruits, from Potassium silicate (Kasil<sup>®</sup>) treated plants

Fruit samples of cv. HYW, from Potassium silicate (Kasil<sup>®</sup>) treated plants, showed the disease initiation on the 5 DAI, whereas that of from control plans was 4 DAI (Table 3). Although, disease reduction was observed in the cv. HYW, there was no significant differences in disease severity was observed between the treated and control fruits (Table 3). However, disease initiation was delayed until 8 DAI, in cv. CA-8 and 87% of the untreated (control) fruits was diseased at this stage but only 54% of the fruit samples, obtained from the treated plants, were diseased at 8 DAI (Table 4).

Also, 100% disease reduction was achieved in treated fruits of cv. CA-8, up to 8 DAI, whereas that of control was only up to 5 DAI (Table 4).

#### Measurement of fruit weight, length and girth

As shown in the table 5, no significant difference was



Figure 3: Antifungal effect of potassium silicate (Kasil®) on germination of conidia of C.acutatum

**Table 3:** Effect of potassium silicate soil-drench treatments (200mg/l) on severity and anthracnose disease incidence in challenge inoculated *C. annum* (cv. HYW) with *C. accutatum*.

Dava	Dise	ase seve	rity [Lesi	on area (	[cm)]	Disease incidence (%)				
Days	4	5	6	7	8	4	5	6	7	8
Control	0.11ª	0.59ª	1.89ª	2.34 ª	2.87ª	8.77ª	42.70ª	74.80ª	88.67ª	98.54ª
Treated % disease	00ь	0.12ª	0.62ª	1.81ª	1.68ª	00ь	27.20ª	69.98ª	81.60ª	96.44ª
Reduction	100	85	65	50	40					

**Table 4:** Effect of potassium silicate soil-drench treatments (200mg/l) on severity and anthracnose disease incidence in challenge inoculated *C. annum* (cv. CA-8) with *C. acutatum*.

Dava	Diseas	se severit	y [Lesion	area (cm)]	Disease incidence (%)			
Days	6	7	8	9	6	7	8	9
Control	0.16ª	0.46ª	1.19	1.69ª	7.67ª	32.70 <sup>a</sup>	87.00ª	97.62ª
Treated % disease	00ь	00 <sup>b</sup>	0.22 <sup>b</sup>	0.84 <sup>b</sup>	00 ь	00 <sup>b</sup>	53.98 <sup>b</sup>	70.86 <sup>b</sup>
Reduction	100	100	100	100				

Table 5: Effect of Silicon soil drench treatment on the number, fresh weight, length and diameter ratio of chili pepper, cv. HYW.

	Control	Treated
Fruit number	42*	47ª
Fresh weight (g)	66ª	58 ª
Fruit Diameter: Length	1.10ª	1.13ª

\*Pairwise mean values, followed by the same letters for yield parameters among treatments, are not significantly different according to Duncan's multiple ranger test (p<0.05). DR- Disease Reduction.



 $TI_2CI_2C_0 \qquad T_0 \ T_2$ 

**Figure 4:** TLC chromatograph of crude ethyl acetate extract of the peel of chili pepper (50 µl spots) treated with potassium silicate (Kasil®).

C0 - Control and non - inoculation, T0 - Treated and 0 days after harvest, T2 - Treated and 2 days after harvest TI2- Treated and 2 days after inoculation with*C.acutatum*, CI2 - Control and 2 days after Inoculation with*C.acutatum*.



Figure 5: β-1,3-glucanase activity of extract of chili pepper treated with potassium silicate (Kasil®).
CI - Control & 4 DAI; TI - Treated & 4 DAI; T - Treated & No Inoculation;
C - Control & No Inoculation

observed in the number of fruits harvested, weight, diameter and length in the Potassium silicate (Kasil<sup>®</sup>) treated fruits, compared with the untreated fruits of either cvs.CA-8 and HYW.

## Assessment of enhanced defense responses due to Potassium silicate (Kasil®) treatment

As per the results of this experiment, potassium silicate (Kasil<sup>®</sup>) treated peel extract contained three antifungal zones, with  $R_f = 0.02-0.06$ , 0.071-0.83 and 0.12. However, the antifungal activity observed at  $R_f = 0.12$  diminished after three days, showing it was relatively less prominent. Whereas, the remaining two antifungal zones at  $R_f = 0.02-0.06$ , 0.071-0.83 were more prominent. However, these two

zones were relatively larger in the peel extract of treated fruits and were challenge inoculated with *C.acutatum* for two days (Figure 4).

Moreover, the non-treated (control) but challenge inoculated peel extract also showed the inhibition zones and but less prominent when compared to the treated and inoculated peel extract. In contrast, the non-treated and noninoculated peel extract did not show any antifungal zones during the bioassay with *C. cladosporioides*. These results indicate the enhanced level of phytoalexin accumulation in the chili pepper fruits, harvested from Potassium silicate (Kasil<sup>®</sup>) treated plants.

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#### Induction of defense related enzymes

The peel extracts of chili pepper fruit samples, harvested from Potassium silicate (Kasil<sup>®</sup>) treated plants showed higher  $\beta$ -1,3-glucanase activity, compared to that of non-treated (control) plants (Figure 5).

#### DISCUSSION

The isolation of pathogen of *C. annuum*, revealed that the species associated with anthracnose in chili pepper (*C.annuum*) was *Colletotrichum acutatum*. Mahendranathan *et al* (2012), reported the association of *C. acutatum* in anthracnose of *C.annuum* in Sri Lanka, as the first time.

The results of this study reveal that the soil amendments with Potassium silicate (Kasil®) could enhance resistance response in chili peppers. As observed in the results, control of anthracnose disease was observed with all concentration of Potassium silicate (Kasil<sup>®</sup>), tried in this experiment (20, 200, 400, 1000 mg/l). However, the concentration that gave the better control was 200 mg/I. This concentration was able to reduce anthracnose lesion area by 100 - 40 % from 4 DAI to 10 DAI, compared to the untreated controls. In addition, the fruit samples, collected from the Potassium silicate (Kasil®) treated plants showed the delay in disease symptom initiation in both cultivars, on challenge inoculation with C. acutatum. Studies by Bowen et al. (1992), Yu and Du (2009) and Abraham (2010) showed that pre-harvest or post-harvest silicon application either inhibiting or delaying the growth and development of the mycelium of the pathogen.

According to Wang *et al.* (2017) potassium silicate could act as a modulator influencing plant defense responses that leads to induced resistance in plants. Nevertheless, Vivancos *et al.* (2015) stated that potassium silicate involves in the metabolic processes of plant–pathogen interaction and thus induces the resistance response in plant diseases by activating defense genes of host plants. Jayawardana *et al.* (2015), reported that the amendment of nutrient solution with silicon, enhanced the resistance in chili pepper due to the concentrations of cell wall-bound phenolic compounds and cuticle thickness in fruit from plants, treated with Si.

Past research evidences also support the effect of silicon in enhancing the host resistance upon challenge inoculation with several fungal pathogens (Rodrigues *et al.*, 2015; Wang, *et al.*, 2017).

As per the result of the TLC bioassay, a variation was observed in inhibition zones compared to that of the control. That is larger inhibition zones were observed in the potassium silicate treated (Si<sup>+</sup>) and challenge inoculated peel extract when compared with the non-treated (Si<sup>-</sup>), challenge inoculated peel extracts and also non-treated and non-inoculated extracts as well. As per the study by Adikaram *et al.* (1982:1983) phytoalxins such as capsidiol and capsicannol, accumulated in chili pepper tissue, with potassium silicate (Kasil<sup>®</sup>) treatment from *Glomerella cingulata* mycelial walls. Disease severity is reduced in the Si-treated plants with higher activity of protective enzymes (POD, PPO and PAL) in leaves of rice (Cai *et al.*, 2008), and cucumber (Liang *et al.*, 2005). These enzymes play an important role in regulating the production of accumulation of antifungal compounds, phytoalexins and pathogenesisrelated proteins in plants. The results of this experiment reveal the possibility of inducing the antifungal compounds by potassium silicate application and might have involved to the reduction in lesion diameter in chili pepper and also delay in disease development.

In addition, as per the results of this study, compared to the extracts of non-treated tissues, there was an increase level of in  $\beta$ -1, 3-glucanase in the extract of the Potassium silicate (Kasil<sup>®</sup>) treated and challenge inoculated fruit peel extracts. Similar observations have also been reported by Li *et al.* (2012), Farahani *et al.* (2013) and Polanco *et al.* (2014). Further, Rahman *et al.* (2015) stated that Sitreatment enhances the of phenolic acids and involved in enhancing resistance to gray leaf spot disease in perennial ryegrass (Magnaporthe oryzae) pathosystems.

Further, as per the field experiments, no significant difference was observed in the number of fruits harvested, weight, fruit diameter and length between the Potassium silicate (Kasil<sup>®</sup>) treated and untreated plants of both the cultivars. Thus, in summary it can be mentioned that preharvest treatment with Potassium silicate (Kasil<sup>®</sup>), as the pre-harvest soil drench, significantly controls anthracnose diseases development of the harvested chili pepper fruits of cvs. HYW and CA-8. This enhanced disease resistance was attributed in chili peppers, through potassium silicate (Kasil<sup>®</sup>) treatment and was found the greater accumulation of phytoalexins and increased level of  $\beta$ -1, 3-glucanases which might have played the role in enhanced resistance against anthracnose disease.

Thereby, there have been enough evidences proved that the pre-harvest or post-harvest of potassium silicate (Kasil<sup>®</sup>), the silicon, application can be an approach in controlling mainly the fungal diseases in plant produce by inhibiting or delaying the growth and development of the mycelium of the pathogen. Further, it has also been shown that the silicon-induced resistance against infections is mainly attributed to the mechanical and chemical defense and can be a promising alternative to the fungicidal application in controlling such diseases.

## CONCLUSION

Soil amendment of Potassium silicate (Kasil<sup>®</sup>) significantly delayed the anthracnose disease development and the disease severity in *Capsicum annum* L. fruits by enhancing the natural disease resistance. Potassium silicate (Kasil<sup>®</sup>)-treated fruits, inoculated with *C. acutatum* after harvest, showed greater accumulation of phytoalexins and increased level of  $\beta$ -1, 3-glucanase in fruit tissue.

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## REFERENCES

- Abraham, A.O. (2010). Integrated use of yeast, hot water and potassium silicate treatments for the control of postharvest green mould of citrus and litchi (Unpublished doctoral dissertation). University of KwaZulu-Natal Pietermaritzburg, Republic of South Africa.
- Abraham, A.O. (2010). Integrated use of yeast, hot water and potassium silicate treatments for the control of postharvest green mould of citrus and litchi (Doctoral dissertation).
- Adikaram, N.K.B., Averil, E. Brown and Swinburne, T.R (1982). Phytoalexin involvement in the latent infection of *Capsicum annuum* L. fruit by *Glomerella cingulata* (Stonem.). Physiological Plant Pathology **21**: 161-170.
- Adikaram, N.K.B., Averil E Brown and Swinburne T.R. (1983). Observations on infection of Capsicum annuum L. fruit by Glomerella cingulata and Colletotrichum capsici. Transactions of British Mycological Society 80(3): 395-401.
- Bi, Y., Tian, S.P., Guo, Y.R., Ge, Y.H. and Qin, G.Z. (2006). Sodium silicate reduces postharvest decay on Hami melons induced resistance and fungistatic effects. *Plant disease* **90**(3): 279-283.
- Bowen, P., Menzies, J. and Ehret, D. (1992). Soluble silicon sprays inhibit powdery mildew development on grape leaves. *Journal of the American Society for Horticultural Science* 117(6): 906-912.
- Cai K., Gao D., Luo S., Zeng R., Yang J., Zhu X. (2008). Physiological and cytological mechanisms of siliconinduced resistance in rice against blast disease. *Physiological Plant* Pathology **134**: 324-333.
- Dann, E.K. and Deverall B.J. (2000). Activation of systemic disease resistance in pea by an avirulent bacterium or a benzothiadiazole, but not by a fungal leaf spot pathogen. *Plant Pathology* **49**(3): 324-332.
- Farahani, L., Etebarian, H.R., Sahebani, N. and Aminian, H. (2013). Induction of defense responses in apple fruit treated with *Pichia guilliermondii* and silicon against *Penicillium expansum*. *International Journal of Agronomy and Plant Production* 4(1): 1-6.
- French-Monar, R. D., Rodrigues, F. A., Korndorfer, G. H. and Datnoff, L. E. (2010). Silicon suppresses *Phytophthora* blight development on bell pepper. *Journal of Phytopathology* 58: 554-560.
- Hadden, J.F. and Black, L.L. (1989). Anthracnose of Pepper Caused by Colletotrichum spp. Proceeding of the International Symposium on Integrated Management Practices: Tomato and Pepper Production in the Tropics; Taiwan: Asian Vegetable Research and Development Centre; 189-199.

Heil, M., Hilpert, A., Kaiser, W. and Linsenmair, K.E.

2000. Reduced growth and seed set following chemical induction of pathogen defence: Does systemic acquired resistance (SAR) incur allocation costs? *Journal of Ecology* **88**: 645-654.

- Huang G.C., Roberts, P.D. and Datnoff, L.E. (2011). Silicon suppresses *Fusarium* crown and root rot of tomato. *Journal of Phytopathology* 159: 546-554.
- Jayawardana, H.A.R.K., Weerahewa, D. and Saparamadu, J. (2015). Enhanced resistance to anthracnose disease in chili pepper (Capsicum annuum L.) by amendment of the nutrient solution with silicon. *Journal of Horticultural Science and Biotechnology* **90**(5): 557-562.
- Klarman and Stanford, 1968. W.L. Klarman, J.B. Stanford. Isolation and purification of an antifungal principle from infected soyabeans. *Life Sciences* 7: 1095-1103.
- Li, W., Bi, Y., Ge, Y., Li, Y., Wang, J. and Wang, Y. (2012). Effects of postharvest sodium silicate treatment on pink rot disease and oxidative stress-antioxidative system in muskmelon fruit. *European Food Research and Technology* 234(1): 137-145.
- Liang Y.C., Sun, W., Si, J. and Römheld, V. (2005). Effects of foliar-and root-applied silicon on the enhancement of induced resistance to powdery mildew in *Cucumis sativus*. *Plant Pathology* **54**: 678-685.
- Ma, J.F. and Takahashi, E. (2002). Soil, fertilizer, and plant silicon research in Japan, 1st edition, Elsevier Science, Amsterdam, Netherlands.5-85.
- Mahendranathan, C., Wijesundera, R.L.C. and Adikaram, N.K.B. (2012). Association of *Colletotrichum acutatum* in the Anthracnose Disease of Chili (*Capsicum annuum* L.) in Sri Lanka. 3<sup>rd</sup> Global Conference, Indian Society of Mycology and Plant Pathology, Udaipur, India: 55 (Abs).
- Mordue, J.E.M. (1971). *Colletotrichum capsici*. [CMI description of pathogenic fungi and bacteria. No.**32**; 317
- Pakdeevaraporn, P., Wasee, S., Taylor, P.W.J., Mongkolpornm, O. 2005. Inheritance of resistance to anthracnose caused by *Colletotrichum capsici* in *Capsicum. Plant Breeding* **124**: 206-208.
- Polanco, L.R., Rodrigues, F.A., Nascimento, K.J., Cruz, M. F., Curvelo, C.R., DaMatta, F.M. and Vale, F.X. (2014). Photosynthetic gas exchange and antioxidative system in common bean plants infected by *Colletotrichum lindemuthianum* and supplied with silicon. *Tropical Plant Pathology* **39**(1): 35-42.
- Rahman, A., Wallis, C.M. and Uddin, W. (2015). Siliconinduced systemic defense responses in perennial ryegrass against infection by *Magnaporthe oryzae*. *Phytopathology* **105**: 748-757.
- Rodrigues, F.A., Polanco, L.R., Duarte H.S.S., Resende, R.S. and Do Vale, F.X.R. (2015). Photosynthetic gas exchange in common bean submitted to foliar sprays of potassium silicate, sodium molybdate and fungicide and infected with *Colletotrichum lindemuthianum*. J. *Phytopathol.* 163: 554-559.
- Van Loon, L.C., Rep, M. and Pieterse, C.M.J. 2006. Significance of inducible defense related proteins in infected plants. *Annual Review of Phytopathology* 44: 135-162.

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- Vivancos, J., Labbe, C., Menzies, J.G. and Belanger, R.R. (2015). Silicon-mediated resistance of Arabidopsis against powdery mildew involves mechanisms other than the salicylic acid (SA)-dependent defence pathway. *Molecular Plant Pathology* 16: 572-582.
- Walters, D.R., Newton, A.C. and Lyon, G.D. 2005. Induced resistance: Helping plants to help themselves. *Biologist* 52:28-33.
- Wang, M., Gao, L., Dong, S., Sun, Y., Shen, Q., and Guo, S. (2017). Role of Silicon on Plant–Pathogen Interactions. *Frontiers in Plant Science* 8:701.

Yu, Y. and Du, X.G. (2009). The inhibitory effect and mechanism of calcium and silicon solutions on cucumber downy mildew. *Northern Horticulture* 10: 041.