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Silicon rich rice hull amended soil enhances anthracnose resistance in tomato

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Abstract

Silicon (Si) has proven to be effective in controlling diseases in many crops and can be used as a substitute of fungicides. It has been shown that rice hull could be applied to the soil as a non-hazardous silicon rich source in crop production. This study was conducted to investigate the effect of half burnt rice hull (HBRH) as a silicon source in suppression of anthracnose in tomato and understanding underlying mechanisms of disease resistance. HBRH was made by burning raw rice hull at 250 °C for 15 minutes. Six different levels of HBRH: 0, 5, 10, 15, 20 and 25g per 1kg of soil were incorporated into soil along with Department of Agriculture (DOA) recommended levels of NPK fertilizers. The resistance against anthracnose disease was evaluated by measuring the lesion area of tomato fruits followed by the challenged inoculation with *Colletotrichum dematium* for 10 days after inoculation. The mechanism behind Si induced resistance was studied by measuring the fruit firmness and cuticle thickness (CT). A significant reduction (at $P \leq 0.05$) of anthracnose disease (87 and 77%) was observed in inoculated fruits of 25 and 20 g HBRH per 1kg of soil treatments. Onset of the disease was delayed by 4 and 3 days after inoculation in fruits obtained from 25 and 20 g HBRH treated plants compared to the control. The increase of fruit firmness and CT were significant in 25 and 20 g HBRH per 1kg of soil provided fruits. Consequently, these results suggest that HBRH is a Si rich source and 20g HBRH per 1kg soil would be sufficient in suppressing tomato anthracnose caused by *C. dematium* and the resistance might have triggered with silicon induced thickened cuticle and increased fruit firmness.

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1. Introduction

The disease anthracnose caused by *Colletotrichum* spp. is one of the major diseases in tomato (*Lycopersicon esculentum* L.). Current management of the disease is achieved mostly by application of fungicides which is limited due to cost, development of resistant pathogen strains and possible environmental and health concerns. Silicon application has proven to have beneficial effects against numerous diseases in many crops¹. Various mechanisms have been proposed being responsible for the Si mediated resistance. Silicon can get deposited beneath the cuticle which creates a physical barrier impeding fungal penetration² and/ or can induce formation of a thicker cuticle against fungal infections. Rice hull (RH) has been considered as a rich organic source of silicon containing about 8% of Si of its dry weight³ and therefore can be considered as a cheaper and natural source of Si. The present study was aimed at investigating the efficiency of utilizing RH (in the form of HBRH) as a silicon source in controlling anthracnose disease in tomato cv. Padma 108 F1 and understating mechanisms of disease suppression.

2. Methodology

2.1. Silicon leaching study from HBRH

Raw rice hull was burnt at 250 °C for 15 minutes using a muffle furnace (AF 11/6B, Lenton thermal designs, England) and was used for the experiment. Samples of 0 (control), 5, 10, 15, 20 and 25g HBRH were mixed with 1kg of soil and suspended in 1 L of sterilized distilled water. Five replicates were maintained for each experiment. The solutions were analyzed for their molybdo-reactive silica concentration using a UV visible spectrophotometer (UVD 3000/3200; Labomed, Inc., Los Angeles, CA, USA) according to Clesteri *et al.*⁴

2.2. Pot experiments with HBRH incorporated in to soil

Mature tomato seedlings of tomato genotype *Lycopersicon esculentum* L. cv. 'Padma 108 F1' were transplanted into pots containing different masses of HBRH (0, 5, 10, 15, 20 and 25 g of HBRH/ kg soil). NPK fertilizers were applied according to the recommendation of DOA, Sri Lanka. Five replicates per treatment were maintained in a complete randomized design.

2.3. Assessment of anthracnose disease resistance against *Colletotrichum dematium*

Harvested tomato fruits were surface sterilized with 70% alcohol and challenged-inoculated with *Colletotrichum dematium* by placing a 20µl drop of conidial suspension (5×10^5 conidia per ml) at three different spots on the fruit surface. Twenty-four fruits were inoculated per treatment and were incubated in a moist chamber (20-30°C and 95-100% relative humidity). The lesion areas were recorded each day until 10 days after inoculation.

2.4. Determination of changes in fruit parameters

A sample of twenty fruits from each treatment was used to measure fruit firmness and cuticle thickness. Firmness was measured using a hand-held penetrometer (FT 40, Wagner Instruments, Greenwich CT, USA). Three transverse sections (0.1mm thick) of each fruit were mounted on a slide with the stain 'Sudan IV' and CT was measured with a calibrated ocular micrometer at 400x. Fruit extracts were prepared by crushing ten fruits per treatment separately using a blender. Homogenized fruit tissue was centrifuged at 14,000 rpm for 10 minutes using a centrifuge (1-14, Sartorius AG, Gottingen, Germany) and the following parameters were measured using the supernatant. Total soluble solid (TSS) content was measured using a Brix refractometer (WZ-113, Zhejiang Top Instruments Co. Ltd., Zhejiang, China) within the range of 0-32% °Brix. pH was measured using a pH meter (IQ150, Spectrum Technologies, Inc. IL, USA).

2.5. Statistical analysis

Collected data were analyzed using one way ANOVA in SPSS 16.0. The means were compared by the Duncan's multiple range tests (DMRT) for determining significant differences among treatments at $P \leq 0.05$ confidence interval.

3. Results and discussion

The present study revealed that the leached Si concentration from HBRH increased with the increased mass of HBRH used for the experiment. The leachates of the highest and lowest average Si levels were detected from 25g HBRH per 1kg of soil (2.11 ppm) and the control (0.212 ppm) respectively, over a period of 20 weeks (Table 01). The anthracnose disease development was found to decrease with increased Si concentration leached from increased levels of HBRH. A significant reduction in anthracnose disease development was observed in fruits obtained from plants treated at 10, 15, 20 and 25g HBRH per 1kg of soil compared to the non-treated control. The highest disease reduction (77 and 87%) and a substantial delay in disease initiation (by 3 and 4 days) were observed in fruits of plants treated at 20 and 25g HBRH per 1 kg of soil respectively. Similarly a reduction of anthracnose disease severity and delay in appearance of anthracnose lesions was reported in bean pods Si-treated plants compared to those of Si-free plants⁵. However, no significant reduction was observed in fruits of plants treated at 5 g HBRH per 1 kg of soil. There was a significant increase of CT in all silicon treated fruits while the highest values were found in 15, 20 and 25g HBRH treated fruits. The highest fruit firmness values were also observed in 20 and 25g HBRH per 1kg soil treated tomato fruits. A significant increase of TSS content was found in the all the silicon treated fruits though the pH was not affected by silicon treatment (Table 1).

Table 1. The leached silicon concentrations from different HBRH and soil compositions; changes in some fruit quality parameters and degree of anthracnose disease reduction on Si application

Treatment HBRH (g/kg of soil)	Leached Si concentration (mg/L)	Total lesion area (mm ²)	Day of disease initiation	Fruit Firmness (N)	Cuticle thickness (μm)	TSS (°Brix)	pH
0 (Control)	0.212 ^f	501.21 ^d	3 ^d	20.96 ^c	7.88 ^d	4.2 ^b	4.2 ^a
5	0.635 ^e	495.54 ^d	3 ^d	21.76 ^c	15.8 ^c	5.7 ^a	4.5 ^a
10	1.293 ^d	325.1 ^c	3 ^d	35.87 ^b	20.25 ^b	5.8 ^a	4.6 ^a
15	1.796 ^c	330.45 ^c	4 ^c	34.51 ^b	23.18 ^a	6.3 ^a	4.4 ^a
20	1.910 ^b	115.3 ^b	6 ^b	49.7 ^a	23.67 ^a	6.1 ^a	4.2 ^a
25	2.107 ^a	63.05 ^a	7 ^a	48.13 ^a	24.11 ^a	6.1 ^a	4.0 ^a

Means in each column followed by different letters are significantly different at $P \leq 0.05$ according to the DMRT. ($n = 5$ for Si leaching experiment, $n = 24$ for total lesion area, $n = 20$ for firmness and cuticle thickness, $n = 10$ for TSS and pH)

The reduction of disease development might be due to thickened cuticle in Si treated fruits assuming that the thicker cuticle possibly might have acted as a pre-existing structural barrier against fungal penetration. Oh *et al.*⁶ observed a negative correlation between CT and anthracnose disease incidence in Si treated pepper fruits. The increased fruit firmness upon increased Si concentration also might have functioned as a rigid physical barrier against *C. dematium* colonisation. In conclusion, HBRH could be utilised as a Si rich natural source and 20g HBRH per 1kg of soil would be sufficient as the Si supplementation in suppressing tomato anthracnose caused by *C. dematium*. The delaying of initiation and development of the disease on tomato fruits might be due to the silicon induced thicker cuticle and increased fruit firmness. However, further studies are required in understanding the reason/s behind the increased cuticle thickness and fruit firmness due to Si application.

Acknowledgements

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