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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  $P \le 0.05$ ) 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 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 thicknes cuticle and increased fruit firmness.

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Keywords: Colletotrichum dematium; rice hull; silicon

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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 crops<sup>1</sup>. 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 penetration<sup>2</sup> 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 weight<sup>3</sup> 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 Clesceri *et al.*<sup>4</sup>

#### 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 ( $5x10^5$  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).

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