



Short paper

Soil amendment with raw rice hull as a source of silicon in enhancing anthracnose disease resistance, plant growth and fruit qualities of chili pepper (*Capsicum annuum* L.)

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Abstract. This study was conducted to investigate the effect of soil incorporation of raw rice hull (RRH) as a source of silicon on plant growth, yield and anthracnose disease development of *Capsicum annuum* L. 'Muria F1'. Experiment was conducted using five different ratios of RRH:soil:sand (T₁-3:2:0, T₂-3:0:2, T₃-3:1:1, T₄-2:3:0, T₅-1:1:3) in the first 5 treatments and applying potassium silicate (PS) as a Si source at the rate of 75 mg/L to the soil replacing RRH in the 6th (positive control). The plants grown in soil medium with no addition of a silicon source were used as the negative control. All treatments were arranged in a completely randomized design with 4 replicates. Anthracnose disease development was assessed as number of days for the appearance of symptoms and the mean lesion areas by challenge inoculation with *Colletotrichum gloeosporioides* on fruits. The highest reduction of disease severity (61%) was observed in crops grown in soils amended with RRH (T₁) compared to control plants. The disease reduction observed in fruits from plants provided with potassium silicate was only about 15%, compared to that of Si-free plants. It was concluded that incorporation of rice hull (60% of the media) in the growing substrate would be an effective solution against anthracnose disease of chili pepper plants.

Keywords. *Capsicum annuum*, silicon, rice hull, soil amendment

1 Introduction

Anthracnose disease caused by *Colletotrichum* species is a major disease of chili pepper (*Capsicum annuum* L.) in tropical and sub-tropical climates and causes severe post-harvest losses (Oanh *et al.* 2004). The disease is controlled by seed treatment or foliar sprays with fungicides. However, continuous use of fungicides can lead to environmental and health hazards and development of fungicide resistance of pathogen populations. Therefore, to address the

current demand in sustainable crop production, environmental friendly alternatives should be investigated to control one of the important diseases, anthracnose. Silicon is beneficial for plants in terms of growth (Kamenidou *et al.* 2008), yield (Ghasemi *et al.* 2013) and resistance to biotic (Huang *et al.* 2011) and abiotic stresses (Savant *et al.* 1999). Silicon can be supplied using both inorganic and organic sources. Rice hull is an agricultural by-product, which is underutilized. It is a natural source of silicon. According to Patel *et al.* (1987) the Si content in raw rice husk is 10.3 (w/w% in wet basis). This study was conducted to investigate the effect of incorporation of rice hull (RRH) and potassium silicate (PS) as silicon sources into potting media on plant growth, fruit quality parameters and anthracnose disease development of chili pepper.

2 Materials and Methods

2.1 Plant material

Seeds of *Capsicum annum* L. 'Muria F1' (East-West seed International Ltd., Thailand) were sown on coir dust and compost medium (1:1) and were maintained in the nursery. Four weeks old chili pepper plants were transplanted in grow bags (300 gauge polythene bags, size 30 cm × 12 cm) filled with equal volumes of the respective growing media according to treatments.

2.2 Treatments and experimental design

RRH: soil: sand in a ratio of (3:2:0, 3:0:2, 3:1:1, 2:3:0, 1:1:3) were used in first five treatments; T₁-T₅ while PS (75 mg/l of Si) was applied to the soil medium instead of adding RRH in T₆ (control 1). The plants grown in soil medium applied with no silicon source were used as the T₇ (control 2). Treatments were arranged in a Completely Randomized Design (CRD) with four replicates having three plants per experimental unit. The data were analyzed using one way ANOVA in SPSS 16 Statistical package.

2.3 Fertilizer Application

Nutrients were supplied using NFV (nutrient formulation for vegetative stage) and NFF (nutrient formulation for fruiting stage) (Jayawardana *et al.* 2015) for all the other treatments except T₆, in which the nutrient formulations were amended with potassium silicate to supply 75 mg/l of Si by making proper adjustment in the nutrient composition (Jayawardana *et al.* 2015). Fertigation (50 ml of nutrient solution per grow bag per day during first 4 weeks and 100 ml per growing bag per day thereafter) was practiced with relevant nutrient

solution according to the treatments. Weeding and watering was practiced when required.

2.4 Pathogen isolation and identification

C. gloeosporioides from anthracnose lesions of diseased chili pepper fruits were cultured on potato dextrose agar (PDA), following surface sterilization with 1% (v/v) NaOCl for 1 min, followed by washing with sterile distilled water. Ten culture plates were incubated at 27°–30°C and observed for mycelial growth, morphology of the cultures, and the shape of conidia were observed using a compound microscope (Daffodil MCX100, Vienna, Australia). *C. capsici* was identified by its sickle-shaped conidia, the presence of prominent setae (Sutton, 1992), and its brown colony colour (Rajapakse and Ranasighe 2002). *C. gloeosporioides* was identified by its orange cotton-like mycelium (Sutton, 1980) and ovoid-shape conidia (Du *et al.* 2005).

2.5 Fruit inoculation and assessment of disease severity

Conidial suspension (10^5 conidia ml⁻¹) of *C. gloeosporioides* or *C. capsici* were prepared by scraping the mycelium from pure 7 days old cultures and suspending them in sterilized distilled water, followed by filtering through glass wool. Harvested fruits were challenge-inoculated by placing a 20 µl drop of conidial suspension at three different points on the fruit surface. Twelve fruits were inoculated per treatment. The inoculated fruits were maintained in a moist chamber (95 – 100% RH) at $28 \pm 2^\circ\text{C}$. Numbers of days for the appearance of disease symptoms in each treatment was recorded. Lesion areas were recorded each day for 10 days and the mean lesion area per fruit was calculated.

2.6 Plant growth and fruit parameters

The shoot length, number of leaves, average leaf area, internodes girth (3-4 nodes) and number of fruits per plant were recorded at 12 weeks after transplanting. Fruit length and fruit fresh weight of each harvested fruit were measured.

3 Results and Discussion

3.1 Anthracnose disease resistance in chili pepper fruits

The highest lesion area after 10 days of pathogen *C. gloeosporioides* inoculation was recorded in the control treatment (77.5 mm²) and the lowest

was recorded in T₁ treatment (29.5 mm²) (Fig. 1.). The significantly high reduction of lesion area compared to the control 2 (T₇) (61%) was shown in crops treated with rice hull in the growing substrate as the Si source (T₁). However, the lesion area observed in fruits from T₁, T₂ and T₃ treatments at 10 days after inoculation was not significant. The reduction of lesion area was significantly lower both in T₄ and T₆ treatments than that of T₁, T₂ and T₃. There was no significant difference observed between the lesion areas of T₅ and control treatments. A comparatively large percentage of rice hull was included in T₁, T₂ and T₃ treatments compared to the other treatments, suggesting that Si supplied by rice hull in the media might have actively contributed for anthracnose disease suppression.

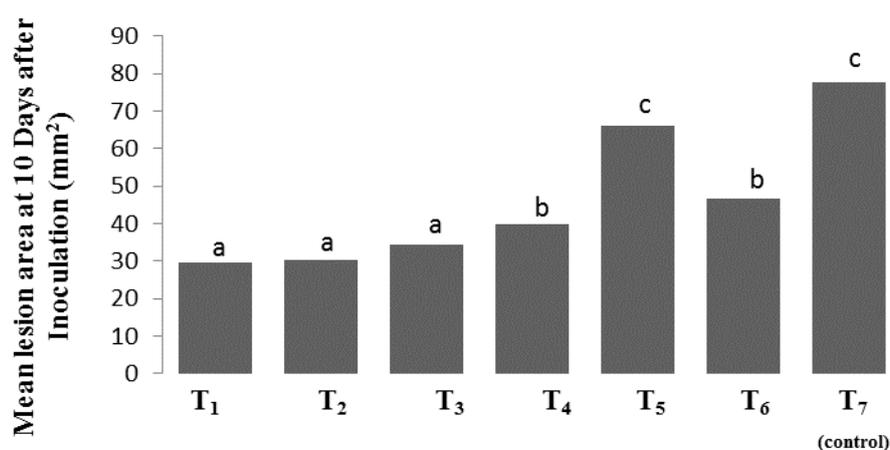


Fig. 1. Mean lesion area (mm²) on chili pepper fruits at 10 days after inoculation of *C. gloeosporioides*. Bars with same letters are not significantly different ($P \leq 0.05$) as determined by Tukey HSD test. (T₁= RRH:Soil:Sand at 3:2:0, T₂= RRH:Soil:Sand at 3:0:2, T₃= RRH:Soil:Sand at 3:1:1, T₄= RRH:Soil:Sand at 2:3:0, T₅= RRH:Soil:Sand at 1:1:3, T₆-soil medium treated with PS and T₇- Si-free medium)

Only a 15% reduction of anthracnose lesion area was observed in fruits from T₆ compared to that of control fruits. However, our previous study indicated that supplementation of 75 mg/L of potassium silicate in hydroponic nutrient solution can significantly reduce the anthracnose disease in chili pepper (Jayawardana *et al.* 2015). There could have been a significant reduction in the anthracnose disease by application of greater concentrations than 75 mg/l of potassium silicate into the soil medium. It has to be further investigated.

Table 1. Plant growth and fruit parameters of chili pepper plants grown in combinations of raw rice hull, soil, sand and PS incorporated substrates (means followed by the same letter within the same row are not significantly different at $P \leq 0.05$ as determined by Tukey HSD test; ns= not significant)

Parameter	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇
Shoot length (cm)	63 ^a	61 ^a	65 ^a	59 ^a	69 ^b	60 ^a	59 ^a
Inter node (3-4) girth (cm)	2.9	2.8	3.1	2.9	2.9	2.8	2.8 ^{ns}
Number of leaves	53 ^a	50 ^a	52 ^a	49 ^a	57 ^b	50 ^a	48 ^a
Number of fruits/plant	9 ^a	9 ^a	10 ^a	9 ^a	12 ^b	8 ^a	8 ^a
Fruit length (cm)	12.8	13.0	12.7	12.2	13.3	12.9	12.0 ^{ns}
Fruit fresh weight (g)	25.0	27.2	24.1	26.3	26.8	27.0	25.5 ^{ns}
Average leaf area (cm ²)	45.2	40.0	43.6	42.9	45.0	44.1	42.2 ^{ns}

T1= RRH:Soil:Sand at 3:2:0, T2= RRH:Soil:Sand at 3:0:2, T3= RRH:Soil:Sand at 3:1:1, T4= RRH:Soil:Sand at 2:3:0, T5= RRH:Soil:Sand at 1:1:3, T6=soil medium treated with PS and T7- Si-free medium)

3.2 Plant growth and fruit parameters

There was no significant effect of potassium silicate applied into the soil medium (T₆) on growth parameters of chili pepper plants compared to the control. However, the shoot length, number of leaves and number of fruits were significantly greater in the plants from T₅ treatment (plants were grown in RH:Soil:Sand at 1:1:3 medium) compared with the rest of the treatments (Table 1). Sand in the medium enhances the porosity. It can be suggested that not only the Si provided by the medium but also the properties of the medium could have affected the results particularly, on the plant growth and fruit parameters. The ratio of 1:1:3 of RRH:Soil:Sand might be a good combination in the substrate to enhance plant growth and yield parameters of chili pepper. However, the disease resistance showed in the T₅ treatment was not significant, which could have been attributed to lower RRH applied. Incorporation of RRH in the form of ash into growing media has improved growth of sunflower plants (Kamenidou 2005; Kamenidou *et al.* 2008). In the current study RRH was used in the growth medium. In a previous study, RRH incorporation in a simplified hydroponic system, showed a significant increase in anthracnose disease resistance, plant growth and fruit qualities of chili pepper plants (Jayawardana *et al.* 2016).

4 Conclusions

Incorporation of rice hull (60% of the media) in the ratios of 3:2:0, 3:0:2 and 3:1:1 of RRH:Soil:Sand in the growing substrate would be effective against anthracnose disease of chili pepper plants. Si provided by RRH in the substrate would be the main factor influencing the disease reduction. However, more sand in combination with RRH in the growing substrate (RRH:Soil:Sand in 1:1:3 ratio) would enhance shoot length, number of leaves and number of fruits of chili pepper plants. Therefore, Combination of RRH with soil and sand in the ratio of 3:1:1 would be better in overall performance of chili pepper crop.

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