Int J Recycl Org Waste Agricult (2016) 5:9–15
DOI 10.1007/s40093-015-0112-4

ORIGINAL RESEARCH

# The effect of rice hull as a silicon source on anthracnose disease resistance and some growth and fruit parameters of capsicum grown in simplified hydroponics

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Received: 16 October 2014 / Accepted: 30 November 2015 / Published online: 5 January 2016


**Abstract**
*Introduction* Silicon is beneficial for many plants for growth, yield and enhancing resistance to biotic and abiotic stresses. In the present study, silicon as a form of rice hull sand mixture (3:2 v/v) was used in simplified hydroponic system to evaluate the performance and disease resistance of *Capsicum annuum* L. The nutrients were supplied by NF (New Formula) or Albert's solution.
*Results* Continuous monitoring of soluble silicon content in the simplified hydroponic boxes revealed that a high amount of silicon was leached by the rice hull sand medium. Disease resistance was assessed by challenge inoculation of harvested fruits with anthracnose-causing fungi, *Colletotrichum gloeosporioides*, and it was observed a significant disease reduction (over 83 %) in fruits harvested from simplified hydroponic system compared to that of liquid hydroponic system. Shoot length, root length, fruit length, fruit weight and fruit firmness were also increased significantly in simplified system compared to the liquid system. However, the results were not significantly affected by the nutrient solutions used in these two systems.
*Conclusions* In conclusion, the simplified hydroponic system composed of rice hull, as a natural silicon supplement could be used as a low-cost environmental friendly growing method of capsicum to enhance resistance against anthracnose disease, and to improve plant growth and fruit quality.

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**Keywords** Anthracnose · *Colletotrichum gloeosporioides* · Rice hull · Silicon · Simplified hydroponics

## Introduction

Capsicum (*Capsicum annuum* L.) is one of the economically important spice/vegetable crops. The anthracnose disease caused by fungi *Colletotrichum* species is one of the major diseases in capsicum in tropical and sub-tropical climates causing postharvest losses (Oanh et al. 2004). The disease is mainly controlled by fungicides. However, fungicides cause environmental and health hazards and emergence of resistance pathogen populations. Recent trend of declining sustainability in agricultural production is appearing as a major threat to most of the Asian countries (Chattopadhyay 2012). Therefore, to address the current demand in sustainable crop production, environmental friendly alternatives should be investigated to control anthracnose disease.

Silicon application in hydroponic systems has been reported beneficial on growth, yield, and also disease resistance of some crops (Epstein 1994). In addition, it has been noticed that omission of Si caused deficiency symptoms in tomato and cucumber plants grown in liquid hydroponic system. (Miyake and Takahashi 1978; Miyaki and Takahashi 1983). Root supplement of Si has been reported to cause significant reductions of many diseases such as, powdery mildew (Adatia and Bestford 1986) and *Phythium* root rot (Cherif and Belanger 1992) of cucumber, blast of rice (Datnoff et al. 1991), *Phytophthora* blight disease of bell pepper (French-Monar et al. 2010), anthracnose of beans (Polanco et al. 2012) and anthracnose of capsicum (Jayawardana et al. 2014a).

Rice hull is an agricultural by-product which is poorly utilized. More than 100 million tons of rice hull is generated annually in the world (Okafor and Okonkwo 2009). The collection and disposal of rice hull is becoming more difficult and expensive and is, therefore, left unused as waste or simply burned in the fields, thereby creating significant environmental problems (Mansaray and Ghaly 1997). It has useful properties as a growing media such as low in weight, inert with respect to adsorption and desorption of nutrients and also has good drainage, aeration and low rate of decomposition (Saparamadu 2008). Ghehsareh (2013) reported some physicochemical properties of rice hull media such as porosity (73 %), water holding capacity (88 %), bulk density (0.09 g/cm$^3$), organic matter content (88.52 %), electrical conductivity (2.24 ds/m) and pH (6.2). The composition of N, P, and K in rice hull is 0.5, 0.08 and 0.4 %, respectively, and the *C/N* ratio of rice hull is 25:1 (Marulanda and Izquierdo 1993). According to Patel et al. (1987), the Si content in raw rice husk is 10.3 (wt%). Si concentration leached into water by rice hull and sand mixture (1:1 v/v) was increased up to 92 ppm within a period of 17 weeks while K, P and N were not increased more than 6 ppm (Saparamadu 2008) which shows that rice hull is a cheap natural source of Si. In a recent study conducted by Jayawardana et al. (2014b) it was found that capsicum grown in nutrient solution incorporated with rice hull leachate showed a significant reduction of anthracnose disease (about 50 %) together with enhanced plant growth and yield.

Rice hull can be mixed with other materials such as coal scoria, saw dust, river sand and volcanic scoria and can be successfully used as media in simplified hydroponic systems (Bradly and Marulanda 2000). Simplified hydroponic is a low-cost aggregate hydroponic system (Fig. 1) where nutrients and water are used efficiently and the labor requirement is less. It can be practiced in natural climatic conditions with any discarded container using waste or low-cost substances as media (Bradley 2000). The plastic tube connected to the simplified box maintains the height of liquid layer comprised with water and nutrients (Saparamadu 2008).

It has been reported that simplified hydroponics system which consisted rice hull:river sand (3:2 v/v ratio) medium enhanced growth of bush beans and tomato (Saparamadu et al. 2008). Therefore, simplified hydroponics system with rice hull as a component in the medium would be an economical way of supplying silicon for plants. Therefore, the objective of this research was to investigate the effect of Si supplied by rice hull in the media on the performance (growth, fruit quality and anthracnose disease resistance) of capsicum grown in simplified hydroponic system in comparison with non-circulating liquid hydroponic system and to determine whether the effect of Si depends on the composition of the nutrient solution.

## Materials and methods

### 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 for 6 weeks. Healthy plants were transferred to simplified hydroponic and non-circulating liquid hydroponic systems in a mesh house under 28–30 °C temperature and 80–85 % relative humidity.

#### *The hydroponics systems and nutrient supplement*

The simplified hydroponic boxes were prepared according to Fig. 1 and were filled with inert media: rice hull (repeatedly soaked and washed for 7 days) and sand (three

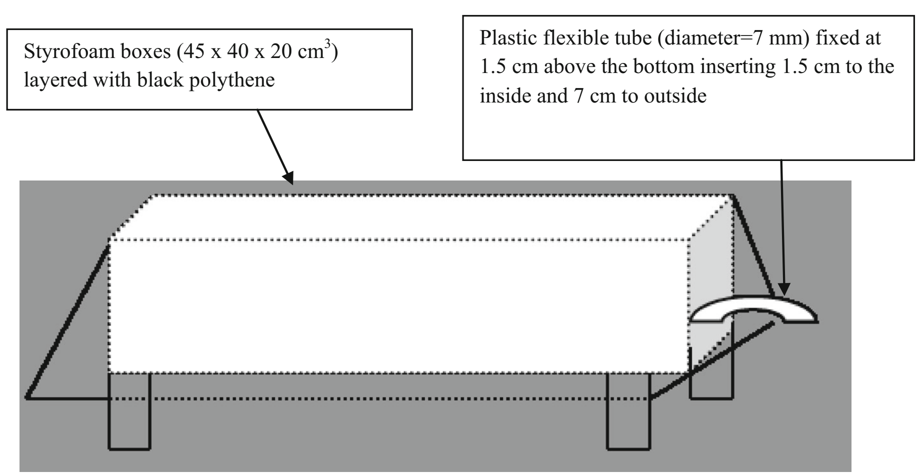


**Fig. 1** Simplified hydroponic system

times washed) in the ratio of 3:2 v/v (Saparamadu et al. 2010).

Nutrients were supplied by two nutrient solutions: NF (New Formula) nutrient solution (Saparamadu 2008) and Albert's solution [Unipower (pvt) Ltd.]. NF consisted with two different nutrient formulations, NFG for growing stage and NFB for blooming stage having the composition (mg/l): NFG: N—313, P—80, K—202, Ca—300, Mg—78 and NFB: N—366, P—80, K—650, Ca—169, Mg—78. Both formulas had Cu—0.01, Fe—3.90, Zn—0.13, Mn—1.20, B—1.00 and Mo—0.13 (Saparamadu 2008). The average composition of Albert's solution was (mg/l) N—83, P—30, K—124, Ca—106, S—226, Mg—20, Cu—0.02, Fe—1.0, Zn—0.1 and Mn—0.27 and Mo—0.05 and it was applied in concentrations 1.6 and 2.6 g/l during growing and blooming stages, respectively (Saparamadu et al. 2010). Nutrients were supplied for simplified hydroponic boxes by manual fertigation with 200 ml of nutrient solution once in 2 days. Non-circulating liquid hydroponic boxes were of the same size as simplified hydroponic boxes and were filled with 20 l of nutrient solution, which was renewed once a week.

## Treatments and experimental design

There were four treatments: simplified hydroponic system supplied with NF nutrient solution (SHNF), simplified hydroponic system supplied with Albert's solution (SHAL), non-circulating liquid hydroponics system supplied with NF nutrient solution (NF) and non-circulating liquid hydroponics system supplied with Albert's solution (AL). Treatments were arranged in Complete Randomized Design (CRD) with three replicates each with four plants. The experiment was repeated once.

## Analysis of molybdo-reactive silica leached by media of the simplified hydroponics system

Sample solutions were sucked using a pipette from several places of the liquid layer in the bottom of simplified boxes without damaging the roots and mixed well and 5 ml was taken from the mixture of each box. Samples were collected once a week diluted and analyzed for molybdo-reactive silica concentration using the procedure given below.

### *Determination of molybdo-reactive silica (Clesceri et al. 1998)*

HCl (0.5 ml) and ammonium molybdate reagent (1.0 ml) were added to the 25 ml of the diluted sample. Then the solution was mixed thoroughly and was allowed to stand for 5–10 min. Oxalic acid (1.0 ml) was added to the same and solution was mixed thoroughly. Calibration curve was drawn using standard solutions in the range of 4–12 ppm. The concentration of Si in the samples was obtained at 410 nm after 2 min using UV visible spectrophotometer (Model Labomed UVD 3000/3200, USA).

## Assessment of disease resistance against anthracnose disease

The anthracnose disease resistance was assessed against one of the main causal organisms *Colletotrichum gloeosporioides*.

### *Pathogen identification and isolation*

Capsicum fruits with anthracnose lesions caused by *C. gloeosporioides* were collected, surface sterilized with 1 % NaOCl for 1 min followed by washing with sterile distilled water, cultured on PDA (Potato Dextrose Agar) media and incubated at 27–30 °C temperature. *C. gloeosporioides* was isolated by sub-culturing. Cultures were observed for morphology and mycelium growth and shape of conidia was observed through a compound microscope. *C. gloeosporioides* was identified based on the Commonwealth Mycological Institute (CMI) descriptions by orange cottony mycelium (Sutton 1980) and typical ovoid shape of conidia (Du et al. 2005) and also with reference to the previous studies of morphological and molecular characterization (Photita et al. 2005).

### *Fruit inoculation and disease assessment*

Fruits harvested at the color breaker stage were used for the inoculation experiment. A suspension of conidia ($10^5$ conidia per ml) was prepared using 7-day-old pure cultures of *C. gloeosporioides*. Ten fruits from each treatment were artificially inoculated by placing drops of (20 μl) conidia suspension at three different places of each fruit. Inoculated fruits were maintained in moist chambers (95–100 % relative humidity) at 28 ± 2 °C. The lesion area was recorded daily for 10 days. The average lesion area was calculated for each fruit. The number of days taken for disease initiation was recorded.

## Measurement of plant growth and fruit parameters

The shoot length, root length, internodes diameter, internodes length, number of leaves, average leaf area and number of fruits were recorded at 15 weeks after transplanting. Fruit length and fruit fresh weight of each harvested fruit were measured. Diameter at the maximum width was measured as the fruit width by a vernier caliper. Fruits were cross sectioned at the maximum width and three measurements of pericarp thickness were taken per cross section by a vernier caliper. Fruit firmness was measured by penetrometer (Model FT 40, Wagner Instruments, Greenwich CT). Fruit extracts were prepared by crushing the fruits separately using a blender and squeezing the pulp through a muslin cloth. Total soluble solids (TSS) of fruit extracts were measured by a refractometer (Model WZ-113, China) within the range of 0–32 % Brix and pH of the extracts was measured using pH meter (Model IQ150, USA). Aliquots (5 ml) of fruit extracts were titrated against 0.1 M NaOH in the presence of phenolphthalein as the indicator and titratable acidity (%TA) for each sample was determined according to Askar and Trepow (1993).

### Sensory evaluation on sensory properties of capsicum fruits

A sensory evaluation was carried out by a trained panel to test sensory properties of harvested fruits; color, hardness, smell, pungency and overall appearance using hedonic scale.

### Data analysis

The data were analyzed using one-way ANOVA in SPSS 16.0 statistical package. Means were compared by Tukey HSD test. Results obtained for the sensory analysis was subjected to non-parametric Kruskal–Wallis Test.

## Results and discussion

### Concentration of Si leached in simplified hydroponics boxes with time

The soluble Si concentration in the simplified hydroponics system appeared to be low up to 3 weeks (<10 ppm). However, the level of silicon was increased up to 30 ppm after 6 weeks and was almost constant up to 12 weeks and further increased up to 50 ppm at the later stage (Fig. 2). However, these observations were similar for both nutrient solutions indicating that the nutrient composition does not affect the leaching of soluble Si. The release of Si from rice hull might be related to decomposition or ion exchange of rice hull.

In a previous study, it has been reported that concentration of Si leached by rice hull was increased with time while concentration of Si leached by sand was lower and was not increased with time (Saparamadu 2008). Therefore, the increase of Si content in simplified boxes may be due to rice hull.

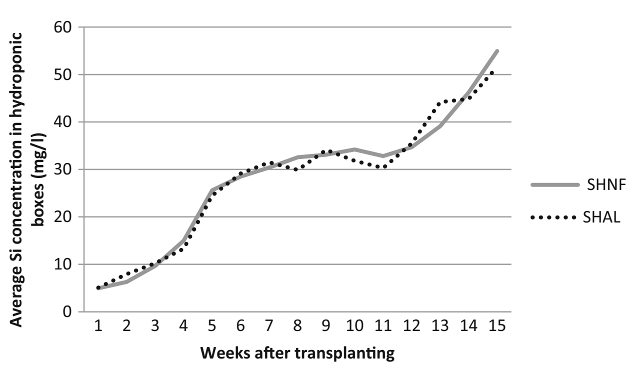


**Fig. 2** Average Si concentration leached in the simplified hydroponics *boxes* with time. (*SHNF* simplified hydroponic system supplied with NF nutrient solution, *SHAL* simplified hydroponic system supplied with Albert's solution)

### Lesion area development of capsicum fruits inoculated with *C. gloeosporioides*

The lesion area observed at 10 days after inoculation was 17.5 and 12.8 $mm^2$ in SHNF and SHAL treatments whereas that was 103.8 and 99.2 $mm^2$ in NF and AL treatments, respectively (Fig. 3). The lesion area observed in fruits from SHNF treatment was 83 % lesser than NF treatment while that was 87 % lesser in SHAL treatment than AL treatment. Further, it was observed that the lesion initiation was delayed by 3 days and the rate of lesion development was slow (average 3.7 $mm^2$/day) in the fruits of capsicum plants grown in simplified hydroponic system compared to that of non-circulating liquid hydroponics system (average 14.5 $mm^2$/day) (Fig. 3).

It was also revealed that the lesion area reduction was not affected by the nutrient solution applied for the treatments. In a recent study, amendment of nutrient solution

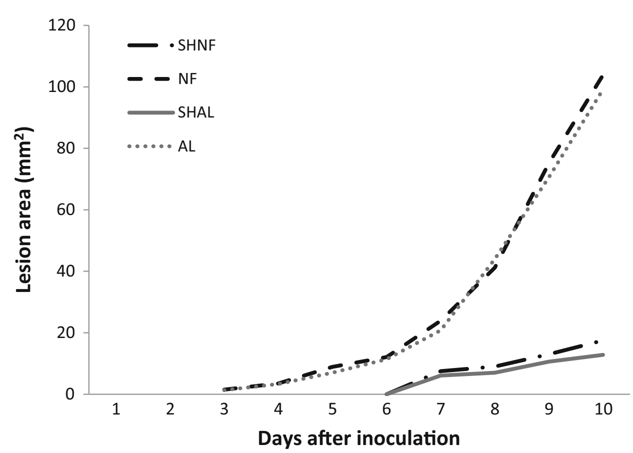


**Fig. 3** Lesion area development ($mm^2$) in capsicum fruits during 10 days after inoculation of *Colletotrichum gloeosporioides* (*SHNF* simplified hydroponic system supplied with NF nutrient solution, *SHAL* simplified hydroponic system supplied with Albert's solution, *NF* non-circulating liquid hydroponics system supplied with NF nutrient solution, *AL* non-circulating liquid hydroponics system supplied with Albert's solution)

with potassium silicate has reduced the anthracnose disease of Capsicum variety Awlegama by over 75 % (Jayawardana et al. 2014a). In the current study, it was revealed that high Si levels were present in the simplified hydroponics boxes during latter stage at blooming and fruiting stage (Fig. 2). Therefore, it could be suggested that the anthracnose disease reduction was due to Si leached by the rice hull and sand mixture in the simplified hydroponic system. It has been reported that rice blast disease caused by *Magnaporthe grisea* was reduced by silicon treatments (Seebold et al. 2001). Causal agents of both rice blast and anthracnose diseases belong to family *Ascomycete* and it can be suggested that silicon has inhibiting action on growth of *Ascomycete* fungi.

The underlying mechanism of disease resistance mediated by silicon has been discussed in different ways. Ma et al. (2006) reported that silicon acts as a physical barrier, which is deposited beneath the cuticle such that the Si layer mechanically impedes penetration by fungi, thereby disrupting the infection process. On the other hand, it is revealed that silicon has a biochemical mechanism in disease suppression. Si could induce defense responses similar to systemic acquired resistance (Cai et al. 2009).

## Growth and fruit parameters of the capsicum plants

The shoot and root length of SHNF treatment (64 and 34 cm, respectively) were significantly higher than that of NF treatment (50 and 20 cm, respectively). Similarly, shoot and root length of SHAL treatment (68 and 40 cm, respectively) was significantly higher than that of AL treatment (59 and 20 cm, respectively). The capsicum plants grown in simplified hydroponic system consisting with rice hull and sand mixture showed significantly higher shoot and root length compared to the plants grown in non-circulating liquid hydroponic system with no Si supplement (Table 1).

In previous studies it was reported that Si treatments increased plant height and stem diameter of sunflower (Kamenidou et al. 2008), plant fresh and dry weight of broad bean (Ghasemi et al. 2013) and also dry weight of leaves and roots of cucumber (Adatia and Bestford 1986). However, there was no significant difference in internodes diameter, internodes length, number of leaves, average leaf area and number of fruits.

The fruit length, fruit fresh and weight fruit firmness of SHNF treatment (13.78 cm, 30.87 g and 24.16 N, respectively) were significantly higher than that of NF treatment (12.25 cm, 25.50 g and 19.50 N, respectively). Similarly, fruit length, fruit fresh weight and fruit firmness of SHAL treatment were also significantly higher than that of the treatment AL (Table 2). In terms of fruit parameters, simplified hydroponic system was better with significant increase in fruit length, fruit fresh weight and fruit firmness than that of non-circulating liquid hydroponic system supplied with either NF or Albert's nutrient solution. However, there was no significant difference in other tested fruit parameters (Table 2). It is also reported that Si treatment increased average pod weight and average seed number of broad bean (Ghasemi et al. 2013) and fruit fresh weight of cucumber (Adatia and Bestford 1986).

The greater fruit firmness in SHNF and SHAL treatments possibly could be due to the strengthening of cell wall structures resulted by Si treatments. Samuels et al. (1994) found that silicon deposition in papillae, in host cell walls, around the haustorial neck and in between host cell wall and plasma membrane as mechanism of defense development by silicon against *Spaerotheca fuliginea* in cucumber. Samuels et al.

**Table 1** Effect of different treatments on growth parameters of the capsicum plants

| Parameter | SHNF | NF | SHAL | AL |
|---|---|---|---|---|
| Shoot length (cm) | $64^a$ | $50^b$ | $68^a$ | $59^b$ |
| Root length (cm) | $34^a$ | $20^b$ | $40^a$ | $20^b$ |
| Internodes diameter (3–4) (cm) | $3.0^a$ | $3.0^a$ | $3.1^a$ | $3.0^a$ |
| Internodes length (5–6) (cm) | $2.9^a$ | $3.0^a$ | $3.0^a$ | $3.1^a$ |
| Num. of leaves | $57^a$ | $54^a$ | $63^a$ | $62^a$ |
| Avg. leaf area ($cm^2$) | $50^a$ | $45^a$ | $48^a$ | $46^a$ |
| Num. of fruits | $10^a$ | $8^a$ | $10^a$ | $8^a$ |

Means followed by the same letter within the same row are not significantly different ($P \leq 0.05$) as determined by Tukey HSD test

*SHNF* simplified hydroponic system supplied with NF nutrient solution, *SHAL* simplified hydroponic system supplied with Albert's solution, *NF* non-circulating liquid hydroponics system supplied with NF nutrient solution, *AL* non-circulating liquid hydroponics system supplied with Albert's solution

**Table 2** Effect of different treatments on fruit parameters of the capsicum

| Parameter | SHNF | NF | SHAL | AL |
|---|---|---|---|---|
| Fruit length (cm) | $13.78^a$ | $12.25^b$ | $13.50^a$ | $13.00^b$ |
| Fruit width (cm) | $2.90^a$ | $2.65^a$ | $2.63^a$ | $2.69^a$ |
| Fruit pericarp thickness (mm) | $4.47^a$ | $3.94^a$ | $4.30^a$ | $3.90^a$ |
| Fruit fresh weight (g) | $30.87^a$ | $25.50^b$ | $34.55^a$ | $24.40^b$ |
| Fruit firmness (*N*) | $24.16^a$ | $19.50^b$ | $24.16^a$ | $20.9^b$ |
| Total soluble solids (°Brix) | $7.4^a$ | $7.5^a$ | $7.5^a$ | $7.4^a$ |
| %TA | $0.55^a$ | $0.54^a$ | $0.54^a$ | $0.52^a$ |
| pH | $5.6^a$ | $5.5^a$ | $5.8^a$ | $5.6^a$ |

Means followed by the same letter within the same row are not significantly different ($P \leq 0.05$) as determined by Tukey HSD test

*SHNF* simplified hydroponic system supplied with NF nutrient solution, *SHAL* simplified hydroponic system supplied with Albert's solution, *NF* non-circulating liquid hydroponics system supplied with NF nutrient solution, *AL* non-circulating liquid hydroponics system supplied with Albert's solution

**Table 3** Mean ranks obtained for the results of sensory evaluation (Hedonic scores) as determined by Kruskal–Wallis test

| Treatments | Fruit color | Fruit hardness | Overall appearance | Pungency | Smell |
|---|---|---|---|---|---|
| SHNF | 20.15[a] | 24.8[a] | 22.35[a] | 20.95[a] | 26.65[a] |
| NF | 17.8[a] | 17.8[a] | 15.25[a] | 20.35[a] | 17.85[a] |
| SHAL | 27.2[a] | 21.9[a] | 26.5[a] | 17.35[a] | 18.6[a] |
| AL | 16.85[a] | 17.5[a] | 17.9[a] | 23.35[a] | 18.9[a] |

Hedonic scores ranged from 1 to 7 where 1 = extremely dislike and 7 = extremely like

[a] There was no significant difference among treatments in any sensory property ($P \leq 0.05$) as determined by Kruskal–Wallis test

(1991) observed coarse texture of fruit surface as a side effect of Si application in cucumber compared to fruit surface of Si-deprived plants and these morphological changes were attributed to deposition of Si in trichomes.

### Sensory evaluation on properties of capsicum fruits

Among the treatments, overall appearance and fruit color of SHAL treatment were highly preferred by the panel while smell and pungency of SHNF treatment were highly preferred. Nevertheless, there was no significant difference observed among treatments in tested sensory properties of fruits: fruit color, fruit hardness, overall appearance, pungency and smell (Table 3).

## Conclusion

The simplified hydroponics system consisting Si sources rice hull:sand (3:2 v/v) media was effective in reducing the anthracnose disease caused by *C. gloeosporioides* by more than 83 % and enhancing shoot and root length, fruit fresh weight, fruit length and fruit firmness of *Capsicum annum* L 'Muria F1' in comparison with non-circulating liquid hydroponic system supplied with either NF or Albert's nutrient solution. Therefore, it could be concluded that simplified hydroponics system with a natural silicon sources, rice hull in the media would be a low-cost and environmental friendly method for growing capsicum to enhance anthracnose disease resistance and other growth and fruit parameters.



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