The Pharma Innovation Journal 2023; 12(3): 5476-5484

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(3): 5476-5484

www.thepharmajournal.com
Received: 05-02-2023
Accepted: 03-03-2023

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Efficacy of silicic acid on severity of powdery mildew (*Oidium neolycopersici*) in tomato

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Abstract
Powdery mildew disease is a serious and debilitating disease of tomato worldwide. The main objective of the study was to find out the efficacy of different concentrations of silicic acid on severity and its disease progress apart from defence responses against powdery mildew disease of tomato cultivated under greenhouse. The application of silicic acid @ 4 mL L^{-1} (35.24 Per cent Diseases Incidence-PDI) and @ 2 mL L^{-1} (59.05 PDI) recorded lowest disease severity when compared to untreated control (80.00 PDI) at 49 days after inoculation. Similarly, the lowest disease progress (Area Under Disease Progress Curve-AUDPC values) was recorded with the application of silicic acid @ 4 mL L^{-1} (690.13) and 2 mL L^{-1} (1016.63), whereas untreated plants recorded highest AUDPC value of 1873.34. The higher dry weight (16.58g), silicon content (1.15%) and increased chlorophyll content recorded with silicic acid @ 4 mL L^{-1} when compared to untreated control plant. The reduced severity and progression of disease on tomato leaves has been attributed to significant increase in activities of defence enzymes *viz*., peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, β-1, 3 glucanase and chitinase upon foliar application of silicic acid @ 4 mL L^{-1} and 2 mL L^{-1}. This clearly illustrate that, foliar application of silicic acid on tomato leaves conferred the resistance against powdery mildew pathogen.

Keywords: Silicon, silicic acid, powdery mildew, tomato, disease

1. Introduction
Powdery mildew disease of tomato caused by an obligate biotrophic fungi, *Oidium neolycopersici*, grouped under order Erysiphales has been recognized as an prominent pathogen on tomato worldwide (Jones *et al*. 2001) [16] and restricting tomato production under both open field and greenhouse (Kiss *et al*. 2001) [19]. Since lack of effective fungicides for management of powdery mildew disease on account of several reports of pathogen isolates either resistant or insensitive to different groups of fungicides (McGrath 2001) [26], breeding of tomato varieties that are resistant/tolerant to powdery mildew disease is seems to be the only viable management strategy. However, the resistance in cultivars achieved by one or two major resistance genes is not stable under greenhouse condition because of existence of more variability of pathogen leading to rapid evolution of virulent races. The breeding of tomato cultivars having high level of durable resistance would be ideal, but little progress has been made in this regard. Therefore, it is needed to develop an environmentally friendly alternative protection methods for powdery mildew disease in tomato. Silicon (Si) application is one such pivotal alternative for eco-friendly disease management.
Silicon (Si) is the second most abundant element in the earth crust (Epstein 1999) [15] and plants take up silicon as soluble silicic acid (H_4SiO_4), is transported *via* the transpiration stream and subsequently accumulates as insoluble amorphous silicon compounds primarily in the apoplast of a plant (Belanger *et al*. 2003) [2]. There are several hypotheses in support of silicon role in inhibiting fungal infection and control of bio-trophic pathogens like powdery mildew disease in oat, wheat, barley, rye, arabidopsis, cucumber, pumpkin, and strawberry by silicon has been reported (Belanger *et al*. 2003; Ghanmi *et al* 2004; Datnoff *et al*. 2007) [2, 13, 10]. So far, many reports have confirmed the preventive effects of silicon mostly against powdery mildew and rice blast (Fauteux *et al*. 2005) [12] was attributed to the accumulation of silicon in the epidermal layer of leaf cells, which were conjecture to interfere with the penetration of pathogen itself into the epidermal cells (Jones and Handreck 1967) [17], and the increased synthesis of defence enzymes in silicon applied plants (Rodrigues *et al*. 2003a, 2004; Remus-Borel *et al*. 2005) [35, 37, 33].

Many findings revealed that the enhanced the plant growth and yield in the silicon applied plant, distinctly when they are subjected to both biotic and abiotic stresses (Ma 2004) [24] due to increased uptake of nutrients (Prakash *et al*. 2011) [31] and photosynthesis.

Despite the potentiality of Si as an alternative strategy to manage powdery mildew, the feasibility of silicon as a foliar application for control of powdery mildew disease is not well studied in tomato. Thus, the present study elaborated the understanding of effect of applications of silicic acid as foliar spray on various disease epidemic components *viz*., powdery mildew disease severity, AUDPC value and activity of defence enzymes in tomato plants. This was combined with studies of quantitative and temporal differences in the infection processes and defence responses against *O. Neolycopersici* with foliar application of silicic acid in comparison with control plants.

2. Material and Methods

2.1 Pot culture experiment

A pot culture experiment was conducted in greenhouse maintained at Department of Plant Pathology, University of Agricultural Sciences (UAS), Bengaluru, India.

2.2 Cropping season: The cropping season was between August to December 2015.

2.3 Planting material

The tomato seeds (Arka Vikas) were used in this experiment were obtained from ICAR-Indian Institute of Horticulture Research, Hessaraghatta, Bengaluru and rinsed thoroughly in distilled water. The rinsed seeds were sown into a sterile peat substrate [1:1 (v/v) mix of coir dust and compost] in pro-trays consisting of eight by twelve compartments and placed them in a nursery until appearance of the four to five true leaf. The greenhouse temperature was maintained at 25- 28 ˚C and 80-85 per cent relative humidity for the three weeks.

2.4 Transplanting of seedlings

Three-week old healthy tomato seedlings were transplanted to three litre capacity plastic pots containing a mixture of compost and sterilized soil (1:1, vol/vol). The sandy clay loam soil was used (54% sand, 29% clay, and 17% silt), with moderate drainage and pH of 7.1. Plants were irrigated every 2-3 days and pots with three holes were used for the experiment for allowing 30% drainage. Plants were kept at 25 to 28 °C in an insect and disease free conditions for three weeks prior to the commencement of the experiments. Experiments were initiated after plants had reached the four to five true leaf developmental stage.

2.5 Pathogen and host plants

O. neolycopersici was collected from young infected leaves of tomato plants grown in a greenhouse at the Department of Horticulture, University of Agricultural Sciences, Bengaluru, India during July 2015. Conidia of the pathogen were collected for artificial inoculation by rinsing infected leaves with sterile distilled water and make a conidial suspension for foliar inoculation by diluting with distilled water. Gelatin/gum (0.1% w/v) was added to the sterile water to aid conidial adhesion to the leaf surface. Seedlings were inoculated by spraying conidial suspension (4 X 10^5 conidia mL^{-1}) as a fine mist on to the leaves by using an aerosol sprayer.

2.6 Foliar treatments

Application of silicic acid

The following foliar treatments were used after dilution by using tap water. The foliar silicic acid (2 mL L^{-1} and 4 mL L^{-1}) was applied thrice at ten days interval starting from a day before artificial inoculation. Foliar silicic acid was obtained from the Department of Soil Science and Agricultural Chemistry, UAS Bengaluru for conducting greenhouse experiments. The material composition was 0.8% Si as silicic acid (H_4SiO_4), 1.2% potassium chloride (KCL), 0.8% boric acid (H_3BO_3), 1.0% hydrochloric acid (HCL), 47.0% Demi water and 48.0% PEG_{400}. The experiment details are as mentioned below.

2.7 Experiment details

Design: Completely randomised design (CRD)
Test crop: Tomato
Variety: Arka Vikas
Test Pathogen: *Oidium neolycopersici*
No. of treatments: 4
No. of replications: 6
Silicon sources: Silicic acid
Application level: 2 mL L^{-1} and 4 ml L^{-1}
Fungicide: Difenoconazole 25% EC @ 0.5 ml L^{-1} as a standard check
Application rate: Thrice at 10 days interval starting from a day before artificial inoculation

2.8 Treatments details

T_1: Untreated control;
T_2: OSAB @ 2 ml L^{-1};
T_3: OSAB @ 4 ml L^{-1};
T_4: Difenoconazole 25% EC @ 0.5 ml L^{-1}

2.9 Disease severity assessment

Disease severity of powdery mildew was visually assessed from 7 days after inoculation (dai) to 49 dai at an interval of 7 days on every leaf, starting from the third true leaf using 0-7 scale (EPPO 2004) based on the percentage of diseased leaf area infection (DLAI) where: 0 = 0% DLAI, 1=0.1-5% DLAI, 2=5.1-10% DLAI, 3=10.1-20% DLAI, 4=20.1-40% DLAI, 5=40.1-60% DLAI, 6=60.1-80% DLAI, and 7=80.1-100% DLAI

2.10 Per cent disease index (PDI)

PDI was calculated by using the following formula (Wheeler 1969) [41].

$$PDI = \frac{\text{Sum of all the numerical disease ratings}}{\text{Total No. of leaves observed X Maximum disease rating scale}} X\ 100$$

The percentage of diseased area was calculated per plant at each assessment date. Data from all assessment dates were used to calculate the Area Under Disease Progress Curve (AUDPC) as described by Campbell and Madden (Campbell and Madden 1990) [4]. Relative AUDPC values were used to compare powdery mildew disease severity between treatments.

2.11 Calculation of Area Under Disease Progress Curve values

$$\text{AUDPC} = \sum_{i=1}^{n} \left(\left\{ \frac{[Yi + Y(i+1)]}{2} \right\} \right) x\, [t(i+1) - ti]$$

Where,
Y_i= Disease severity at time t_i
$T_{(i+1)}$ - t_i= Time (days) between two disease severity scores
n= Total number of observations

During observation period the biometric parameters *viz*., shoot fresh and dry weight, and silicon content (%) were measured at 70 days after planting and chlorophyll content was measured at 55 days after planting.

2.12 Biochemical analysis of tomato plant samples to understand the mechanism of resistance

2.12.1 Preparation of leaf sample for enzyme analyses

For determination of peroxidase (POD), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), β- 1, 3 glucanase and chitinase (CHI) activities, five to six leaves were collected randomly at 0, 24, 72, 96 and 120 hours after inoculation (hai) of *O. neolycopersici* from both silicic acid applied and non-applied plants from each treatment. Collected leaves were weighed and used for extraction of antifungal compounds and stored in clean cellophane bags at -20 °C. The detailed procedure for enzyme assays is described below.

2.12.2 Enzymatic assay of peroxidase (POD)

The activity of peroxidase in the leaf extract was recorded at room temperature by measuring the appearance of pink/brown color resulting from oxidation of guaiacol in presence of hydrogen peroxide (Dann and Deverall 2000) [8]. The activity was detected as change in optical density measured at 470 nm and expressed in min^{-1} mg of $protein^{-1}$.

2.12.3 Enzymatic assay of polyphenol oxidase (PPO)

The activity of PPO was also recorded colorimetrically at 398 nm (A398) by following the method proposed by Qin and Tian (2005) [32], and expressed as A398 min^{-1} mg of $protein^{-1}$. Protein content present in crude sample extracts was estimated by using Bradford protein assay (Bradford 1976) [3].

2.12.4 Assay of phenylalanine ammonia lyase (PAL)

PAL activity was recorded colorimetrically at 290 nm by following the method of Qin and Tian (2005) [32] and was defined as nanomole cinnamic acid produced at A290 nm h^{-1} mg^{-1} $protein^{-1}$. The protein content of the leaf enzyme extracts was determined by the procedure given by Bradford (1976) [3].

2.12.5 Assay of β-1, 3-glucanase

Glucanase activity was recorded colorimetrically at 610 nm by adopting the method described by Dann and Deverall (2000) [8]. The glucanase activity was detected as a change in optical density at 610 nm and expressed in µmol min^{-1} mg of $protein^{-1}$.

2.12.6 Assay of chitinase

Chitinase activity was recorded calorimetrically at 410 nm by following the method described by Roberts and Selitrennikoff (1988) [34] modified by Harman *et al.* (1993) [14]. The absorbance of the final product released by the chitinase was determined at 410 nm. The chitinase activity was expressed as nmol min^{-1} mg of $protein^{-1}$.

2.12.7 Assay of chlorophyll content

Soil and Plant Analysis Development-SPAD meter (SPAD-502, 1989 Minolta Co., Ltd) was used to measure the chlorophyll content of leaves (Inada 1985) [15]. The fully expanded young leaf of a plant was used for SPAD measurement. A mean of 10 leaves per plant were taken for SPAD value.

2.13 Determination of silicon in plant samples

2.13.1 Digestion of plant sample

The top leaves at fourth and fifth position from each plant were collected replication wise in each treatment and washed in deionized water. The samples were then dried in an oven at 70 °C for 2 to 3 hours prior to analysis and ground with a pestle and mortar until the contents could pass through a 40-mesh screen. The sample (0.1 g) was digested in acid mixture consists of 7 ml of HNO_3 (70%), 2 ml of H_2O_2 (30%) and 1 ml of HF (40%) using microwave digestion system (Milestone-Start D) with following steps: 1000 watts for 17 minutes with a ramping rate of 7 °C per minute and 1000 watt for 10 minutes at holding temperature of 150 °C and venting for 10 minutes (Narayanaswamy and Prakash 2010) [29]. The digested samples were made up to 50 ml by addition of 4 per cent boric acid.

2.13.2 Estimation of silicon content in plant samples

The concentration of Si in the digested plant sample solution was determined by transferring 0.5 ml of digested aliquot to a plastic centrifuge tube and 3.75 ml of 0.2 N HCl, 0.5 ml of 10 per cent ammonium molybdate ($(NH4)_6Mo_7O_2$), 0.5 ml of 20 per cent tartaric acid and 0.5 ml of reducing agent (Amino naphtholsulphonic acid - ANSA) was added, and the final volume was made to 12.5 ml with the addition of distilled water. After an hour, the absorbance was recorded at 600 nm with a UV-visible spectrophotometer (Shimadzu) (Ma and Takahashi 2002) [25]. Similarly, standards (0, 0.2, 0.4, 0.8 and 1.2 ppm) were prepared by following the above procedure.

2.14 Experimental design and statistical analysis

Data generated were statistically analyzed using the XLSTAT software *via* one-way ANOVA by using Fisher's test at $p \leq 0.01$ and $p \leq 0.05$.

3. Results

3.1 Efficacy of foliar application of silicic acid in reducing the disease severity of powdery mildew in tomato

In greenhouse experiment, the spray application of silicic acid at 4 mL L^{-1} and 2 mL L^{-1}, significantly (CD < 0.01) reduced the disease severity in tomato (Table 1) over untreated control plants. Foliar applications of silicic acid (4 mL L^{-1}) and fungicide difenoconazole 25% EC @ 0.5 mL L^{-1} applied thrice at 10 days interval were significantly controlled disease severity as compared with the untreated control.

The results shown that, foliar silicic acid @ 4 mL L^{-1} recorded lowest powdery mildew disease severity (35.24 per cent disease index-PDI) with 55.95 per cent reduction over untreated control (80.00 PDI), whereas, foliar silicic acid @ 2 mL L^{-1} recorded powdery mildew disease severity of 46.67 PDI with 41.67 per cent reduction over untreated control at 49 days after inoculation.

Table 1: Efficacy of silicic acid on severity of powdery mildew disease in tomato

Treatments	Per cent Disease Index (PDI) Days after inoculation (DAI)							Disease reduction (%) at 49 DAI
	7	14	21	28	35	42	49	
Control (Untreated)	12.38a	20.00a	29.52a	43.81a	55.24a	66.67a	80.00a	-
Foliar silicic acid @ 2 ml L^{-1}	5.71b	10.48b	16.19b	21.90b	28.57b	39.05b	46.67b	41.67
Foliar silicic acid @ 4 ml L^{-1}	1.90c	4.76c	10.48d	14.29c	20.95d	28.57c	35.24c	55.95
Difenoconazole 25% EC @ 0.5 mL^{-1}	3.81bc	8.57b	12.38c	16.19c	25.71c	28.57c	36.19c	54.76
S.E.M	0.74	0.85	0.60	0.74	0.77	1.35	0.90	-
CD @ 0.01	2.97	3.43	2.42	2.99	3.09	5.42	3.63	-

PDI values are mean of six replications. The different alphabets in the same column indicate significance different according to Duncan's multiple range test ($p \leq 0.01$) applied after an ANOVA.

3.2 Area under disease progress curve (AUDPC)

Lowest AUDPC value was recorded with silicic acid applied @ 4 mL L^{-1} (690.13) followed by standard fungicide check, Difenoconazole 25% EC @ 0.5 mL L^{-1} (793.92) whereas, untreated control recorded highest AUDPC value of 1873.34 (Fig.1).

3.3 Efficacy of silicic acid on silicon content (%) in tomato leaves

Foliar application of silicic acid at 2 ml L^{-1} and 4 ml L^{-1} was significantly increased silicon content in leaves over control plants (Fig. 1). The silicon content (1.15%) was found maximum in plants treated with silicic acid at 4 ml L^{-1} as compared with untreated control (0.72%).

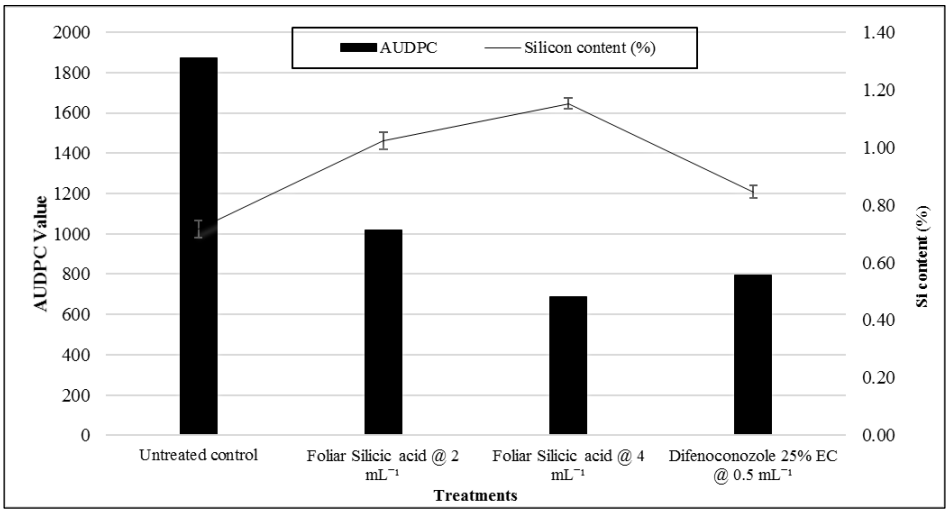

Fig 1: Relationship between powdery mildew disease progress as measured by area under disease progress (AUDPC) and silicon content (%) in tomato leaves. Vertical lines represent standard errors of means (n=5) of 6 replications

3.4 Efficacy of foliar application of silicic acid on growth and chlorophyll content of tomato

Foliar application of silicic acid @ 2- and 4-mL L^{-1} significantly increased both fresh weight and dry weight of shoot (Table 2) and chlorophyll content in leaves as measured by SPAD reading (Fig. 2) compared to untreated control plants. However, significantly highest fresh (69.55±1.93a) and dry (16.58 ± 0.69a) shoot weight and highest chlorophyll content was recorded in foliar silicic acid @ 4 mL L^{-1} treatment.

Table 2: Efficacy of silicic acid on fresh weight and dry weight of shoots in tomato plants inoculated with *O. Neolycopersici*.

Treatment	Fresh shoot weight (g)	Dry shoot weight (g)
Untreated control	36.55 ±1.63d	8.16 ± 0.37 d
Foliar silicic acid @ 2 ml L^{-1}	56.89 ± 1.98b	14.13 ± 1.01b
Foliar silicic acid @ 4 ml L^{-1}	69.55 ± 1.93a	16.58 ± 0.69a
Difenoconazole 0.05% @ 0.5 mL^{-1}	53.00 ± 0.59c	11.89 ± 0.73c
SEM	0.73	0.33
CD @ 0.01	3.02	1.36

Values are means ± standard error (n=5). The different alphabets in the same column indicate significance different according to Duncan's multiple range test ($P \leq 0.01$) applied after an ANOVA.

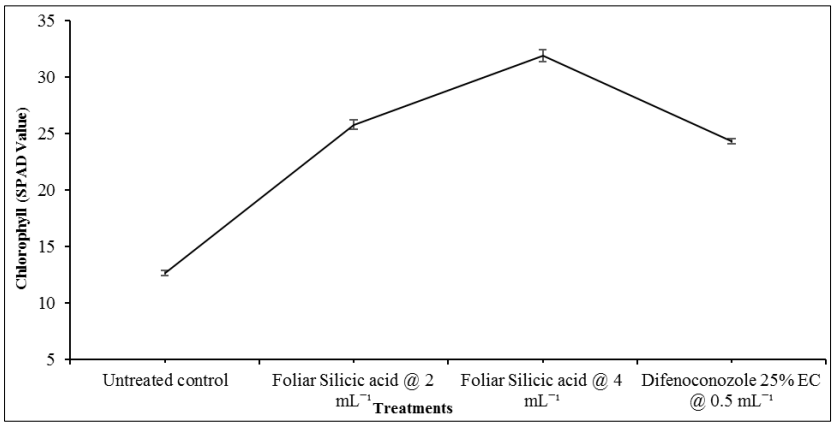

Fig 2: Efficacy of silicic acid on chlorophyll content of tomato leaves (SPAD values) inoculated with *O. Neolycopersici*. The values are mean of five replications. Vertical lines represent standard errors (n=5).

3.5 Activities of PO, PPO, PAL, ß 1, 3-glucanase and chitinase

In pot culture experiment, peroxidase activity in the leaves was significantly higher in the silicic acid applied plant over untreated plants upon inoculation with *O. Neolycopersici* (Fig. 3). The observations revealed that the foliar application of foliar silicic acid @ 4 ml L^{-1} was highly effective in enhancing the peroxidase activity (0.179 min^{-1} mg of $protein^{-1}$ at 96 hai) followed by silicic acid @ 2ml L^{-1} (0.112 min^{-1} mg of $protein^{-1}$ at 96 hai). The PO activity was initially increased till 72 hai and decreased thereafter in foliar silicic acid applied plants, whereas it was started decreasing early (24 hai) in untreated plants subsequent to inoculation with *O. Neolycopersici*.

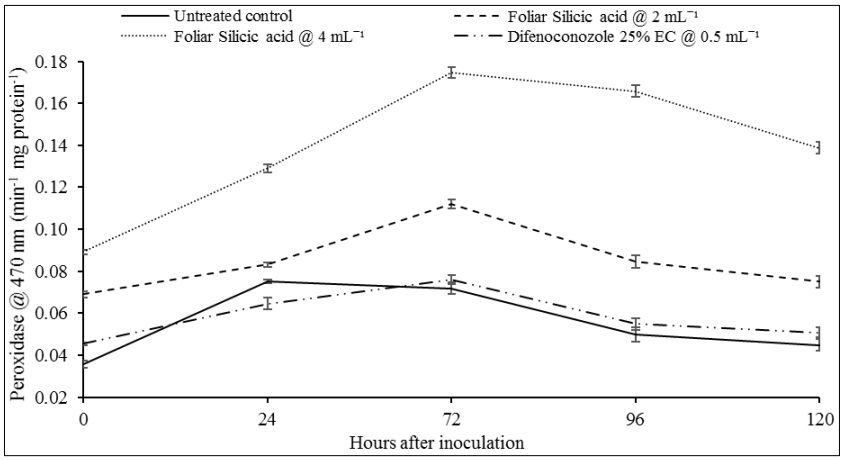

Fig 3: Efficacy of silicic acid on peroxidase (PO) activity in leaves inoculated with *O. neolycopersici.* The values are mean of five replications. Vertical lines represent standard errors.

Foliar application of silicic acid has increased the activity of PPO in tomato plants inoculated with *O. Neolycopersici* compared to untreated control plants (Fig. 4). Among the treatments, silicic acid applied at @ 4 ml L^{-1} showed the highest level of PPO activity (1.88 min^{-1} mg of $protein^{-1}$ at 96 hai) followed by silicic acid @ 2 ml L^{-1} (1.25 min^{-1} mg of $protein^{-1}$ at 96 hai).

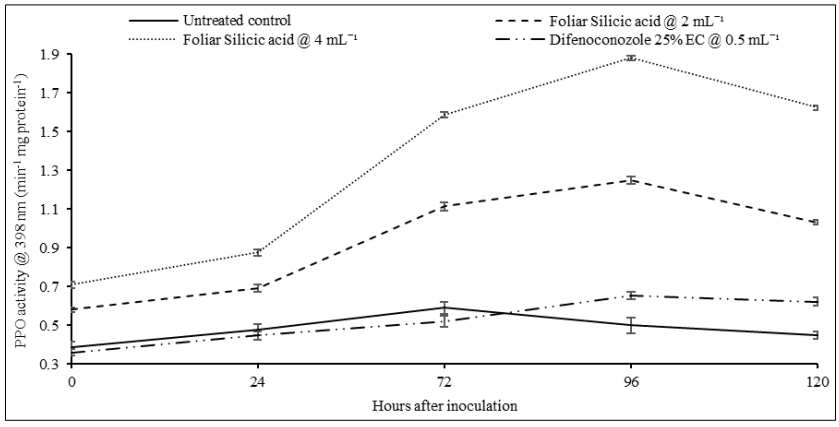

Fig 4: Changes in activity of polyphenol oxidase (PPO) in silicic acid applied tomato plants inoculated with *O. Neolycopersici*. The values are mean of five replications. Vertical lines represent standard errors (n=5)

The activity of PAL was significantly higher in foliar silicic acid (2- and 4-ml L^{-1}) applied plants than in untreated control plants upon inoculation with *O. Neolycopersici* (Fig. 5). Among the treatments, silicic acid applied @ 4 ml L^{-1} recorded highest level of PAL activity (5.99 µmol min^{-1} mg of $protein^{-1}$ at 96 hai) followed by foliar silicic acid @ 2 ml L^{-1} (5.02 µmol min^{-1} mg of $protein^{-1}$ at 96 hai). Further, the PAL activity found increasing till 96 hai and decreased thereafter.

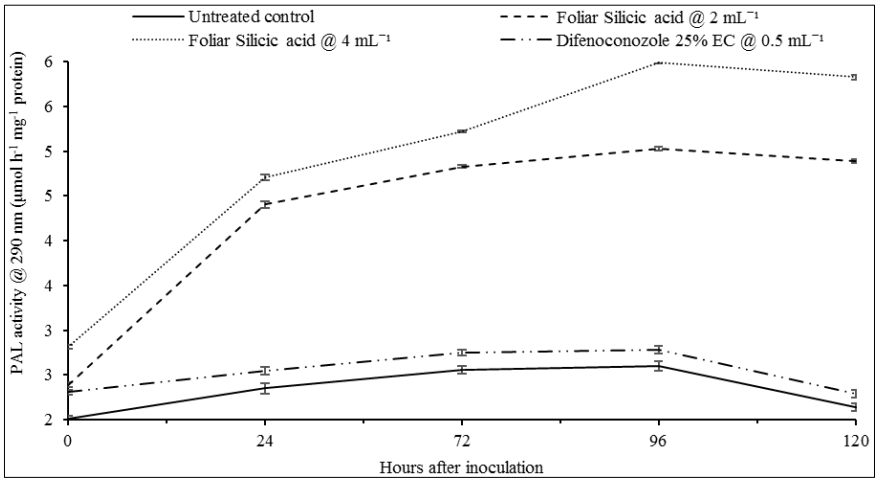

Fig 5: Changes in activity of phenylalanine ammonia-lyase (PAL) post application of foliar silicic acid in leaves of tomato plants inoculated with *O. Neolycopersici*. The values are mean of five replications. Vertical lines represent standard errors (n=5)

The β-1, 3 glucanase activity was significantly higher in silicic acid applied plants compared to untreated control plants (Fig.6). Among the treatments, silicic acid applied @ 4 ml L^{-1} showed the highest level of β-1, 3 glucanase activity (10.75 µmol min^{-1} mg of $protein^{-1}$ at 96 hai) compared silicic acid applied @ 2 ml L^{-1} (10.28 µmol min^{-1} mg of $protein^{-1}$ at 96 hai). Further, the β-1, 3 glucanase activity was observed increasing till 96 hai and decreased thereafter in both silicic acid and fungicide treated plants, whereas it was observed decreasing from 72 hai itself in untreated plants subsequent to inoculation with *O. Neolycopersici*.

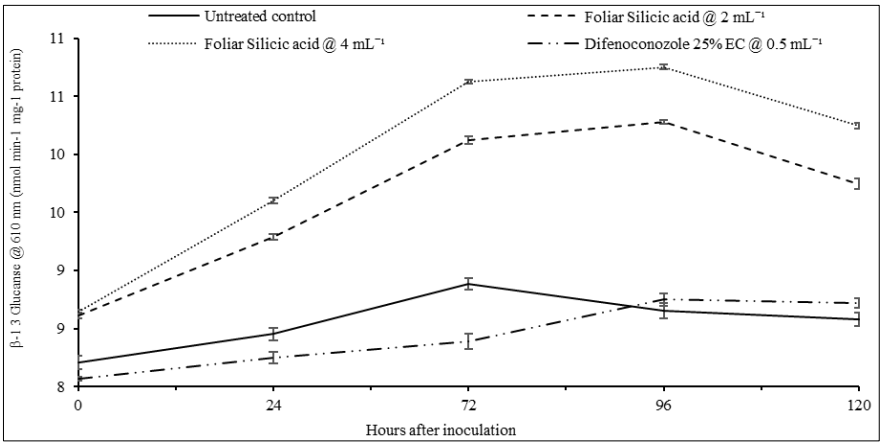

Fig 6: Changes in the activity of β-1, 3 glucanase in silicic acid applied tomato plants inoculated with *O. neolycopersici*. The values are mean of five replications. Vertical lines represent standard errors (n=5).

The activity of chitinase increased during subsequent inoculation with *O. Neolycopersici* and reached its maximum at 96 hai in silicic acid applied plants compared to foliar silicic acid non-applied plants (Fig. 7). Among the treatments, foliar application of silicic acid @ 4 ml L^{-1} showed the highest level of chitinase activity (235.2 nmol min^{-1} mg of $protein^{-1}$ at 96 hai) followed by foliar silicic acid @ 2 ml L^{-1} (195.30 nmol min^{-1} mg of $protein^{-1}$ at 96 hai). Further, chitinase activity increased till 96 hai and decreased thereafter, whereas it was observed decreasing from 72 hai itself in fungicide treated and untreated plants subsequent to inoculation of *O. Neolycopersici*.

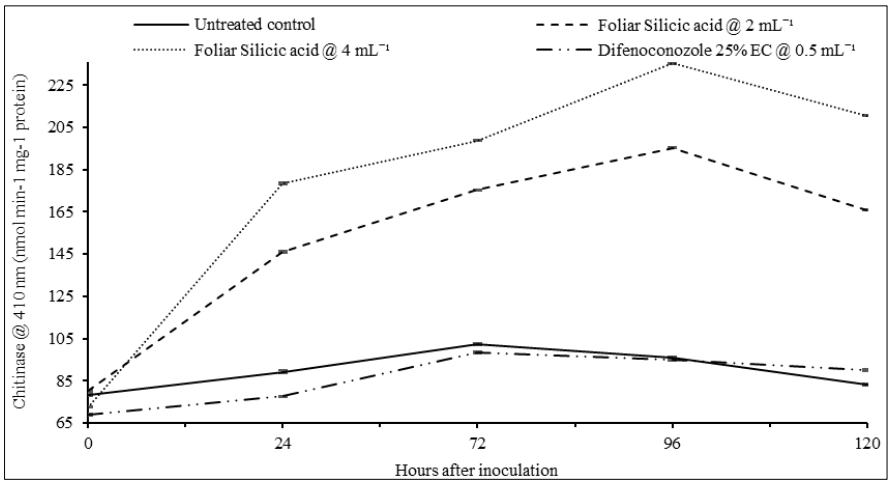

Fig 7: Activity of chitinase during post application of foliar silicic acid in leaves of tomato plants inoculated with *O. Neolycopersici*. The values are mean of five replications. Vertical lines represent standard errors (n=5).

4. Discussion

Powdery mildew in tomato is an economically important disease due to its direct negative impact on plant growth and yield. In the present study, results showed that the application of silicic acid at 2 and 4 mL^{-1} as foliar spray decreased the powdery mildew disease severity (*O. Neolycopersici*) and lowest disease progress in tomato upon artificial inoculation (Table 1). In addition to disease reduction, it was also enhanced the fresh shoot weight and dry shoot weight of tomato plants, and chlorophyll content. The physiological and nutritional effects of silicon in plants has been well documented for years. For example, silicon can increase both growth and mechanical properties of the plant thereby enhance resistance to biotic or abiotic stresses (Liang *et al*. 2005a; Liang *et al*. 2005b; Liang *et al*. 2007; Ma 2004) [20-21, 23]. The results further supported the fact that silicon had a positive role not only on disease resistance to powdery mildew, but also on plant growth and yield.

Silicon application could also able to enhance host resistance against fungal infection (Rodrigues *et al*. 2003b; Sun *et al*. 2010; Liang *et al*. 2005a; Cherif *et al*. 1992) [37, 38, 23, 6]. Winslow *et al*. (1997) [42] reported that there was a negative correlation between silicon concentration in plant and severity of plant disease. There are recent hypotheses concerning the role of silicon in suppression of infection by fungal pathogens. Additional accumulation of silicon beneath the cuticle layer is one of the well-known and documented mechanism involved in silicon mediated host plant resistance (Kim *et al*. 2002) [18]. This is most effective in preventing invasion of fungal pathogen and also in reducing the effect of degradation enzymes of secreted by the fungal pathogens on cell walls of tomato plants. In the present study, foliar application of silicic acid resulted in higher shoot silicon content and recorded lowest disease severity in treated plant compare to untreated control plants. This indicates that the potentiality of silicic acid as a promising control measure for powdery mildew disease in tomato plants. Similar results reported from the studies conducted against blast disease in rice and powdery mildew in cucumber when high concentrations of fungal inoculum were inoculated onto the test plant (Sun *et al*. 2010; Liang *et al*. 2005a) [38, 23] and also found that, the silica deposited on the leaf cell walls could able to restrict the penetration of fungal hypha and thus protect plants from fungal infection.

However, many studies revealed that plants treated with silicon responded more promptly to fungal infection by increased synthesis of phenolic compounds, and the activity of POD, PPO, PAL, β-1,3-glucanase (Liang and Sun 2002; Dann and Muir 2002; Piperno 2006; Waewthongraka *et al*. 2015) [22, 9, 30, 40] and chitinase (Cherif *et al*. 1994; Dallagnol *et al*. 2011) [5, 7]. In this study, peroxidase, polyphenoloxidase, phenylalanine ammonia-lyase, β-1, 3 glucanase and chitinase activities were significantly higher in silicon applied plants as compared to untreated control plants (Fig. 3 to 7). Elevated production of these defence-related enzymes helped to enhance the resistance in tomato plants against powdery mildew leading to reduction in disease severity and progression besides playing role in essential metabolism of tomato plants. Chitinase and β 1,3-glucanase found having potential to hydrolyse the major components of fungal cell wall *viz*., chitin and β 1,3 glucan activity, respectively leading to inhibition of growth of several fungi. Our study recognized the presence of pre-formed chitinases in silicic acid applied plants. This observation is confirmed by the findings of Adikaram *et al*. (2010) [1] who observed pre-formed chitinases in mango fruit peel. The results presented here above are clearly represent the induction of biochemical changes in tomato plant post silicon application are playing significant role in inhibition of powdery mildew pathogen by inducing host plant resistance.

Foliar applied silicic acid played a active physiological role in enhancing resistance besides direct physical protection against infection process by powdery mildew pathogen, *O. Neolycopersici* in tomato by depositing below cell wall. Therefore, foliar application of foliar silicic acid @ 4 mL^{-1} might be an effective alternate option for managing powdery mildew disease of tomato by enhancing resistance to pathogen *O. Neolycopersici*.

5. Conclusion

In this study, we applied silicic acid @ 4 mL L^{-1} and silicic acid @ 2 mL L^{-1} to evaluate their antifungal activity against powdery mildew disease of tomato under green-house condition. The reduced disease severity and progression of disease on tomato leaves has been attributed to significant increase in activities of defence enzymes *viz*., peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, β-1, 3 glucanase and chitinase upon foliar application of silicic acid

@ 4 mL L^{-1} and 2 mL L^{-1}. This clearly illustrate that, foliar application of silicic acid on tomato leaves conferred the resistance against powdery mildew pathogen. This different concentration of silicon source has the potential to be used as an antimicrobial agent in antifungal remediation or as an additive in conventional formulations.

6. Acknowledgement

The author is thankful to Department of Plant Pathology, Department of Soil Science and Agricultural Chemistry, UAS, GKVK, Bengaluru, and Department of Science and Technology, Government of India for having awarded an INSPIRE Fellowship to the first author for pursuing doctoral degree program. Also, disease severity data has been presented previously and provide details of this.

7. Conflict of interest statement

No conflict of interest

8. References

1. Adikaram NKB, Karunanayake LC, Abayasekara CL.The role of pre-formed antifungal substances in the resistance of fruits to postharvest pathogens. In: Gullino L, Prusky D (eds) Postharvest pathology. Springer New York; c2010. p. 1-13.
2. Belanger RR, Benhamou N, Menzies JG. Cytological evidence of an active role of silicon in wheat resistance to powdery mildew (Blumeria graminis f. sp. tritici). J Phytopathol. 2003;93:402-12.
3. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-254.
4. Campbell CL, Madden LV. Introduction to plant disease epidemiology. John Wiley Sons, New York; c1990. p. 532.
5. Cherif M, Asselin A, Belanger RR. Defense responses induced by soluble silicon in cucumber roots infected by Pythium spp. Phytopathology. 1994 Mar 1;84(3):236-242.
6. Cherif M, Benhamou N, Menzies JG, Belanger RR. Silicon induced resistance in cucumber plants against Pythium ultimum. Physiol. Mo. Plant Pathol. 1992;41(69):411-425.
7. Dallagnol LJ, Rodrigues FA, Damatta FM, Mielli MV, Pereira SC. Deficiency in silicon uptake affects cytological, physiological, and biochemical events in the rice-Bipolaris oryzae interaction. Phytopathol. 2011 Jan;101(1):92-104.
8. Dann EK, Deverall BJ. Activation of systemic disease resistance in pea by an avirulent bacterium or a benzothiadiazole, but not by a fungal leaf spot pathogen. Plant Pathol. 2000;49:324-332.
9. Dann EK, Muir S. Peas grown in media with elevated plant-available silicon levels have higher activities of chitinase and β-1,3glucanase are less susceptible to a fungal leaf spot pathogen and accumulate more foliar silicon. Australasian Plant Pathol. 2002 Mar;31:9-13.
10. Datnoff LE, Rodrigues FA, Seebold KW. Silicon and plant disease. In: Datnoff LE, Elmer WH, Huber DM, eds. Mineral Nutrition and Plant Disease. St Paul, MN, USA: APS Press; c2007. p. 233-46.
11. Epstein E. Silicon. Annu Rev Plant Physiol Plant Mol Biol. 1999;50:641-664.
12. Fauteux F, Remus-Borel W, Menzies JG, Belanger RR. Silicon and plant disease resistance against pathogenic fungi. FEMS Microbiol Let. 2005 Aug 1;249(1):1-6.
13. Ghanmi D, McNally DJ, Benhamou N, Menzies JG, Belanger RR. Powdery mildew of Arabidopsis thaliana: a pathosystem for exploring the role of silicon in plant-microbe interactions. Physiol Mol Plant Pathol. 2004 Apr 1;64(4):189-99.
14. Harman GE, Hayes CK, Lorito M, Broadway RM, Pietro AD, Peterbauer C, *et al*. Quitinolytic enzymes of Trichoderma harzianum: purification of quitobiosidase and endoquitinase. Phytopathol. 1993 Mar 1;83(3):313-318.
15. Inada K. Spectral ratio of reflectance for estimating chlorophyll content of leaf. Japanese J Crop Sci. 1985;54:261-265.
16. Jones H, Whipps JM, Gurr SJ. The tomato powdery mildew fungus Oidium neolycopersici. Mol Plant Pathol. 2001;2:303-309.
17. Jones LHP, Handreck HA. Silica in soils, plants and animals. Adv Agron. 1967;19:107-149.
18. Kim SG, Kim KW, Park EW, Choi D. Silicon-induced cell wall fortification of rice leaves: a possible cellular mechanism of enhanced host resistance to blast. Phytopathology. 2002 Oct;92(10):1095-1103.
19. Kiss L, Cook RTA, Saenz GS, Cunnington JH, Takamatsu S, Pascoe I, *et al*. Identification of two powdery mildew fungi, Oidium Neolycopersici sp. Nov and O. lycopersici, infecting tomato in different parts of the world. Mycol Res. 2001;105:684-697.
20. Liang Y, Sun W, Zhu YG, Christie P. Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: A review. Environ Pollut. 2007;147:422-428.
21. Liang YC, Si J, Romheld V. Silicon uptake and transport is an active process in Cucumis sativus L. New Phytologist. 2005b;167:797e804.
22. Liang YC, Sun WC. Resistance of cucumber against anthracnose induced by soluble silicon and inoculated Colletotrichun lagenarium. Scintia Agricultura Sinica. 2002;35:267-271.
23. Liang YC, Sun WC, Si J, Roomheld V. Effect of foliar- and root-applied silicon on the enhancement of induced resistance in Cucumis sativus to powdery mildew. Plant Pathol. 2005a;54:678-685.
24. Ma JA. Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. Soil Sci Plant Nutrit. 2004;50:11-18.
25. MA JF, Takahashi E. In: Soil, fertilizer and Plant silicon research in Japan. Elsevier Amsterdam; c2002. p. 1-20.
26. McGrath MT. Fungicide resistance in cucurbit powdery mildew: experiences and challenges. Plant Dis. 2001;85:236-245.
27. Menzies JG, Ehret DL, Glass ADM, Helmer T, Koch C, Seywerd F. Effects of soluble silicon on the parasitic fitness of Sphaerotheca fuliginea on Cucumis sativus. Phytopathol. 1991b;81:84-89.
28. Menzies JG, Ehret DL, Glass ADM, Samuels AL. The influence of silicon on cytological interactions between Sphaerotheca fuliginea and Cucumis sativus. Physiol Mol Plant Pathol. 1991a;39:403-14.
29. Narayanaswamy C, Prakash NB. Evaluation of selected extract ants for plant-available silicon in rice soils of

Southern India. Commun Soil Sci Plant Anal. 2010;41(8):977-989.
30. Piperno DR. The production, deposition, and dissolution of phytoliths. Phytoliths: a comprehensive guide for archaeologists and paleoecologists. Piperno, D. R., (ed); c2006. p. 5-22.
31. Prakash NB, Chandrashekar N, Mahendra C, Patil S, Thippeshappa GN, Laane HM. Effect of foliar spray of soluble silicic acid on growth and yield parameters of wetland rice in hilly and coastal zone soils of Karnataka, South India. J Plant Nutri. 2011;34:1883-1893.
32. Qin GZ, Tian SP. Enhancement of biocontrol activity of Cryptococcus laurentii by silicon and the possible mechanisms involved. Phytopathology. 2005;95:69-75.
33. Remus-Borel W, Menzies JG, Belanger RR. Silicon induces antifungal compounds in powdery mildew-infected wheat. Physiol Mol Plant Pathol. 2005;66:108-115.
34. Roberts WK, Selitrennikoff CP. Plant and bacterial chitinases differ in antifungal activity. J Gen Microbiol. 1988;134:169-176.
35. Rodrigues FA, Benhamou N, Datnoff LE, Jones JB, Belanger RR. Ultrastructural and cytochemical aspects of silicon-mediated rice blast resistance. Phytopathology. 2003a;93:535-546
36. Rodrigues FA, McNally DJ, Datnoff LE, Jones JB, Labbe C, Benhamou N, *et al*. Silicon enhances the accumulation of diterpenoid phytoalexins in rice: a potential mechanism for blast resistance. Phytopathology. 2004;94:177-183.
37. Rodrigues FA, Vale FXR, Datnoff LE, Prabhu AS, Korndorfer GH. Effect of rice growth stages and silicon on sheath blight development. Phytopathology. 2003b;93:256-261.
38. Sun WC, Zhang J, Fan Q, Xue G, Li Z, Liang Y. Silicon-enhanced resistance to rice blast is attributed to silicon-mediated defence resistance and its role as physical barrier. Eur J Plant Pathol. 2010;128:39-49.
39. Sundarraj N, Nagaraju S, Venkataramu MN, Jagannath ML. Design and analysis of field experiments. University of Agricultural Sciences Bangalore India; c1972.
40. Waewthongraka W, Pisuchpenb S, Leelasuphakul W. Effect of Bacillus subtilis and chitosan applications on green mold (Penicilium digitatum Sacc.) decay in citrus fruit. Postharvest Biol and Technol. 2015;99:44-49.
41. Wheeler BEJ. An introduction to plant diseases, John Wiley and Sons Ltd. London; c1969. p. 301.
42. Winslow MD, Okada K, Correa-Victoria F. Silicon deficiency and the adaptation of tropical rice ecotypes. Plant Soil. 1997;188:239-248.