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In vitro efficacy of soluble silicon against sesame (*Sesamum indicum* L.) charcoal rot disease caused by *Macrophomina phaseolina* (Tassi) Goid

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Abstract
Charcoal rot caused by *Macrophomina phaseolina* is one of the important diseases of sesame causing considerable yield loss globally. The present study investigated the effect of four different silicon *viz*., magnesium silicate, potassium silicate, sodium silicate and sodium meta silicate on the growth of charcoal rot pathogen *M. phaseolina* under *in vitro*. Results showed that the mycelial growth of the *M. phaseolina* was significantly inhibited on the PDA medium amended with silicates *viz*., potassium silicate (5%) and sodium silicate (0.2%), followed by sodium meta silicate (0.1%) over the control.

Keywords: *Sesamum indicum*, charcoal rot, *Macrophomina phaseolina*, silicon, poison food technique

Introduction
Sesame (*Sesamum indicum* L.) is one of the important oil seed crops in India. In India sesame crop is grown in an area of 19.47 lakh hectares with a production of 8.66 lakh tons. In Tamil Nadu the crop covers over an area of 28.23 thousand hectares with a production of 10.28 thousand tons (Indiastat, 2017) [5]. Sesame seeds are rich in protein (17 – 19%) and carbohydrate (16 – 18%) and have high amount of unsaturated fatty acids (Kiranmayi, 2007) [8]. The crop was infected by many fungal and bacterial diseases. Among the fungal diseases, charcoal rot caused by *M. phaseolina* is one of the important diseases causing severe yield loss ranging from 50 to 60 per cent (Dinakaran and Mohammed, 2001) [3]. Many studies reported that silicon (Si) applied plants expressed high yield and vigour growth, while main role of silicon is increased résistance against pest and diseases due to physical and biochemical mechanism. Maekawa *et al.,* (2003) [11] reported that the hyphal growth of rice blast fungus was very slow on agar plates containing soluble potassium silicon. Bekker *et al.,* (2006) [1] found that liquid potassium silicate inhibit the mycelial growth of *A. solani, C. gloeosporoides, C. lunata, F. solani, P. capsici, S. rolfsii under in vitro* condition. Shen *et al.,* (2010) [15] reported that potassium silicate control soil borne phytopathogenic fungi *in vitro* and observed inhibition of *Rhizoctonia solani, Pestalotiopsis clavispora* and *F.oxysporum* f. sp. *fragariae*. Fayadh and Aledani (2011) [4] tested different concentration of silicon elements (30, 200, 500 ppm) against *R. solani,* who have found that the mycelial growth of fungus gradually inhibited at high concentration of silicon application. Khan *et al.,* (2013) [7] reported that sodium silicate was effectively reduced the mycelial growth of *M. phaseolina*. With a view to understand the role of silicon in sesame charcoal rot disease control, *in vitro* experiment was performed to study the effect of different silicon sources on the mycelial growth of *M. phaseolina* in sesame.

Materials and methods
Collection and isolation of *Macrophomina phaseolina*
A survey was conducted in major sesame growing areas of Tamil Nadu. The typical charcoal rot symptoms showing plants were collected (Mohanapriya *et al.,* 2017) [12]. Diseased roots of sesame plants showing characteristic charcoal rot symptoms were used for the isolation of pathogen by tissue segment method. Disease incited sesame roots were cut into small pieces and sterilized with 70% ethanol for 1 min and washed thrice with sterilized water. Then the root bits were placed on sterilized potato dextrose agar (PDA) medium and incubated at room temperature for seven days. The pure cultures of ten isolates were obtained by single hyphal tip method and stored at 4°C for further study (Raja Mohan and Balabaskar, 2012) [14].

Pathogenicity test

The ten purified isolates of *M. phaseolina* were tested for pathogenicity under *in vivo*. The isolates of *M. phaseolina* were mass multiplied in sand-maize medium (1900 g of sand and 100 g of maize powder (19:1) was mixed, moistened with 400 ml of water kg^{-1}) and then packed in polythene bags. These bags were sterilized in autoclave at 1.4 kg/cm^2 pressure for 20 minutes. Each bags were inoculated with nine mm culture disc of 7 days old *M. phaseolina* fugal culture and incubated for 15 days at room temperature (28 ± 2°C), till the sclerotia turned black indicating maturity. Then the sand maize medium containing fully grown fungal culture (*M. phaseolina*) was mixed thoroughly with sterilized soil and used for pathogenicity test.

In vitro* efficacy of silicon on the growth of *M. phaseolina

The poison food technique (Shravelle, 1961) [16] was followed to study the antifungal activity of four different sources of silicon *viz*., potassium silicate, magnesium silicate, sodium silicate and sodium meta silicate. The required quantity *viz*., 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4 and 5 per cent of four different sources of silicon were added separately into 100 ml of autoclaved PDA medium. Later 20 ml of the poisoned media was poured into sterilized Petri plates and allowed to solidify at room temperature. Then nine mm of seven days old actively growing fungal disc of *M. phaseolina* was placed onto center of the poisoned medium. The Petri plates containing PDA medium without silicon served as control. Three replications were maintained for each treatment. Then the plates were incubated at room temperature for seven days and the radial mycelial growth was measured when the fungus attained maximum growth in the control plates and per cent inhibition of mycelial growth was calculated by using the following formula

$$\text{Inhibition rate (\%)} = \frac{C-T}{C} \times 100$$

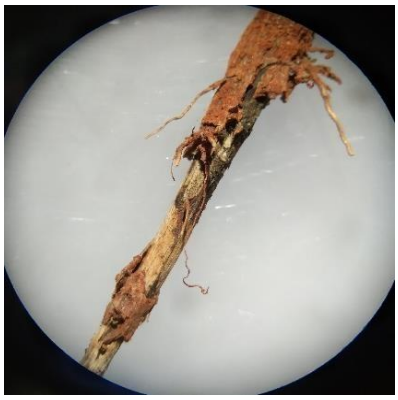
C = Radial growth of fungus in the control plates

T = Radial growth of fungus in silicon treated plates

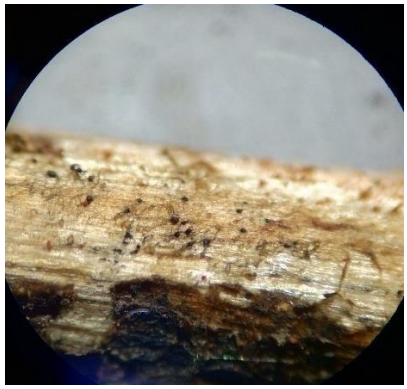
Results and discussion

Collection and Isolation of the pathogen

The data obtained during the survey conducted in major sesame growing areas of Tamil Nadu was presented in the table 1. Isolates of *M phaseolina* was isolated from diseased plants showing the typical symptoms of root bark shredding and presence of sclerotia on the root surface (Fig: 1.a & b) using PDA medium. The fungi were observed under microscope, identified as *M. phaseolina* based on the morphological characters of mycelium and sclerotial structures. The isolated fungus produced dark, black coloured fluffy mycelium and irregular sclerotia on the PDA medium. (Fig: 2.a & b) Purified fungal culture sent to the Indian Type Culture Collection, PUSA, IARI, also confirmed the isolated fungal culture as *M. phaseolina* (I.D NO.10,024.19).

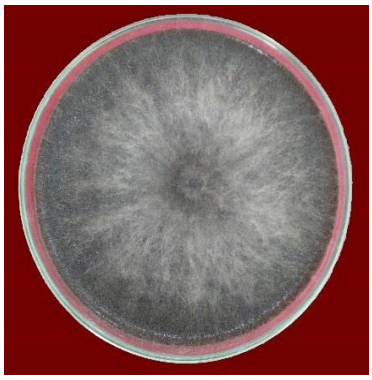


a. Bark shredding symptom on root

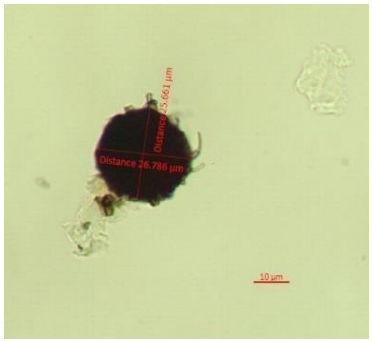


b. Sclerotia on infected root

Fig 1: Symptoms of sesame charcoal rot



a. Mycelium



b. Sclerotia

Fig 2: Morphological characters of *M. phaseolina*

Pathogenicity test

The isolated pathogen was proved to be pathogenic on sesame plants. Inoculated plant produced rotting and root bark shredding symptoms and sclerotia on the root surface. No such symptoms were observed on the uninoculated control plants. Among the different isolates tested, the isolate from Virudhachalam was found to be the most virulent in inducing the charcoal rot symptoms (Fig.3).

Fig 3: Pathogenicity test

Effect of silicon on the mycelial growth of *M. phaseolina* under *in vitro*

Different silicon sources were tested at different concentrations by poison food technique showed an inhibitory effect on *M. phaseolina* over untreated control. Inhibition of *M. phaseolina* was found increased with increase in the concentration of silicon sources tested in the medium (Table 2, Fig. 4.). Among the silicon sources, 0.1 and 0.2 per cent of sodium meta silicate showed maximum inhibition (Table 2, Fig.4.d) (81.1% &100 % respectively) followed by sodium silicate (0.2 % & 0.3 %) (Table 2, Fig 4.c). Potassium silicate (5%) completely inhibited the mycelial growth (Table 2, Fig 4.b). Magnesium silicate did not show any inhibitory effect on the mycelial growth of the *M. phaseolina.* In the present study, the inhibition of *M. phaseolina* by different sources of silicon was mainly due to the antifungal activity of silicon tested.

The results obtained were similar to Kaiser *et al.,* (2005) [6], who reported that 40ml and 80ml of potassium silicate amended PDA medium completely inhibited the hyphal growth of *Phytophthora cinnamomi*, *Phytophthora capsici*, *Sclerotinia sclerotiorum*, *S. rolfsii*, *Pythium* sp, *Mucorpusillus*, *Drechslera spp*, *Fusarium oxysporum*, *F. solani*, *A. solani*, *C. coccodes*, *Verticillium fungicola*, *C. lunata* and *Stemphylium herbarum.*

Li *et al.,* (2009) [9] reported that sodium silicate inhibited F. *sulphureum* mycelial growth and spore germination. He also examined the sodium silicate at different concentrations (0, 25, 50, 100, 200 mM) and the maximum inhibition was recorded at 200mM. He concluded that increase in silicon concentration increases the inhibitory effect on mycelial growth and observed silica treated hyphae and spore under scanning electron microscopy, where he found morphological changes like mycelial sparsity and asymmetry, hyphal swelling, curling and cupping.

Bi *et al.,* (2006) [2] reported that 100mM sodium silicate completely inhibited the mycelial growth of *Alternaria alternata*, *Fusarium semitectum* and *Trichothecium roseum* under *in vitro* condition. Rachniyom and Jaenaksorn (2008) [13] reported that sodium and potassium silicates significantly reduced mycelial growth and sporangial production of *Pythium aphanidermatum.*

Liu *et al.,* (2009) [10] found that the mycelial growth and spore germination of *Fusarium* spp was inhibited by treatment with silicate. The effect of potassium silicate was studied against *R. solani*, *F. oxsporum* f. sp. *fragariae* and *Pestalotiopsis clavispora* (Shen *et al*., 2010) [15].

Fig 4.a.: *In vitro* efficacy of Magnesium silicate amended PDA on the growth of *M. phaseolina*

1	Magnesium silicate - 0.1%	6	Magnesium silicate - 1%
2	Magnesium silicate - 0.2%	7	Magnesium silicate - 2%
3	Magnesium silicate - 0.3%	8	Magnesium silicate - 3%
4	Magnesium silicate - 0.4%	9	Magnesium silicate - 4%
5	Magnesium silicate - 0.5%	10	Magnesium silicate - 5%

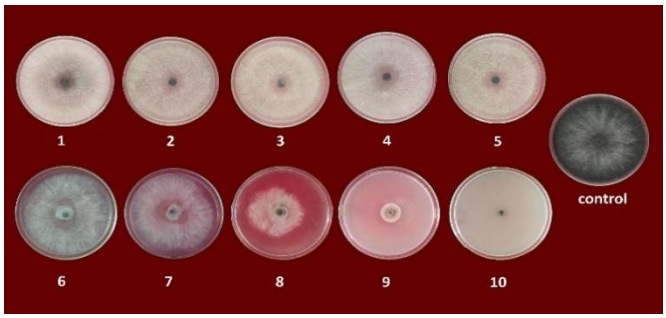


Fig 4.b.: *In vitro* efficacy of potassium silicate amended PDA on the growth of *M. phaseolina*

1	Potassium silicate - 0.1%	6	Potassium silicate - 1%
2	Potassium silicate - 0.2%	7	Potassium silicate - 2%
3	Potassium silicate - 0.3%	8	Potassium silicate - 3%
4	Potassium silicate - 0.4%	9	Potassium silicate - 4%
5	Potassium silicate - 0.5%	10	Potassium silicate - 5%

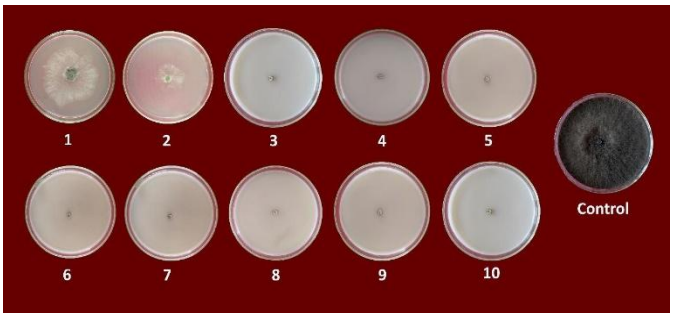


Fig 4.c.: *In vitro* efficacy of Sodium silicate amended PDA on the growth of *M. phaseolina*

1	Sodium silicate - 0.1%	6	Sodium silicate - 1%
2	Sodium silicate - 0.2%	7	Sodium silicate - 2%
3	Sodium silicate - 0.3%	8	Sodium silicate - 3%
4	Sodium silicate - 0.4%	9	Sodium silicate - 4%
5	Sodium silicate - 0.5%	10	Sodium silicate - 5%

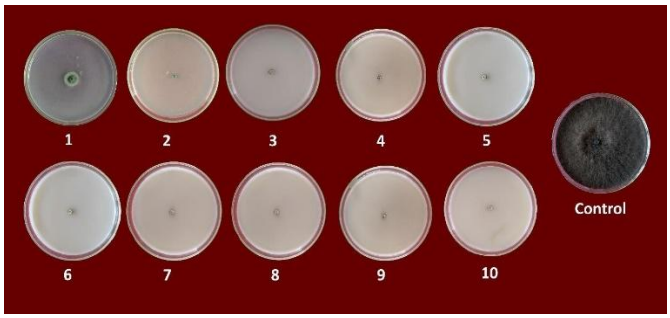


Fig 4.d.: *In vitro* efficacy of Sodium meta silicate amended PDA on the growth of *M. phaseolina*

1	Sodium meta silicate - 0.1%	6	Sodium meta silicate - 1%
2	Sodium meta silicate - 0.2%	7	Sodium meta silicate - 2%
3	Sodium meta silicate - 0.3%	8	Sodium meta silicate - 3%
4	Sodium meta silicate - 0.4%	9	Sodium meta silicate - 4%
5	Sodium meta silicate - 0.5%	10	Sodium meta silicate - 5%

Table 1: Survey for assessing the charcoal rot disease incidence in sesame

S. No	Place of collection	Districts	Isolate code	Geo co ordinate	
				Latitude	Longitude
1	Pulavankadu	Thanjavur	Mp 1	100 55N	790 32E
2	Pulavankadu	Thanjavur	Mp 2	100 57N	790 26E
3	Neivasal	Thanjavur	Mp 3	100 15N	780 67E
4	Karakottai	Thanjavur	Mp 4	100 10N	790 19E
5	Aathikkottai	Thanjavur	Mp 5	100 45N	790 34E
6	Sathyam	Cuddalore	Mp 6	110 53N	790 22E
7	Virudhachalam	Cuddalore	Mp 7	110 52N	790 34E
8	Vilankattur	Cuddalore	Mp 8	110 52N	790 24E
9	Chithoor	Cuddalore	Mp 9	110 59N	790 63E
10	Agricultural College and Research Institute	Madurai	Mp 10	100 03N	780 33E

Table 2: *In vitro* efficacy of soluble silicon on the growth of *M. phaseolina*

Concentration (%)	Mycelial growth (mm)				Percent of inhibition over control			
	Magnesium silicate	Potassium silicate	Sodium silicate	Sodium meta silicate	Magnesium silicate	Potassium silicate	Sodium silicate	Sodium meta silicate
0.5	90	90	59	14	0.0	0.0	34.44	84.44
0.6	90	90	19	0.0	0.0	0.0	78.99	100
0.7	90	90	0.0	0.0	0.0	0.0	100	100
0.8	90	90	0.0	0.0	0.0	0.0	100	100
0.9	90	90	0.0	0.0	0.0	0.0	100	100
1	90	79	0.0	0.0	0.0	12.22	100	100
2	90	69	0.0	0.0	0.0	23.33	100	100
3	90	55	0.0	0.0	0.0	38.88	100	100
4	90	17	0.0	0.0	0.0	81.11	100	100
5	90	00	0.0	0.0	0.0	100	100	100
Control	90	90	90	90	0.0	0.0	0.0	0.0
CD 5%	NS	2.18	1.08	0.29				
SEd		1.07	0.53	0.14				

Conclusion

There are numerous reports available for the control of various phytopathogens both under *in vitro* and *in vivo* conditions by different silicon sources. Results obtained from the present study revealed that the silicon exhibited antifungal activity against the charcoal rot fungi *M. phaseolina*. In future, the use of silicon along with fertilizers not only increases the plant growth but also increases the resistance in plants against diseases.

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