Int.J.Curr.Microbiol.App.Sci (2021) *10*(03): 233-240

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 10 Number 03 (2021)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1003.031

Invitro Investigation of Potassium Silicate on Mycelial Growth of *Fusarium* Isolate

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Keywords

Potassium silicate, Mycelial growth, Fusarium

Article Info

Accepted:
04 February 2021
Available Online:
10 March 2021

A B S T R A C T

Mango malformation (MM) is an ambiguous mango disease with tremendous economic importance throughout the mango growing regions. Several studies reported that *Fusarium* species especially *Fusarium mangiferae* is associated with mango malformation. Silicon is an essential element, which plays a vital role in plant microbial interaction. It deposits under the cuticle and acts as a physical barrier that protects the plant from fungal infection. The present study was conducted to observe the in-vitro application of various silicon (potassium silicate) concentrations on mycelial growth of *Fusarium* isolates. The mycelial growth of *Fusarium* was significantly inhibited on PDA medium amended with various concentration of potassium silicate. Results suggested that inhibition of mycelial growth was dose dependent with 100% inhibition at 5% (pH 5.8, 6, 6.2, 6.6, 9.6 and 11.5) and 2% (at pH 9.6 and 11.7) soluble potassium silicate per litre of agar. The inhibition of mycelial growth of *Fusarium* species was found to be pH dependent and was more in basic pH as compared to acidic pH.

Introduction

Mango is one of the finest fruits with great religious and cultural significance and acknowledged as "king of fruits". Out of 1500 varieties of mango, 1200 are found in India (Krishnan *et al.,* 2009). India occupies the first rank in an area of about (2.209 hectares) and production about 21.378 thousand million tonnes/hectares in the world (NBH, 2019). Although the area and production of mango are high, still productivity is very low (6.3million tonnes/hectare). It may be due to various biotic and abiotic factors which affect its growth and productivity. Among these factors mango malformation is a severe threat to its productivity, leading to a reduction of about 40-80% per year (Kumar and Misra, 2016; Raj *et al.,* 2017).

According to various reports, malformation is a fungal disease caused by *Fusarium* species and leads to irregular vegetative growth and inflorescence, results in direct reduction in fruit yield (Kvas *et al.,* 2008; Kumar *et al.,* 2016). Silicon is the second most essential element in the lithosphere (27.70%) and it is a major inorganic constituent of plants, in

which silicon content varies from 0.1% to 10% of dry matter (Epstein 1994). Soluble silicates are well-established synthetic chemicals that have been used in a range of applications in humans and the environment, such as soap production, soil remediation, water treatment and agriculture (Baehr and Koehl, 2007). Both potassium and sodium silicates have been classified as safe by the Food and Drug Administration (21CFR 182.90 and 21CFR 182.1711). Silicates act as bioactive elements and have beneficial effects in physiological mechanisms (Epstein 2001). The role of silicon in defense mechanisms against various pathogens in plants was recorded in 1997 when various researchers identified that increase in silicon concentration reduces pathogen infection (Atta *et al.,* 2019). Silicate has been reported as a controlling agent for various plant diseases of pear (Spotts and Cervantes, 1989), apple (Biggs 2004), muskmelon (Bi *et al.,* 2006), strawberry (Kanto *et al.,* 2006), and sweet cherry fruits (Qin and Tian, 2005). Si also fights against various pathogens, which demonstrate a wide range of lifestyles and different modes of action (Van Bockhaven *et al.,* 2013). Growth of *Monilinia fructicola* (G.Wint) which causes brown rot of peach fruit can be reduced upto 65% by application of potassium silicate (Biggs *et al.,* (1997). In vitro inhibition of mycelial growth of various phytopathogenic fungi has also been reported when grow on potassium silicate amended PDA media (Bekker *et al.,* 2006).

To better characterise the role of silicates in controlling the mango malformation, in vitro experiment was performed to determine the effect of various concentration of potassium silicate on mycelial growth, of *Fusarium* species which causes malformation disorder. The inhibitory effect of potassium silicate treatment on *Fusarium isolate* was also investigated.

Materials and Methods

Potassium silicate used for this experiment is of CDH (Central drug house) grade. pH of concentrated potassium silicate solution is 12.7, which when added to PDA media increases pH of media (Bekker *et al.,* 2006).

PDA media preparation

200g of peeled potatoes were cut into fine pieces and boiled in 800 ml of distilled water for 30 mins. The potato infusion was filtered through a muslin cloth. In the filtrate, 20 g dextrose was added and dissolved in the beaker using magnetic stirrer. The pH of media was adjusted to 5.6±0.1 with the help of 0.1N HCl and 0.1N NaOH and made up to 1L. Now broth was transferred in the 1L conical flask and 20 g of agar added in it. Flasks were then plugged with cotton and autoclaved at 121oC and 15 psi for 20 minutes. Potassium silicate was added to the PDA media after cooling. PDA media having pH 5.6 upon adding different concentration of potassium silicate (0.25%, 0.50%, 0.75%, 1%, 2% and 5%) per litre of agar, the pH is raised to (5.8, 6, 6.2, 6.6, 9.6 and 11.5) respectively. The potassium silicate-agar solution was mixed with magnetic stirrers to ensure even distribution of potassium silicate. Then, it was poured into Petri dishes and incubated for seven days at room temperature to ensure no contamination occurs.

Pathogen Isolation and identification

Pathogen was isolated from malformed tissues of two mango cultivars Dashehari and Amrapali grown at Horticultural Research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar. According to previously described procedures, for obtaining a pure culture of *Fusarium* a single spore culture and hyphal tip isolation technique were used (Goh, 1999; Britz *et al.,*

2002). 5mm long malformed tissue was sterilized for 5 min in a 0.1% solution of HgCl2. After that, tissue was rinsed three times with double distilled water and kept on PDA (Potato dextrose agar) plates or slants containing an antibacterial streptomycin sulphate solution. Thereafter, for proper growth these plates and slants were kept in BOD incubator at 27oC for 4-5 days. The identification of *Fusarium* isolates was made based on macro and microconidia and the presence of purple orange colour on PDA media. The pure culture was kept at 4oC for future use.

Invitro measurement of mycelial growth of *Fusarium* isolate

The effect of potassium silicate on mycelial growth of *Fusarium* isolate was assayed according to Yao and Tian (2005). The mycelial disks (5mm in diameter) from 2 week old fungal cultures were placed in the centre of the petri plates (9 cm in diameter) with 20ml of PDA containing different concentration of potassium silicate (0, 0.25%, 0.50%, 1%, 2% and 5%). To observe effect of different pH (5.8, 6, 6.2, 6.6, 9.6 and 11.5) on the mycelial growth, the pH of PDA media was adjusted with 0.1N HCL and 0.1N NaOH. Higher pH is known to supress the fungal growth. All plates inoculated with isolates of *Fusarium* were kept in a BOD incubator at 270C. Plates with sterile water were used as control. The mycelial growth was determined by measuring colony diameter one week after inoculation. Each treatment was replicated three times.

Antifungal activity assay

Silicon ameliorated agar plates were inoculated with each fungus by placing a 5 mm diameter disc from an actively growing culture in the centre of each plate. Three replicates were used per treatment. Fungi were also grown on non-ameliorated PDA (i.e. with no silicon) as a control. All fungi were incubated at 27°C for seven days in the dark. Fungal growth (colony diameter) was measured and percentage inhibition calculated according to the formula:

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

C = Radial growth of fungi in control condition

T = Radial growth of fungi in silicon treated condition

Statistical analysis

All Statistical analyses were performed with SPSS 22. Data were analysed by one-way analysis of variance (ANOVA). Mean separations were performed by Duncan's multiple range tests. Differences at $P < 0.05$ were considered to be significant.

Results and Discussion

Colony colour

The *Fusarium* isolate exhibited velvety and cottony morphology with different colony colour on potato dextrose agar (PDA). The isolate initially exhibited white then gradually turned to light orange colour from upper view and orange purple colour from lower view. *Fusarium* isolates produce macro and micro-conidia on carnation leaf agar (Figure 1).

Effect of potassium silicate on mycelial growth of *Fusarium* isolate at different pH

PDA media having pH 5.6, upon adding the potassium silicate of concentration (0.25, 0.50, 0.75, 1, 2 and 5%) changes the pH to (5.8, 6, 6.2, 6.6, 9.6, 11.7) respectively. Under the control condition, as the pH changes from acidic to basic, the mycelial growth decreases

(Fig. 2). The maximum mycelial growth was shown at acidic pH, 5.8 (8.7cm) and minimum growth was shown at basic pH 11.7 (5.03cm) under control conditions. As compared to control, as the concentration of potassium silicate increases in the media, mycelial growth decreased gradually. At the concentration of 2% PS (at pH 9.6 and 11.7) and 5% PS (5.8, 6, 6.2, 6.6, 9.6 and 11.5) the mycelial growth was completely inhibited (Table 1 and Fig. 3).

Among all the silicon concentration, 5% potassium silicate showed maximum (100%) inhibition of mycelial growth of *Fusarium* at all the pH (5.8, 6, 6.2, 6.6, 9.6 and 11.5) however potassium silicate of concentration 2% also showed 100 percent inhibition at pH 9.6 and 11.7 respectively. Results also suggested that as the concentration of potassium silicate increases in the growth media, the percentage inhibition also increases (Table 2).

Table.1 Effect of potassium silicate on mycelial growth of *Fusarium* isolate at different pH

Treatment	Mycelial growth at different pH (cm)					
	pH 5.8	pH 6	pH 6.2	pH 6.6	pH 9.6	pH 11.7
Control	8.70±0.0	8.10±0.1	7.4±0.	6.33±0.0	5.7±0.2	**5.03±0.**
0.25 % PS	7.83±0.2	7.03±0.4	6.5±0.2	5.93±0.	5.23±0.0	**4.33±0.1**
0.50% PS	7.03±0.3	6.16±0.5	5.87±0.1	5.06±0.	4.37±0.	**2.83±0.2**
0.75% PS	5.80±0.6	5.36±0.5	4.83±0.4	4.33±0.	3.50±0.4	**1.56±0.2**
1 % PS	5.13±0.8	4.23±0.7	3.50±0.6	3.20±0.	2.40±0.4	**1.43±0.1**
2 % PS	3.63±0.9	2.93±0.9	2.03±0.6	1.27±0.	0.00±0.	**0.00±0.**
5 % PS	**0.00±0.**	**0.00±0.**	**0.00±0.**	**0.00±0.**	**0.00±0.**	**0.00±0.**

Values are Mean±S.E., n=21(3 from each replicate)
Alphabatic subscripts (a,b,c…f) indicates significant difference within a row. Numeric subscripts (1,2,3….7) indicates significant difference within a column (p≤0.05)

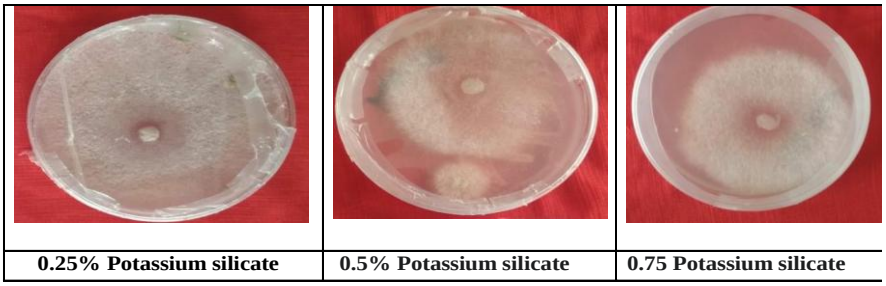
Table.2 Antifungal activity of potassium silicate against *Fusarium* isolate under *in vitro* condition

Treatments	% inhibition						Percentage mean inhibition
	pH 5.8	pH 6	pH 6.2	pH 6.6	pH 9.6	pH 11.7	
0.25 % PS	9.96	13.1	12.1	6.3	8.18	13.8	**10.59**
0.50% PS	19.15	23.8	20.7	20	23.3	43.67	**25.13**
0.75% PS	33.33	33.7	34.6	31.5	38.5	68.85	**40.12**
1 % PS	40.99	47.7	52.7	49.4	57.8	71.50	**53.38**
2 % PS	58.23	63.7	72.5	80	100	100	**79.08**
5 % PS	**100**	**10**	**100**	**100**	**100**	**100**	**100**

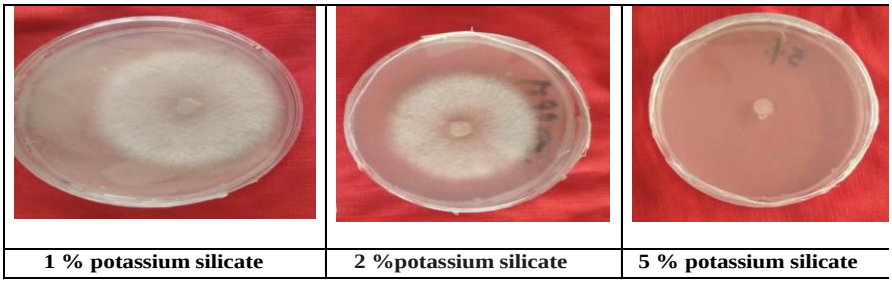
Fig.1 Colony morphology of *Fusarium* isolate on PDA media at pH 5.6

Control (Upper view)	Control (Lower view)	Conidia

Fig.2 Effect of different concentration of potassium silicate on mycelial growth of *Fusarium* isolate in response to 0.25%, 0.50%, 0.75%, 1%, 2%, and 5% soluble potassium silicate per litre of potato dextrose agar (PDA) media

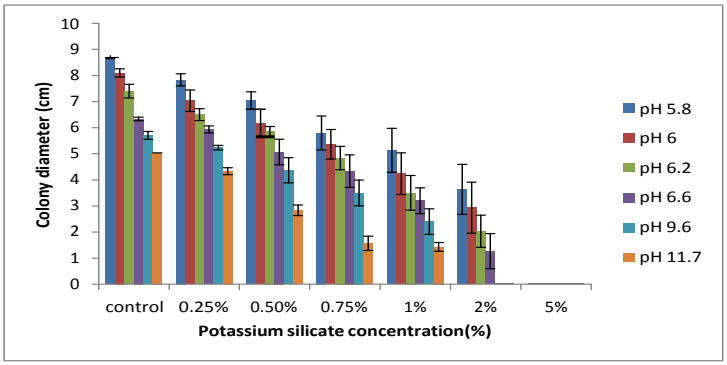


0.25% Potassium silicate	0.5% Potassium silicate	0.75 Potassium silicate



1 % potassium silicate	2 %potassium silicate	5 % potassium silicate

Fig.3 Effect of potassium silicate on colony diameter at different pH



The results obtained from this study suggested that potassium silicate significantly reduces the mycelial growth of *Fusarium* isolate because silicon causes reduction in turgor pressure of fungal cell as a result there is shrinkage and collapse of spores and hyphae (Guevel *et al.*, 2007). This collapse and shrinkage of hyphae could be associated with the formation of cavities within the cells, induction of abnormal thickening of cell wall of the fungus, disintegration of the cells that irregularly contracted and complete hyphal damage at higher silicon concentration (Li *et al.,* 2009). It was suggested that damage in plasma membrane play vital role in antifungal activity since leakage of sugars and proteins from silicon treated spores was significantly higher as compared to control (Liu *et al.*, 2000). The results obtained were similar to Rachniyom and Jaenaksorn (2008) who reported that soluble silicon significantly reduced mycelial growth and sporangial production of *Pythium aphanidermatum.* In vitro investigation of potassium silicate on the growth of five soil borne plant pathogenic fungi showed that mycelial growth of fungal isolates (*Pestalotiopsis clavispora, Rhizoctonia solani* and *F. oxysporum* f. sp. *Fragariae)* significantly ($p<0.05$) reduces on PDA media amended with potassium silicate (Shen *et al.*, 2010). Fayadh and Aledani (2011) investigated that silicon having concentration 30, 200, 500 ppm were completely reduces the growth of the pathogenic fungus (*R. solani).*

Kaiser *et al.*, (2005), who suggested that 40ml and 80ml of potassium silicate amended PDA medium completely inhibited the mycelial growth of *Fusarium oxysporum, Sclerotinia sclerotiorum, Phytophthora cinnamomi, Phytophthora capsici, Stemphylium herbarum, S. rolfsii, Pythium sp, Mucorpusillus, A. solani, C. coccodes, Verticillium fungicola, F. solani, C. lunata* and *Drechslera spp.* Siddiq *et al.,* (2019) reported silicon in the form of sodium silicate was found to be effective in reducing mycelial growth of *Macrophomina phaseolina* and also observed gradual reduction of mycelia growth with increase concentration of silicon. Maekawa *et al.,* (2003) reported that hyphal growth of fungus causing rice blast was retarded on agar plates having soluble silicon (silicic acid).

In conclusion there are various reports available for the control of various plant pathogens both under in vivo and in vitro conditions by using different silicon sources. Results obtained from the present study revealed that the silicon (potassium silicate) exhibited antifungal activity. Our *in-vitro* experimental finding suggests that exogenous application of silicon (potassium silicate) could inhibit the infection of *Fusarium* species in host plant tissues and might be very helpful in controlling mango malformation. In future, silicon (potassium silicate) can be used for increasing the resistance in plants against various pathogens.

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How to cite this article:

Ritika Yadav and Gurdeep Bains. 2021. *Invitro* Investigation of Potassium Silicate on Mycelial Growth of *Fusarium* Isolate. *Int.J.Curr.Microbiol.App.Sci.* 10(03): 233-240.
doi: https://doi.org/10.20546/ijcmas.2021.1003.031