

USE OF SILICON IN INHIBITING THE GROWTH OF *MACROPHOMINA PHASEOLINA*

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Abstract

Silicon in the form of sodium silicate was found to be effective in reducing *in vitro* growth of *Macrophomina phaseolina*. Silicon was effective against *M. phaseolina* when added to the medium before autoclaving. There were no significant effects observed on growth of *M. phaseolina* when silicon was added to Potato Sucrose Agar (PSA) after autoclaving and before boiling. There was much more inhibition of pathogen observed when silicon was added to water agar with (2%) sucrose. Gradual reductions of growth were seen with increase concentration of silicon with different components of Potato Sucrose Agar.

Introduction

Macrophomina phaseolina is the charcoal rot fungus which affects more than 500 species of plants (Shahzad & Ghaffar, 1995; Sinclair, 1982). *M. phaseolina* causes root rot, collar rot, seedling blight, stem rot, leaf blight, pod and seed infection in mung and urdbeans in different parts of India (Vidhyasekaran & Arjuman, 1978; Simiappan & Vidhyasekaran, 1981; Kaushik *et al.*, 1981). Yield losses to the tune of 10.8% have been reported due to seed infection in mungbean (Kaushik *et al.*, 1981) and 6.75 to 15.5% in soybean (Haq *et al.*, 2012). *Macrophomina phaseolina* is reported as a high temperature pathogen since the disease spreads more rapidly and become severe under high temperature and dry conditions (Grover & Sakhuja, 1981).

Silicon is the second most abundant element in the earth crust and also present in most soils (Epstein, 1994; Datnoff *et al.*, 1997; Marschmer, 1995). It is not an essential but major micronutrient that is readily taken up by the plants, often present in higher concentration in some plant tissues and its concentration sometimes exceeds even the concentration of nitrogen and potassium (Epstein, 1994). The beneficial effects of adequate silicon include decreased susceptibility to fungal pathogens and insects, amelioration of abiotic stresses and increased growth in some plants (Epstein, 1994; Marschmer, 1995; Nanayakkara *et al.*, 2008). Silicon has also been shown to reduce the incidence of powdery mildew in Kentucky bluegrass (Hamel & Heckman, 2000). Gray leaf spot of St. Augustine grass was reduced by silicon applications and its addition increased the effectiveness of fungicide treatments (Brecht *et al.*, 2001). The spherical silica nanoparticles inhibited the growth of *Candida albicans* to hyphal transition (Cousins *et al.*, 2007). Silicon also reduced the effect of salinity on cotton plants (Khan & Ismail, 1997). Silicon application can significantly regulate plant growth and yield if applied at proper time with feasible concentration (Kim *et al.*, 2012). Fungicides like Cabendazim, Thiophenate-methyle and Benomyle gave 90% control of foliage blight when applied as foliar treatments. Thiophenate-methyle was the most effective soil drench fungicide as compared to others (Hooda & Grover, 1983). However, hazardous effects of fungicides on environment and health are well documented (Fiume & Fiume, 2006).

The present report describes the effect of silicon in the form of sodium silicate on *In vitro* growth of *M. phaseolina*.

Materials and Methods

Silicon on radial growth of *Macrophomina phaseolina*:

Silicon in the form of Sodium silicate (Na₂SiO₃, Merck) was added into the potato sucrose agar (PSA: potato 200g, sucrose 20g, Agar 20g, water 1L) medium before autoclaving to get the final concentration of 10,000 ppm. The medium was sterilized @ 15 p.s.i. for 20 minutes and poured into 9 cm diam., Petri plates. Penicillin @ 100,000 units L⁻¹ and streptomycin @ 0.2 g L⁻¹ were added to the media just before pouring. Media without sodium silicate served as control.

Culture of *Macrophomina phaseolina* was grown on PSA for 5 days. A 5 mm diam. inoculum disc from the periphery of the colony was cut with the help of a flame sterilized cork borer and placed in the centre of each Petri plates. There were 5 replicates for each treatment. The Petri plates were incubated at room temperature *i.e.*, 28±2°C and colony diam., were recorded daily.

Efficacy of silicon on heating: Sodium silicate was added to PSA @ 10,000ppm before or after autoclaving. In another treatment autoclaved medium was amended with Sodium silicate and boiled for 20 minutes on a Bunsen burner. Medium not amended with Sodium silicate was used as control. The media were poured into 9cm diam., Petri plates; an inoculum disc of *M. phaseolina* was placed in the centre of each Petri plate. The plates were incubated at room temperature and radial colony growth was recorded daily.

Different concentrations of silicon on radial growth of *Macrophomina phaseolina*:

Sodium silicate was added in to PSA medium @ 1000, 100, 10 and 1ppm and also added in PSA before autoclaving @ 1, 10, 100, 1000, 3000, 5000, 7000 and 9000 ppm. The sterilized media were poured in 9 cm diam., Petri plates and a 5 mm diam., inoculum disc of *M. phaseolina* was placed in the center of each Petri plate. The Petri plates were incubated at room temperature and colony diam., were recorded daily.

Effect of different component of PSA on efficacy of Na_2SiO_3 : Media containing different components of PSA were prepared. It included water agar (2%), water agar (2%) with sucrose (2%), potato (200 g L^{-1}) decoction agar and PSA. Sodium silicate was added to each medium to get final concentrations of 0 (control), 1, 10, 100, 1000, 3000, 5000, 7000, 9000 and 10,000ppm. The media were sterilized at 15p.s.i. for 20 minutes and poured in 9cm diameter. Petri plates and a 5mm inoculum disc of *M. phaseolina* was placed in the centre of each plate. The plates were incubated at room temperature and radial growth of the fungus was recorded daily.

Results

Small colony growth of *Macrophomina phaseolina* was observed in PSA amended with Sodium silicate @ 10,000ppm before autoclaving but not significant increase as the time pass whereas in control treatment plates were filled after 3 days of incubation (Fig. 1).

Silicon significantly suppressed the growth of *M. phaseolina* when it amended in PSA before autoclaving but when it was added after autoclaving and before boiling it was not effective against pathogen (Fig. 2). Sodium silicate inhibit the growth of *M. phaseolina* in PSA, Potato extract agar, Sucrose agar and Water agar as compared to without sodium silicate amended PSA, Potato extract agar, Sucrose agar and Water agar. Negative correlation between the growth of *M. phaseolia* and the concentration of sodium silicate were observed in water agar treatment. Growth of the pathogen appears after 2 days of incubation in 10,000ppm of Sodium silicate concentration (Fig. 3a). Maximum inhibition was observed in Sucrose agar with Sodium silicate (Fig. 3b). Similarly, the growth of *M. phaseolina* was also inhibited in sucrose agar and no growth was observed in first 3 days of incubation in 10,000ppm concentration of silicon and 2 days of 9000ppm and 7000ppm of Sodium silicate, gradual decline of growth was seen as concentration of Sodium silicate increases but the plates were filled after 4th days of incubation in non-silicon amended media (Fig. 3c). Some growth was observed in 1ppm Sodium silicate treatment but as the concentration of Sodium silicate increases the growth of pathogen was also suppressed in PSA control showing no

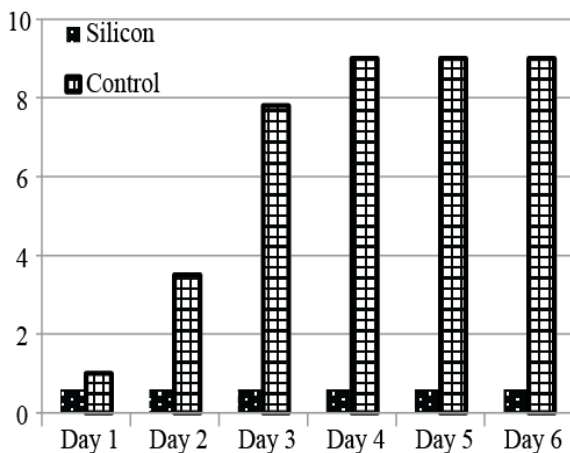


Fig. 1. Effect of silicon on radial growth of *Macrophomina phaseolina*.

inhibition of growth and plates were filled after 4th day of incubation (Fig. 3d). As the concentrations of Sodium silicate were increased, reduction in radial colony growth of *M. phaseolina* was seen, as compared to control (Fig. 4). When water agar on amended with Sodium silicate, reduction in radial growth of *M. phaseolina* was observed with the increase in concentration of Sodium silicate up to 10,000ppm (Fig. 5).

In water agar there is a marked decrease in the growth of *M. phaseolina* with increasing concentration of Sodium silicate in without sucrose (2%) maximum reduction was observed after 5000 ppm but when we used 2% sucrose in water agar there was gradual reduction of the growth of *M. phaseolina* as concentration of Sodium silicate is increased. Control plates show maximum growth with or without 2% sucrose (Fig. 6).

Discussion

M. phaseolina, the charcoal rot fungus is the most pathogenic and affects several important crops (Wyllie, 1993). Some soil-borne root infecting fungi like *Macrophomina phaseolina* are difficult to eradicate because they produce resting structure like sclerotia, chlamydo spores etc., for their survivor for a longer period of time under adverse environmental conditions (Baker & Cooke, 1974).

Most of the chemical fungicides are not only producing some health hazardous effects on human but also on crops as well as important fungi present in the soil (Dłużniewska, 2003). Use of fungicides were providing control of soil borne plant pathogen but these hazardous chemical were harmful for important crops, field workers as well environment (Kerry, 2001).

Silicon stimulated the growth of young maize plants exposed to Cd and influenced the development of Casparian bands and suberin lamellae as well as vascular tissues in root without affecting the distribution of apoplasmic and symplasmic Cd in maize roots, but considerably decreased symplasmic and increased apoplasmic concentration of Cd in maize shoots (Vaculik, 2012).

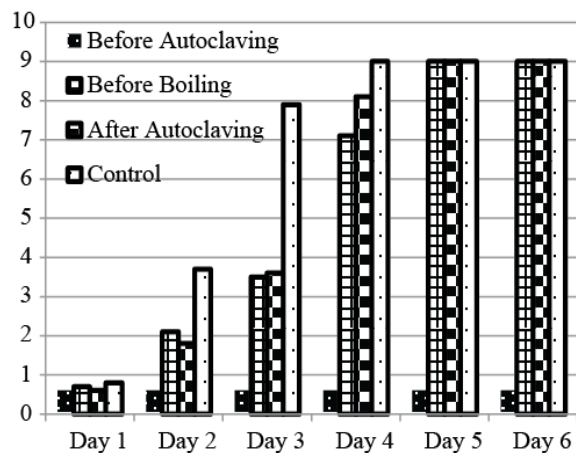


Fig. 2. Effect heating on efficacy of silicon against *in vitro* growth of *Macrophomina phaseolina*.

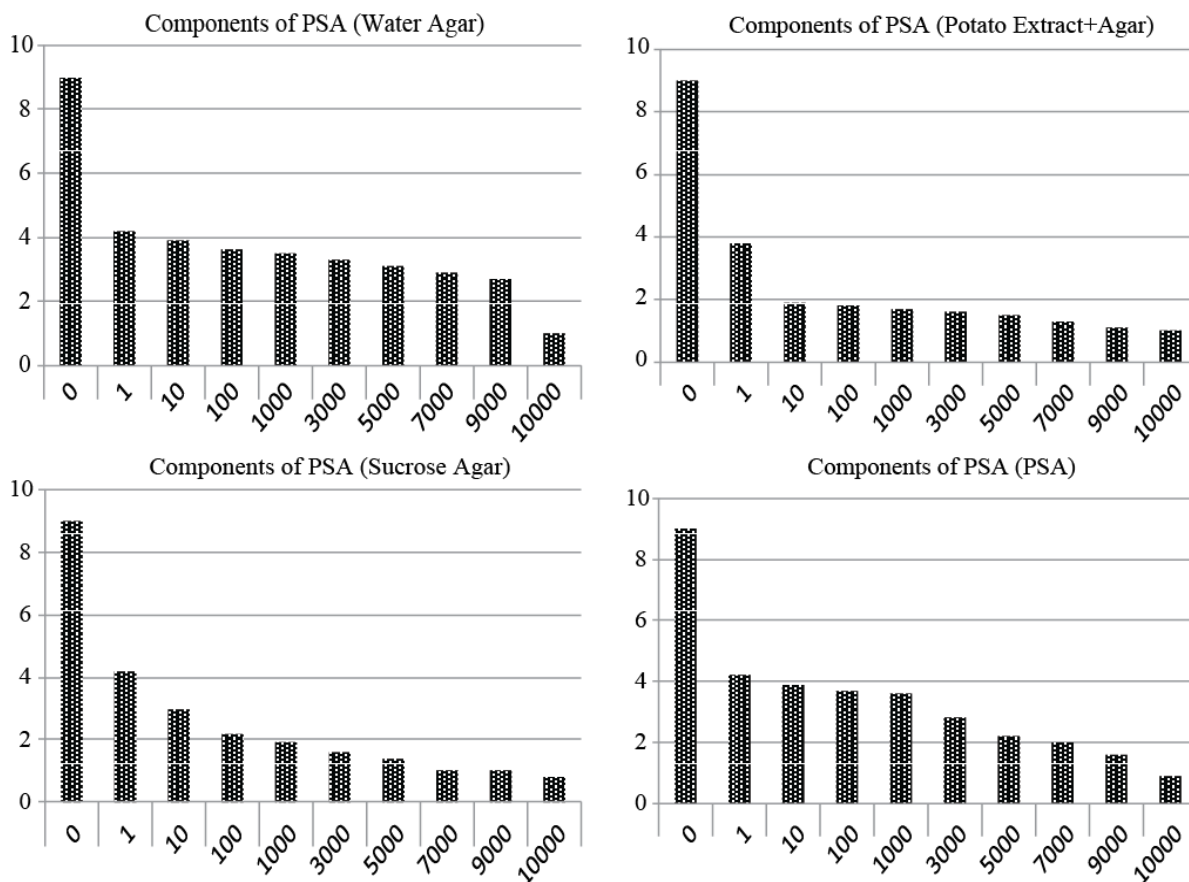


Fig. 3 (a, b, c, d). Effect of sodium silicate with different components of PSA on the growth of *Macrophomina phaseolina* (0-10000 ppm of silicon).

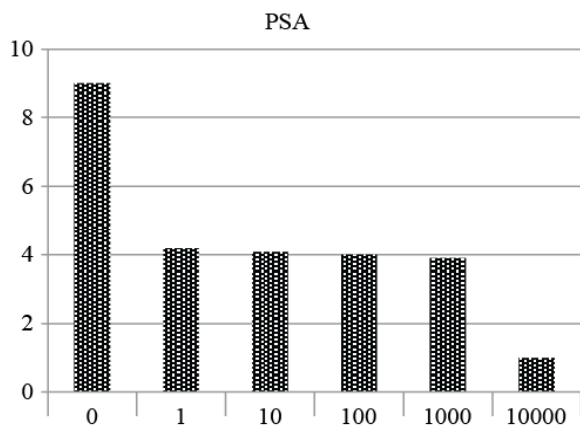


Fig. 4. Effect of different concentrations of sodium silicate on the growth of M.P on PSA.

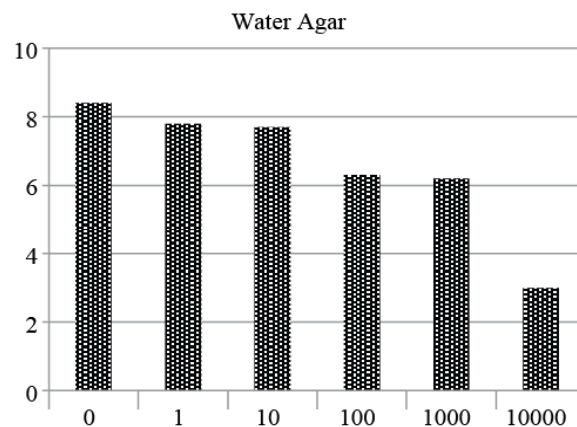


Fig. 5. Effect of different concentrations of sodium silicate on the growth of M.P on water agar.

Silicon was found effective for the suppression of plant pathogenic fungi either soil borne or foliar in cucumber, rice, sugar-cane, turf and several other plant species (Datnoff *et al.*, 2001). Silicon amended PDA @ 0.6% or more completely inhibited the growth of *Penicillium expansum* (Ebrahimi *et al.*, 2012). As we increased the concentration of Sodium silicate specially after autoclaving treatments the growth of *Macrophomina phaseolina* was hampered and also

sucrose is the component in PSA which somehow promotes the growth of *M. phaseolina* when we exclude it the growth declined sharply. Similar results were obtained in the field, and silicon appears to be as effective as fungicides in controlling gray leaf spot development (Brecht *et al.*, 2001). It is therefore suggested to use silicon with sucrose for the better control of pathogenic fungi specially soil borne root infecting fungi like *M. phaseolina*.

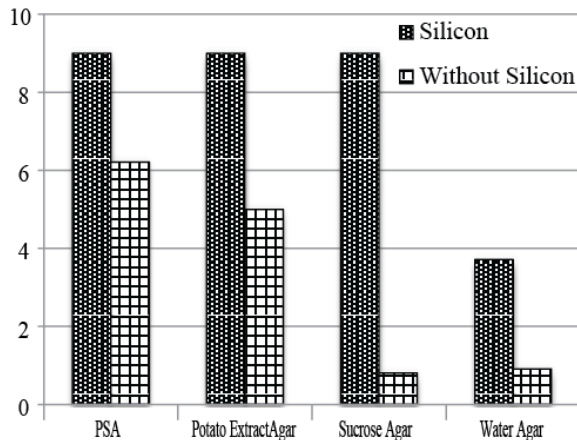


Fig. 6. Effect of different component of potato sucrose agar with and without sodium silicate on the growth of *Macrophomina phaseolina*.

Acknowledgements

One of the authors Muhammad Omer Khan is greatly Mr. Muhammad Ather Khan and Mrs. Fehmida Abdullah for their moral support.

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