

## Effectiveness of Potassium Silicate in Suppression White Rot Disease and Enhancement Physiological Resistance of Onion Plants, and its Role on the Soil Microbial Community

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### ABSTRACT

In this study, field and greenhouse experiments were carried at Agricultural Research farm, El Khatatba location, Menofia governorate and Agriculture Research Center in Giza, respectively to estimate the effect of soluble potassium silicate (PS) application on inducing onion resistance against *Sclerotium cepivorum* and the rhizosphere of soil microbial communities. Application treatments included dipping, soil drench and combining dipping and soil drench with three concentrations (0.1, 0.2 and 0.4 %) was done. The combined treatments of dipping and soil drenching followed by soil drench of potassium silicate (PS) were more efficient for suppressing onion white rot disease and increasing the plant fresh weight (FW) as well as onion bulb yield under green house and field conditions, respectively, whereas dipping only was the lowest effective one in this respect. PS at 0.4% applied as combined treatment of dipping and soil drenching and the soil drenching only, as well as the combined treatment at 0.2% of potassium silicate were the most superior treatments for decreasing onion white rot and increasing plant fresh weight under greenhouse conditions. However, the integration treatment of dipping & soil drenching of PS at 0.4% was the greatest treatment for reduction white rot disease and increasing onion bulb yield under field conditions. PS amendments increased the amount of soil bacteria, actinomycetes and reduced the soil fungi counts significantly. The treatment of dipping and soil drenching of potassium silicate at 0.2% significantly increased soil dehydrogenase and nitrogenase activities under naturally infested conditions with *Sclerotium cepivorum*. Also, the treatments of PS increased the physiological activity such as photosynthetic pigments, osmoregulation solutes, and enzyme activity, while decreased the malondialdehyde and membrane leakage of onion plant. Moreover, PS at 0.4% applied as the combined treatment of dipping & soil drenching was the highest effectiveness of our treatments. Our results suggested that Si amendment is an efficient approach against *S.cepivorum*. Furthermore, Si-mediated resistance in onion against *Sclerotium cepivorum* is associated with the changes of soil microbial such as microorganism amounts, enzyme activity of the soil, as well as physiological effect on plant leaves as photosynthetic pigments, compatible solutes, and enzyme activity.

**Key words:** Onion, White rot disease, Potassium silicate, Dipping, Soil drench.

### Introduction

Onion (*Allium cepa* L.) commonly called “queen of kitchen” is an important crops in which can grow in Egypt and other countries of the world. Moreover, it has been reported that it has medicinal flavonols (Javadzadeh *et al.* 2009). Egyptian onion is greatly desired for its good quality and early appearance in the foreign markets. The cultivated area by onion in Egypt was 196968 fed in 2014/2015, this area can produce 2,888,791 tons with average of 14.67 tons/fed.

In the last decade, Onion production has been decreased significantly due to white rot disease caused by *Sclerotium cepivorum* Berk (Jones, 2010). Onion white rot disease is broadly distributed in Egypt and it can be considered as a limiting factor mainly in the Upper Egypt for onion cultivation, production and exportation (Khalifa *et al.*, 2012). The pathogen can produce numerous long-lived

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small size survival structures (sclerotia) in the soil and considered as a primary source of inoculum (Jones, 2010), which are able to germinate in the presence of *Allium* root exudates, specially alkyl cysteine sulphoxides (Coley-Smith *et al.*, 1990).

Silicon (Si) concentration ranges from 1% to 10% or higher in plant dry matter and its highly abundant in the soil. But, it is not necessary element for the majority of plants, with an exception of some Equisitaceae members (Epstein, 1993). The useful effects of Si were discovered in the grasses and a few plant species (Jones and Handreck, 1967). Si has been described as a non-essential of plant nutrient, but it can play a vital role to improve disease resistance in plants (Forbes & Watson, 1992). Silicon (Si) has important potential in the plant growth and development (Datnoff *et al.*, 2001). PS is the main source of soluble potassium and silicon. In general, plants require silica to resist against biotic and abiotic stress (Ma, 2004).

Plants usually absorb silicon in the forms of monosilicic and polysilicic acids. The presence of silicon can reduce the harmful effect of metal elevation and improve water-use efficiency and photosynthesis rate in plants. In addition, Si can acts as a bioprotectant against fungal attack (Datnoff *et al.*, 1997). Si amended to the soil as nutrient solution exhibited enhancement in plant growth, yield, increased the disease and insect resistance and reduced the harmful of mineral toxicities (Belanger *et al.*, 1995). Soluble Si has shown a potential impact for increasing resistance to fungal diseases such as powdery mildew and root rot (Belanger *et al.*, 1995). Application of silicic acid to crops (such as rice) can control diseases and also decrease the amount of fungicides that released into the environment, (Ma *et al.*, 2004). *Allium* white rot severity can be reduced by several methods such as: use of fungicides, use of synthetic *Allium* oil (Delgadillo *et al.*, 2004), adding of composts (Entwistle, 1990), crop rotation and soil flooding (Banks and Edgington, 1989), solarization and mulching (Delgadillo *et al.*, 2004), incorporation of cruciferous residues (Zavaleta-Mejia *et al.*, 1992b), and application of antagonists (Ulacio-Osorio *et al.*, 2004).

In Egypt and other countries, several attempts were done upon fungicides as the main strategy for controlling onion white rot disease, and then increase the quantity and the quality of onion yield (Abd El-Moity *et al.* 1997). The harmful side effects of fungicides were reported on humans and environment (Garcia, 1993). With increasing locally and globally demand of onion and the emphasis laid on export of onion, there is greater require to increase the production of onion. In recent years, the increase of health consciousness among the people can strict the exportation of the onion to other countries. The new focus has been shifted to find out safer alternative way to chemical fungicides in managing the plant diseases. Thus, the development of nontoxic alternative methods to fungicides would be useful in reducing these harmful effects (Khalifa *et al.*, 2013). Such management would help prevent the pollution and also health hazards (Kumar, 2007).

Since Si play a vital role in leaves stability and can expose more leaves to light so, it could cause an increase of plants canopy photosynthesis efficiency (Quanzhi and Erming 1998). Si can cause leaf development and can cause photosynthesis improvement. Plants exposed to different environmental stresses including disease infections exhibit changes in membrane leakage that lead to loss of membrane integrity (Blokhina *et al.*, 2003).

The combination of white rot infection with silicon nutrition in the form of PS via the nutrient solution can improve the impact of disease infection on membrane leakage and lipid peroxidation. The addition of PS can mediated a reduction in lipid peroxidation that is attributed to its regulation of antioxidant activity in plants (Lamb and Dixon 1997). Both osmoregulators, proline and soluble carbohydrate, content were significantly increased. Shekari *et al.*, (2015) reported that application of Si to the dill plants that grown under abiotic stress can increase soluble carbohydrates content. Higher content in soluble carbohydrates as osmotic adjustment and can be a protective mechanism under abiotic stress (Munns and Tester, 2008). Accumulation of proline normally takes place in the cytoplasm where it can cause cytosolic osmotic adaptation (Munns and Tester, 2008). Several studies have reported a link between Si supply and an improvement in the antioxidant activity of plants when they are infected by plant pathogens. An increase in the activities of ROS-scavenging enzymes, such as ascorbate peroxidases (APX), glutathione reductases, superoxide dismutases (SOD) and catalases in plants receiving Si restricted the ROS-dependent cellular damage that was indirectly linked to the high concentration of malonaldehyde (Domiciano *et al.*, 2015).

The aim of this study was to examine the effect of potassium silicate at different concentrations and applications as a tool for disease management of onion white rot disease and their effects on soil biological microbiology and physiological activities.

## Materials and Methods

### 1. Greenhouse Experiment:

A pot experiment was carried out on the second week of December under greenhouse conditions at Agri. Res Cent., Giza. Potassium silicate ( $K_2SiO_3$ , 99% purity), were procured from Sigma-Aldrich Chemie (USA) and were used without any further purification.

Three different treatments from PS were used in this experiment as follows:

1. Dipping treatment of onion transplants before planting in PS as concentration of 0.0, 0.1, 0.2 and 0.4%
2. Soil drench only with potassium silicate at four different concentrations i.e. 0.0, 0.1, 0.2 and 0.4% at the time of planting and after planting two times by intervals one month .
3. Dipping treatment of onion transplants before planting combined with soil drench with potassium silicate at four different concentrations i.e. 0.0, 0.1, 0.2 and 0.4% at the time of planting and after 30 and 60 days of planting respectively.

Pots (30 cm-diameter) were sterilized by immersed in 5.0% formalin solution for 15 minutes, left to dry for two days to remove formalin residues, then filled with nearly 3 kg soil previously sterilized by formalin solution (5.0%) for 2 weeks and left for another 2 weeks to get rid of formalin residues. Fungal inoculation of *S. cepivorum* (previously isolated from infected onion plants and confirmed their pathogenic capabilities by the authors) and then it was prepared using sorghum-coarse sand water (2:1:2 v/v) medium. The ingredients were mixed, bottled and autoclaved for 1h at 1.5 air pressure. The autoclaved media in glass bottles were inoculated separately using agar discs obtained from the periphery of five days old colony of the tested fungi and incubated at ( $20\pm 2^\circ C$ ) for two weeks and used for soil infestation. Fungal propagules of *S. cepivorum* were added to the potted clay loam soils (3 kg soil/pot) at the rate of 10.0 g/kg soil (w/w), mixed with the soil surface of each pot then irrigated with water and left for one week for the inoculum establishment.

Apparently healthy onion transplants of Giza 20 cultivar were dipped for 1h in each particular solution of PS at different concentrations then raised and transplanted in infested potted soil with *S. cepivorum* immediately at the rate of 5 transplants per pot. Control treatment of dipping and soil drenching treatment (0.0%) was applied by dipped onion transplants in tap water and added water to the soil, respectively.

Infected plants that having a symptoms of white rot disease were counted after two and four month from planting and their percentage were calculated according to Hovius and Goldman (2004) as well as white rot reduction% was recorded as follows:

$$\text{Infection (\%)} = \frac{\text{No. of transplants infested with white rot}}{\text{Total No. of transplants}} \times 100$$

$$\text{White rot reduction (\%)} = \frac{\text{White rot\% in each treatment} - \text{White rot\% in control}}{\text{White rot\% in control}} \times 100$$

Fresh weight of onion plants from each pot of different treatments was also recorded directly after harvest as g/pot.

### 2- Field Experiment:

Field experiment was carried out during the growing season 2015/ 16 in sandy loam soil naturally infested with *S. cepivorum* at Agricultural Research farm, El Khatatba location, Menofia governorate (latitude of  $30^\circ 29'N$  and longitude of  $30^\circ 97'E$ ). Randomized complete block design was used in this study. Three replicates were used and the plot area was  $3.0 \times 3.5 \text{ m}$  ( $10.5 \text{ m}^2 = 1/400$  feddan (feddan =  $4200 \text{ m}^2$ ). Each plot included 6 rows (each 3.0 m length and 50 cm width). Sixty day-old transplanting of onion cultivar Giza 20 were planted per each plot at spacing 10 cm X 10 cm, within each row on the first week of December. Onions were grown to maturity under irrigation, fertilizer

and pest management practices standard with commercial production in the area. The physico-chemical properties of the experimental soil were estimated according to (Black *et al.*, 1982). The soil texture was sandy loam having the following characteristics, sand 60.5%, silt 24.2%, loam 15.5 %, pH 7.6, EC 1.36 ds/m, Organic matter 0.85 %, total nitrogen 0.13%, total phosphorus 0.025% and available phosphorus 0.005%.

The treatments used under greenhouse conditions were used under field conditions. White rot incidence as a percentage of bulbs with symptoms was assessed at harvest by pulling and observing all onion bulbs in each plot. Also, Onion white rot reduction was estimated as previously mentioned. Onion bulbs from each plot were harvested and weighed (kg/plot) for yield assessment, as well as and onion% of bulb yield increase was estimated as mentioned above. Onion plants and soil samples were taken after 60 and 90 days from planting to determine microbial counts and some plant and soil enzyme activities and other determinations as follow.

### **Plant and Soil Biological activities:**

#### *1- Microbial populations:*

For total microbial count determination, after 60 and 90 days of planting, rhizospheric soil samples were collected and kept at 4°C in plastic bags to stabilize the microbiological activity distributed during soil sampling and handling. Plate count technique was applied using potato dextrose agar medium (PDA) and nutrient agar medium (Difco, 1985) to enumerate total fungi and bacterial count in respective order. Total actinomycetes were estimated by the standard procedure of Rolf and Bakken (1987). The isolation and enumeration of phosphate solubilizing and silicate bacteria from onion rhizospheric soil was carried out by using serial dilution and standard count technique using Pikovskaya's agar medium for phosphate solubilizers (Pikovskaya, 1948) and Aleksandrov's agar medium for silicate bacteria (Zahra, 1969).

#### *2- Soil enzymes activities:*

Dehydrogenase activity in soil was determined according to the method described by Skujins (1976). Nitrogenase activity in plant rhizosphere was assayed by the acetylene reduction according to Somasegaran and Hoben, (1994).

#### *3- Soil PH:*

The initial pH of the soil as well as after adding potassium silicate was analysed using benchtop pH meter (Orion 2-Star; Thermo Scientific, USA), as described by Margesin and Schinner (Margesin and Schinner 2005)

#### *4- Photosynthetic pigments measurement:*

Chlorophyll a, b and carotenoids were performed using Lichtenthaler method. 0.2g of fresh leaves was grinded with 80% acetone and the extract was centrifuged for 10 minutes at 10000g and then, the extract was measured using a spectrophotometer (-UV1901PC, Phenix, China) at wavelengths of 646, 663 and 470 nm. Chlorophyll content was calculated using formula (Lichtenthaler and Wellburn, 1983).

#### *5- Assessment of electrolyte leakage (EC):*

At the end of the field experiment (after 6 weeks), the fully expanded leaves were cut into 5-mm-long fragments and positioned in test tubes filled with 8-mL distilled water. Electrolytes leakage (EC) was measured using a conductivity meter (DDS-307). Ten replicates were used for each treatment which was placed in a vial containing 20 mL of deionized water. After the vials were shaken slightly, then immediately the conductivity of the solution was measured. Again after 1 hour, the conductivity of the solution was measured. Finally, each vial was placed in boiling water for 1 h,

then cooled to the room temperature (about  $20\pm 2^{\circ}\text{C}$ ) and then shaken, after which total conductivity was measured. Leakage rate of electrolytes (expressed in  $\mu\text{S}\cdot\text{cm}^{-1}\text{FW}\cdot\text{h}^{-1}$ ) was calculated as the net conductivity of the solution with leaf sample immersed for 1 h divided by the total conductivity after boiling.

#### 6- Determination of Malondialdehyde (MDA) Content:

MDA was determined according to the methods described by Heath and Packer (1968) and Dhindsa *et al.*, (1981) with slight modifications. Briefly, 1 mL of extracted enzyme solution was added to 2 mL of a reaction solution containing 20% (v/v) trichloroacetic acid and 0.5% (v/v) thiobarbituric acid. The solution was placed in a water bath at  $95^{\circ}\text{C}$  for 30 min and then transferred to an ice water bath. After the solution was centrifuged at  $10,000\text{ g}$  for 10 min, the absorbance of the supernatant was read at 532 and 600 nm. Nonspecific absorbance at 600 nm was subtracted from that at 532 nm, and MDA content was calculated using this adjusted absorbance and the extinction coefficient of  $155\text{ mm}^{-1}\text{cm}^{-1}$  (Heath and Packer, 1968).

#### 7- Compatible solutes:

Total free amino acids (TAA) and total soluble sugars (TSS) were extracted from the plant materials by 80% ethanol. TAA was determined spectrophotometrically by the methods of Dubey and Rani (1989b) and total sugars were determined by phenol-sulphoric acid method as described by Sadasivam and Manickam (1996). Free proline was determined using the method of Bates *et al.* (1973).

#### 8- Antioxidant Enzyme Assays:

All procedures were carried out at  $0-4^{\circ}\text{C}$ . The fresh leaves were grind in a cold mortar using specific buffers for each enzyme extraction. The homogenates were centrifuged at  $12,000\text{ rpm}$  for 15 min, and the obtained supernatants were stored at  $-20^{\circ}\text{C}$  for later determination of enzyme activity. Protein concentrations were determined according to Bradford (1976), using bovine serum albumin as a standard. The specific activity of the enzymes was expressed in units as function of protein content; 1 unit =  $1\text{ }\mu\text{mol}$  substrate metabolized  $\text{mg}^{-1}\text{ protein min}^{-1}$ .

##### A- Super Oxide Dismutase (SOD) Assay:

SOD (EC 1.15.1.1) was extracted with 50 mM phosphate buffer (pH 7.0), containing 1 mM EDTA, 0.05% Triton X- 100, 2% (w/v) PVP and 1 mM ascorbic acid (AsA). SOD activity was assayed in terms of its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT), according to the method of Beauchamp and Fridovidsh (1971) as described in Donahue *et al.*, (1997).

##### B- Catalase (CAT), Ascorbate Peroxidase (APX) and Glutathione Reductase (GR):

CAT, APX and GR were extracted with 50 mM Tris-HCl (pH 7.5), containing 20% (v/v) glycerol, 1 mM AsA, 1 mM DTT, 1 mM EDTA, 1 mM GSH, 5 mM  $\text{MgCl}_2$  and 1% (w/v) PVP. CAT (EC 1.11.1.6) activity was determined by directly measuring the decomposition of  $\text{H}_2\text{O}_2$  at 240 nm ( $0.04\text{ mM}^{-1}\text{ cm}^{-1}$ ) as described by Aebi (1983). APX (EC 1.11.1.11) activity was assayed as the decrease in absorbance due to ascorbate oxidation observed at 290 nm ( $2.8\text{ mM}^{-1}\text{ cm}^{-1}$ ) and  $25^{\circ}\text{C}$ , according to the method of Nakano and Asada (1981). GR (EC 1.6.4.2) activity was determined by monitoring NADPH oxidation by glutathione disulfide (GSSG) as a substrate for GR to regenerate glutathione (GSH) at  $25^{\circ}\text{C}$  through the decreased absorbance at 340 nm ( $6.2\text{ mM}^{-1}\text{ cm}^{-1}$ ) (Halliwell and Foyer 1978). The assay mixture contained 50 mM Tris-HCl (pH 7.5), 5 mM  $\text{MgCl}_2$ , 0.5 mM GSSG, 0.2 mM NADPH, and 20  $\mu\text{L}$  enzyme sources in 1 mL final volume.

#### Statistical analysis:

The obtained data were statistically analyzed by analysis of variance (ANOVA) using MSTAT-C program version 2.10 (1991). The least significance difference (LSD) test (0.05) was used to find out the significance of mean difference of various treatments. (Gomez and Gomez, 1984).

## Results and Discussion

### 1- Effect of PS treatments on white rot disease and plant fresh weight of onion under greenhouse conditions:

Data tabulated in (Table, 1) showed that, most of potassium silicate treatments caused a significant decreasing in onion white rot disease incidence and increasing the plant fresh weight compared to control treatment (0%). In general, the combined treatment of dipping and soil drenching was the best treatment in this regard, followed by soil drenching with insignificant differences between them, whereas dipping only was the least effective one at different concentrations *i.e.* 0.0, 0.1, 0.2 and 0.4%. The conc. 0.4% of different application was the most significant effective one followed by 2% with insignificant differences between them, while 0.1% of different application was the least significant one.

**Table 1:** Effect of PS treatments on white rot disease and plant fresh weight of onion under soil infestation with *S. cepivorum* under greenhouse conditions in season 2014/2015.

Potassium silicate treatments		White rot %	White rot reduction%	Plant fresh weight g/pot	Fresh weight Increasing %
Application	Conc.				
Dipping only	0%	73.3	0.0	45.7	0.0
	0.1%	46.7	36.3	151.3	231.1
	0.2%	26.7	63.6	213.1	366.3
	0.4%	13.3	81.9	230.8	405.0
Mean	-	40.0	45.5	160.2	250.6
Soil drenching	0%	73.3	0.0	45.7	0.0
	0.1%	26.7	63.6	173.9	280.5
	0.2%	20.0	72.7	220.5	382.5
	0.4%	6.7	90.9	263.5	476.6
Mean	-	31.7	56.8	175.9	284.9
Dipping + Soil drenching	0%	73.3	0.0	45.7	0.0
	0.1%	13.3	81.9	242.4	430.4
	0.2%	6.7	90.9	254.1	456.0
	0.4%	6.7	90.9	309.7	577.7
Mean	-	25.0	65.9	213.0	366.0
LSD 0.05 for:		White rot %		Plant fresh weight	
Application (A):		10.34		43.76	
Conc. (C):		11.94		50.53	
A X C interaction		20.68		NS	

The most superior treatments for decreasing onion white rot and increasing plant fresh weight were potassium silicate at 0.4% applied as the combined treatment of dipping & soil drenching and soil drenching only, as well as the combined treatment at 0.2% of potassium silicate which recorded 6.7% of white rot disease incidence and 90.9% disease reduction (%), as well as recorded 309.7, 263.5 and 254.1 of plant fresh weight g/pot with increasing (%) 577.7, 476.6 and 456.0%, respectively compared to untreated control (0%) of different treatments which recorded 73.3% of white rot disease incidence and 45.7 g/pot of plant fresh weight.

### 2. Effect of PS treatments on white rot disease and bulb yield of onion under field conditions:

Data presented in (Table, 2) stated that, in general, most potassium silicate treatments caused significant decreasing in onion white rot disease incidence and increasing in onion bulb yield compared to control treatment (0%) under natural infection with white rot pathogen. The highest concentration *i.e.* 4% of different applications was the best concentration in this regard, followed by 2% with insignificant differences between them of incidence of white rot disease only, whereas conc. of 1% was the least significant effective one compared with untreated control (0%). Regarding for potassium silicate applications, the combined treatment of dipping and soil drenching at different

concentrations was the best treatment in this regard, followed by soil drenching with insignificant differences between them for both decreasing white rot disease incidence and increasing onion bulb yield, whereas dipping only was the least significant effective one in this respect.

The most superior treatment for decreasing white rot disease incidence and increasing bulb yield of onion was the combined treatment of dipping & soil drenching and soil drenching only of potassium silicate at 4%, followed by the combined treatment at 2%, which recorded 5.3, 9.1 and 11.9% of white rot disease incidence and 87.7, 79.0 and 72.6% disease reduction (%), respectively as well as recorded 30.2, 25.9 and 22.7 kg/plot of onion bulb yield with increasing (%) 190.4, 149.0 and 118.3%, respectively compared to untreated control (0%) of different treatments which recorded 43.4% of white rot disease incidence and 10.4 kg/plot of onion bulb yield.

Under our investigation, all potassium silicate treatments tested have suppressive effects on disease incidence of onion white rot compared to control (0%) under artificial and natural infection with *S. cepivorum*. The different concentrations of combined treatment of dipping and soil drenching followed by soil drenching of potassium silicate was the best treatment in this respect, whereas dipping treatment only was the least effective one compared with untreated control (0%). The integration treatment of potassium silicate *i.e.* dipping & soil drenching at 0.4% was the most superior treatment in this respect, followed by soil drenching only at 0.4%. On the other hand, the most treatments of potassium silicate improved onion plant growth and onion bulb yield under infestation in pots and in field infection, respectively.

**Table 2:** Effect of potassium silicate treatments on white rot disease and bulb yield of onion under natural conditions in field season 2015/2016.

Potassium silicate treatments		White rot %	White rot reduction%	Yield Kg/Plot (10.5m <sup>2</sup> )	Bulb yield Increasing %
Application	Conc.				
Dipping only	0%	43.4	0.0	10.4	0.0
	1%	25.1	42.2	14.8	42.3
	2%	21.5	50.5	15.6	50.0
	4%	15.6	64.1	20.5	97.1
Mean	-	26.4	39.2	15.3	47.4
Soil drenching	0%	43.4	0.0	10.4	0.0
	1%	22.3	48.6	16.4	57.7
	2%	17.2	60.4	19.2	84.6
	4%	9.1	79.0	25.9	149.0
Mean	-	23.0	47.0	18.0	72.8
Dipping + Soil drenching	0%	43.4	0.0	10.4	0.0
	1%	16.7	61.5	18.9	81.7
	2%	11.9	72.6	22.7	118.3
	4%	5.3	87.8	30.2	190.4
Mean	-	19.3	55.5	20.6	97.6
LSD 0.05 for:		White rot %		Bulb yield	
Application (A):		4.41		4.02	
Conc. (C):		5.09		4.65	
A X C interaction		NS		NS	

These results in harmony with Nada *et al.* (2014), who stated that, PS was the most effective treatment than the other silicon sources tested *i.e.* calcium silicate and sodium silicate in reducing damping-off incidence and in improving plant growth parameters as well as seed yield and seed oil yield. Inhibition effects of silicon against pathogens in the soil culture of plant have been reported since the 1920s (Kanto *et al.*, 2006). Miyake and Takahashi (1983b) reported that both potassium silicate and calcium silicate suppressed Fusarium wilt of cucumber for 3 years more than sodium silicate. Kanto *et al.*, 2006 tested liquid PS as soil drench to control the powdery mildew of strawberry in the soil and they found that, the soluble PS suppressed the powdery mildew disease more efficiently as a protective control than as a control to diminish initial incidence. Also they measured strawberry leaf hardness for the control and silicate-treated leaves and found that leaves which treated by silicate was harder than control leaves. Jayawardana *et al.*, 2014 studied the root and the foliar application of soluble silicon as PS on the plant growth, fruit quality parameters and anthracnose



silicate 0.4% treated as soil dipping > potassium silicate 0.4% treated as soil drench > potassium silicate 0.4% treated as dipping + soil drench. Potassium silicate addition to soil through irrigation practice can affect microorganisms that interact with soil environment. Microbial populations in both diversity as well as numbers in soil are influenced by the amount and type of various compounds entering soil through plant litter, root exudates and management factors like mineral and organic fertilizers. This in turn affects crop production and sustainability of soil health (Nashwa *et al.*, 2015). The results show that bacterial CFU increased in treatments with 0.1% and 0.2% PS because of it can enhance the bacterial populations in the soil. A similar observation was reported by Wainwright *et al.*, (2003). PGPR in soil plays a key role in maintaining soil fertility by recycling the nutrient and promoting growth by acting as biofertilizer, biostimulant and bioprotectant (Gholami *et al.*, 2009). Hence, increasing the growth rate of these beneficial bacteria can enhance the plant growth.

**Table 3:** Effect of potassium silicate treatments on total bacterial, fungal and Actinomycetes count (colony forming unit) in onion rhizosphere during different growth intervals in field season 2015/2016

Potassium silicate treatments		After 60 days			After 90 days		
Application	Conc.	Bacteria (CFU/10 <sup>6</sup> )	Fungi (CFU/10 <sup>4</sup> )	Actinomycetes (CFU/10 <sup>4</sup> )	Bacteria (CFU/10 <sup>6</sup> )	Fungi (CFU/10 <sup>4</sup> )	Actinomycetes (CFU/10 <sup>4</sup> )
Control	0.0%	6.66	34.00	2.13	9.50	39.533	3.80
Dipping only	0.1%	14.70	26.33	2.07	21.26	24.16	7.06
	0.2%	11.56	21.33	2.40	25.36	14.96	5.73
	0.4%	15.26	8.86	1.53	19.57	11.53	2.23
Soil drenching	0.1%	22.03	14.33	3.06	26.13	8.63	6.06
	0.2%	24.00	10.33	2.40	30.56	6.70	7.46
	0.4%	21.03	8.66	1.53	14.06	2.90	1.60
Dipping + Soil drenching	0.1%	15.7	14.00	2.73	28.00	7.26	5.80
	0.2%	21.33	11.33	4.46	36.33	4.00	7.56
	0.4%	12.50	6.40	1.40	9.16	2.00	1.86

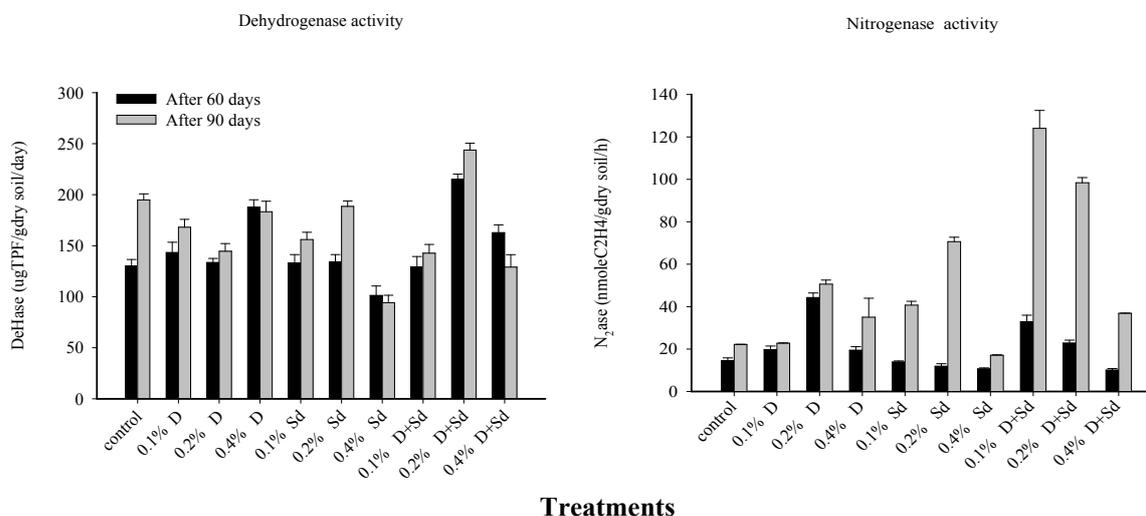
Several authors reported the capability of some bacterial species to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate. The populations markedly increased in 90 days more 60 days. The phosphate solubilizing activity is significantly affected by mineral input. As shown in table (4) Significantly high populations of phosphate dissolving bacteria were found in soil treated with 0.2% Potassium silicate treated as soil drench after both 60 and 90 days (78.3x10<sup>4</sup> and 137.67x10<sup>4</sup> respectively). The lowest populations recorded in 0.4% potassium silicate treated as dipping + Soil drench treatment (36.22. x10<sup>4</sup> and 57.00 x10<sup>4</sup>). The highest populations of silicate bacteria recorded with 0.2% Soil drench treatment (62.33 and 71.33 X 10<sup>4</sup> CFU after 60 and 90 days respectively). The observed low population of silicate bacteria represented mainly on *Bacillus circulans* group is an indicator of the sandy and alkaline pH nature of soil in this area. A comprehensive description of K solubilization from feldspar was investigated with 36 different K solubilizing isolates which indicated the K solubilizing efficiency that increases with decrease in pH and increase in viscosity and viable cell count (Anjanadevi *et al.*, 2016). Some bacterial isolates can solubilize silicates, phosphates and potash into soluble form by production of organic acids such as 2 keto-gluconic acid, alkalis and polysaccharides (Joseph *et al.*, 2015). Despite of the abundance of microorganisms in the soil, only a few are capable of solubilizing silicates. Naureen *et al.*, 2015 found that out of a total of 111 bacterial isolates, only 29 were able to solubilizing silicates. Efficient silicate solubilizing bacteria can release other essential nutrients in the soil. This could be due to solubilization of other minerals by silicate solubilizing bacteria directly or indirectly due to solubilized silicon itself. It has been earlier reported that the solubilized Si can improve the availability of phosphorus to plants by competing with P fixation sites in soil (Muralikannan and Anthomiraj, 1998).

**Table 4:** Effect of potassium silicate treatments on phosphate dissolvers and silicate bacteria population in the rhizosphere of onion plants during different growth intervals in field season 2015/2016

Potassium silicate treatments		After 60 Days		After 90 Days	
Application	Conc.	Phosphate dissolvers (CFU/10 <sup>4</sup> )	Silicate bacteria (CFU/10 <sup>4</sup> )	Phosphate dissolvers (CFU/10 <sup>4</sup> )	Silicate bacteria (CFU/10 <sup>4</sup> )
Control	0%	54.00	38.33	73.00	95.33
Dipping only	0.1%	67.00	53.34	101.67	62.00
	0.2%	71.34	37.66	95.67	60.67
	0.4%	55.00	30.66	93.66	76.00
Soil drenching	0.1%	63.34	52.65	108.32	51.33
	0.2%	78.00	62.33	137.67	71.33
	0.4%	63.34	32.00	54.34	32.67
Dipping + Soil drenching	0.1%	63.33	32.00	54.34	32.67
	0.2%	66.34	53.34	74.67	45.00
	0.4%	36.34	35.34	57.00	33.33

### 3.2. Soil enzymatic activities:

It considered as indirect indicator of microbial performance which are directly correlated with soil microbial dynamics. Enzyme activities in the soil ecosystem are considered to be a main contributor of soil microbial activity (De Forest *et al.*, 2012). Results in Figure (1) show that treatment with potassium silicate 0.2% treated as dipping + soil drench obtained the highest activity of dehydrogenase enzyme after 60 and 90 days, it recorded 214.82 and 243.4 µg TPF/g dry soil/day, respectively. Whereas treatment with potassium silicate 0.4% treated as dipping & soil drench treatment induced the lowest dehydrogenase activity in both periods (153.33 and 129.00µg TPF/g dry soil/day). The reduction of dehydrogenase activity in the soil treated with 0.4% PS treated as dipping + soil drench treatment), may be due to the increase of soil pH.



**Fig. 1:** Microbial biomass activity of Dehydrogenase and Nitrogenase as affected by Potassium silicate treatments, Dipping only (D), Soil drenching (Sd) and Dipping + Soil drenching (D+Sd)

In concern nitrogenase enzyme activity, the obtained results revealed that the activity of nitrogenase in soil rhizosphere at first period (60 days) is very low with all treatments, whereas in the second period (90 days) the activity increased markedly with 0.2% potassium silicate treated as dipping + soil drench treatment, it attained 78.50 nmole C<sub>2</sub>H<sub>4</sub>/g dry soil/h., followed by treatment with 0.1% potassium silicate treated as dipping + soil drench treatment (73.01 nmole C<sub>2</sub>H<sub>4</sub>/g dry soil/h) nitrogenase activity increased with increasing plant growth in all treatments compared with control.

This treatment could have a positive effect of PS in nitrogen fixation in soil. The role of K in numerous physiological and biochemical processes in the plant including photosynthesis, the translocation of assimilates, protein synthesis, maintenance of water balance, and promoting enzyme activities - are well-known (Marschner, 2012). In terms of practical importance of K for onion yield and quality has been reported (Yadav *et al.*, 2002). Also an sufficient K content of the bulb is important for storage quality of the crop. K deficiency in onion can cause the appearance of brown tips in older leaves and poor bulb formation. The application of an suitable quantity and source of K to onion at critical growth stages is thus essential for improving onion growth and quality (Raut, 2007).

### 3.3. Soil pH:

It is important to analyze the nature of soil by measuring its pH value before any cropping practices. Optimal pH facilitates microbial growth and improves soil nutrient value. It is well known that if the pH is in the extreme (alkaline or acidic), microbial population will be affected, which in turn affects soil nutrient value. The pH value can be used as biomarker for growth-based measurements in the bacteria and fungi as well as soil nutritional value (Rousk *et al.*, 2009). Figure (2) shows that there is no significant increase of soil pH was observed. In this study, no soil pH adjustments were made thus continuous application of increasing amount of potassium silicate to the soil may have increased soil pH leading to reduction of Si absorption. The concentration of Si in the soil solution is controlled by a pH dependant reaction and due to the adsorption of monosilicic acid at high pH by sesquioxides. When the pH increased the total amount of soluble Si decreased. The difference in Si accumulation varies with the plant species, and it has been attributed to the ability of the roots to take up Si from the soil (Ma and Takahashi, 2002).

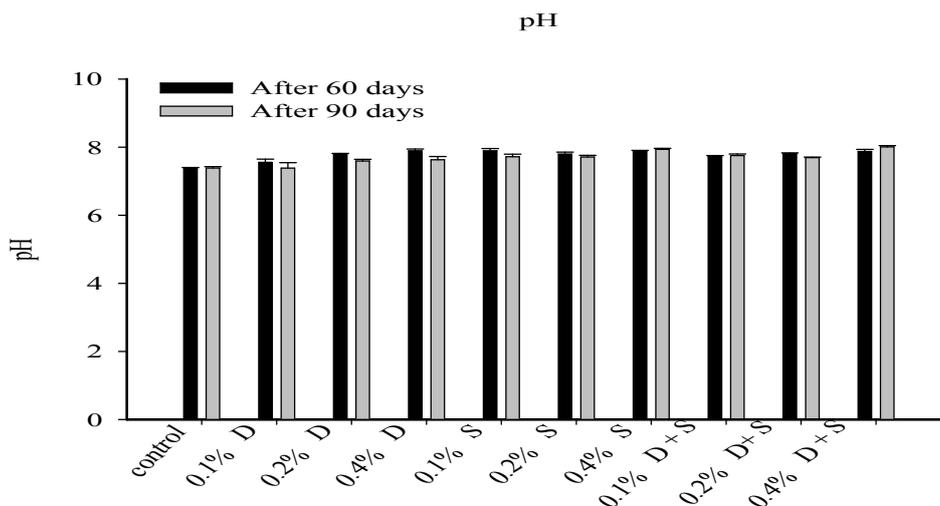


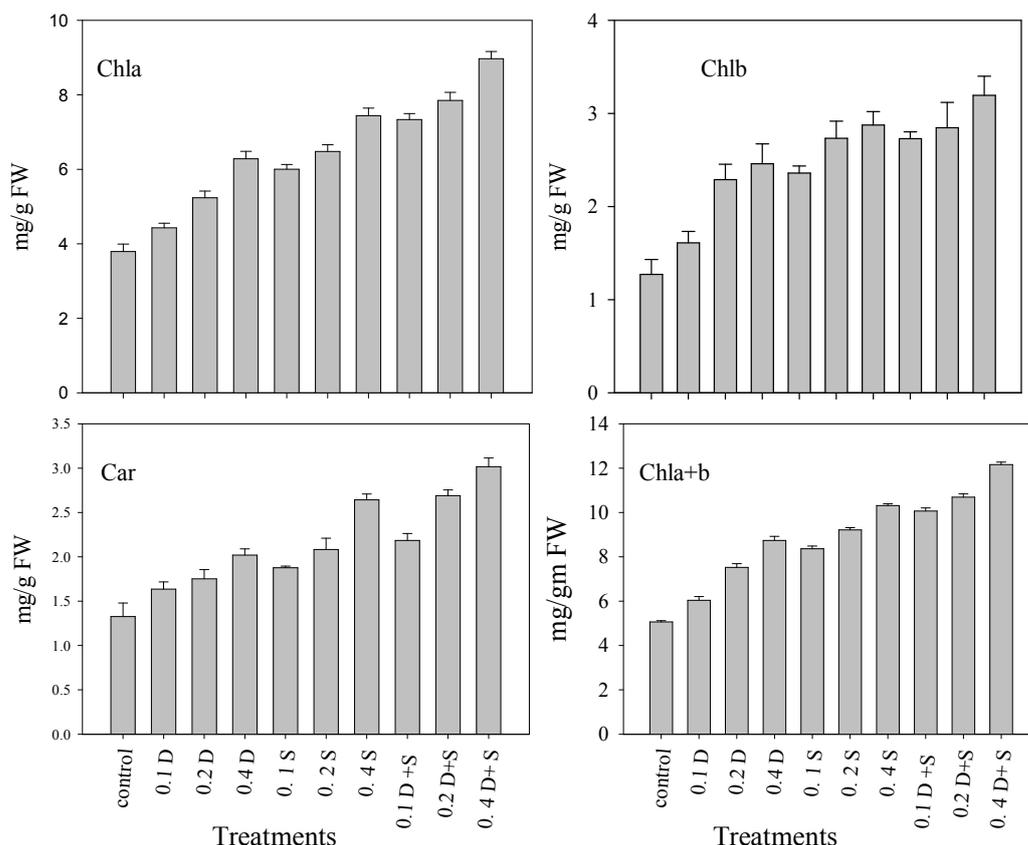
Fig. 2: Soil pH as affected by potassium silicate treatments. Dipping only (D), Soil drenching (Sd) and Dipping + Soil drenching (D+Sd)

## 4. Plant Biological activities of onion plants under white rot disease stress.:

### 4.1. Effect of potassium silicate treatments on photosynthetic pigments in onion plant:

Figure 3. shows the effect of PS treatments on photosynthetic pigments. In general, the application of potassium silicate was found to be effective in increasing the Chl a, b, carotenoids and total Chl concentrations in onion leaves. (potassium silicate 0.4% treated as dipping and soil drench treatment) significantly increased the activity of photosynthetic pigments compared to control. Since

silicon has an important role in leaves stability and able to expose more leaves to light so, it cause to increase of plants canopy photosynthesis efficiency (Quanzhi and Erming 1998). Quanzhi and Erming, 1998 found that silicon can cause leaf development and can cause improve photosynthesis. Agarie 1993 reported that silicon has a significant effect on photosynthetic rate and prevent the destruction of chlorophyll. Silicon also increased leaf area extends, that cause make more light available for photosynthesis implement. Whereas, the silicon shortage can cause reduction of chlorophyll amount in the plant leaves (Agarie 1993). This could be interpreted as possible effect of Si on the biosynthesis of new chlorophylls and the protection mechanisms of existing chlorophylls against salinity-induced oxidative stress (Shekari *et al.*, 2015).

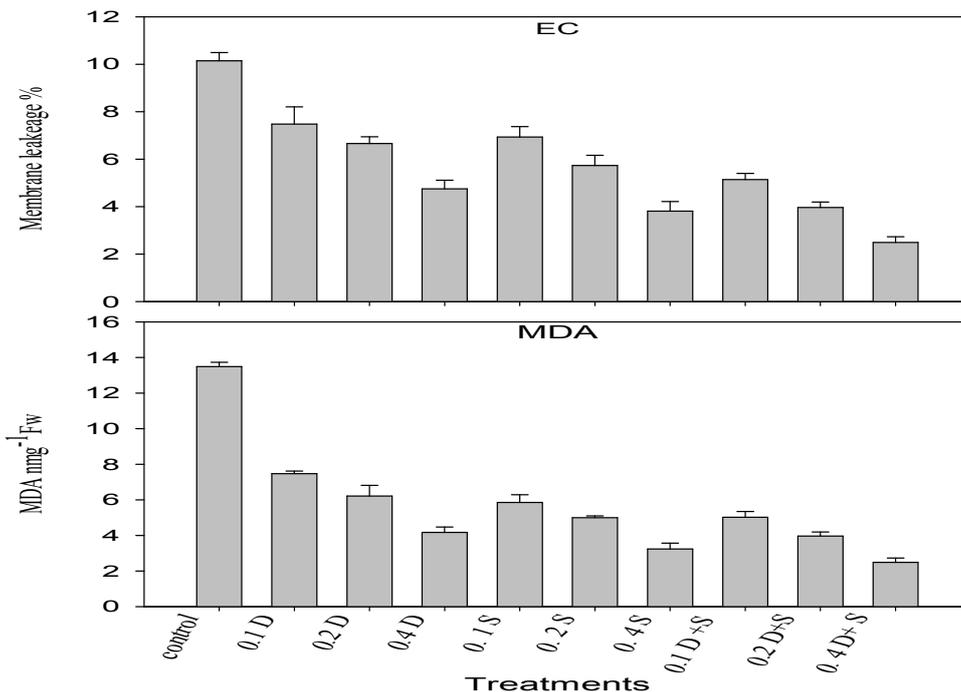


**Fig. 3:** Effect of potassium silicate treatments on photosynthetic pigments Dipping only(D), Soil drenching (S) and Dipping + Soil drenching (D+S). .

#### 4.2. Effect of potassium silicate treatments on malondialdehyde (MDA) and assessment of electrolyte leakage (EC) of onion plant:

In general, there is an increment of malondialdehyde (MDA) and other aldehydes interpreted as reason for an increased lipid peroxidation under white rot infection. As was expected the dipping and soil drench of potassium silicate ( $K_2O_3$  Si) 0.4% treatments on onion plants exhibited the highest reduction level of MDA. The data indicated that white rot infection induced an increase in the amount of MDA and other aldehydes (Figure 4). In order to investigate, the effect of white rot infection on membrane permeability, membrane leakage was measured after treatment. Base on the obtained results, Dipping and soil drench of ( $K_2O_3$  Si 0.4%) caused a highest decreasing on electrolyte leakage to intercellular space and reduced this leakage at all of potassium silicate ( $K_2O_3$  Si) levels (Figure 4). Plants exposed to different environmental stresses including disease infections exhibit changes in membrane permeability that lead to loss of membrane integrity (Blokina *et al.*, 2003). The combination of white rot infection with silicon nutrition in the form of potassium silicate via the

nutrient solution significantly ameliorated the impact of disease infection on membrane integrity and lipid peroxidation. The addition of potassium silicate-mediated a decrease in lipid peroxidation is attributed to its regulation of antioxidant defense in plants.



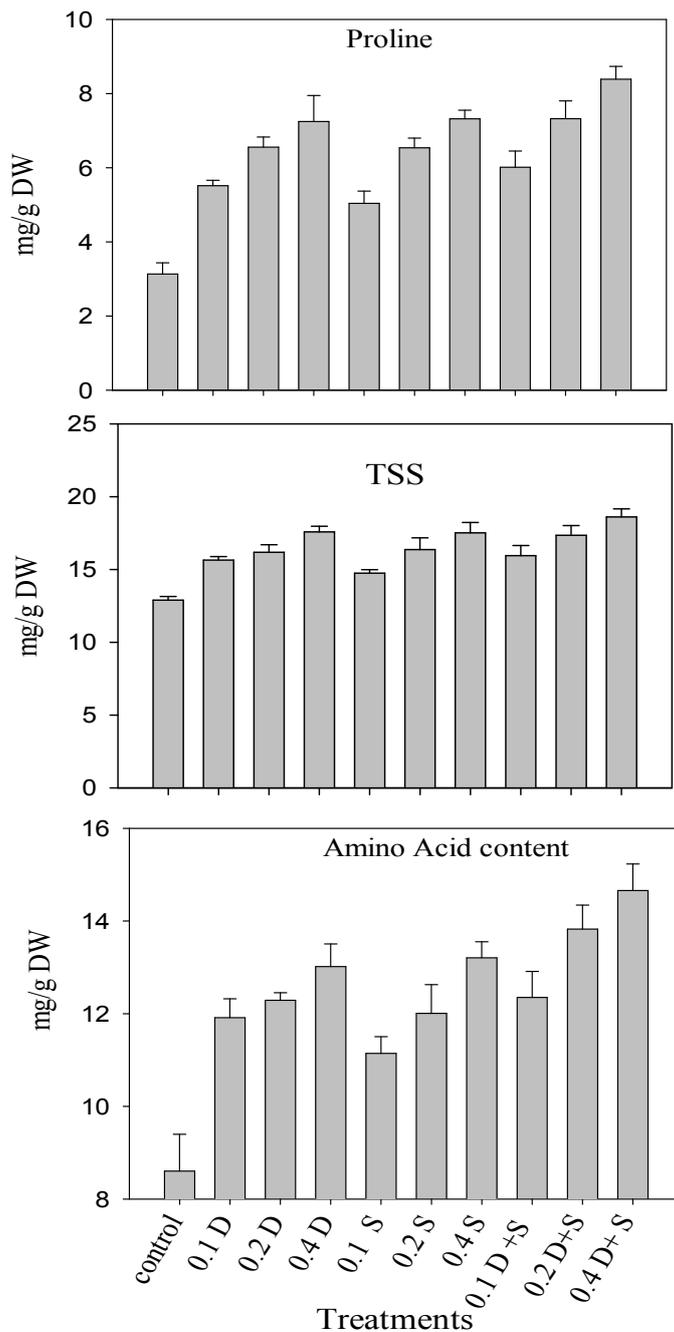
**Fig. 4. :** Effect of potassium silicate treatments on electrolyte leakage and malondialdehyde (MDA) under white rot disease stress.

#### 4.3. Effect of potassium silicate treatments on compatible solutes:

In this study, in response to white rot infection, supplementation of potassium silicate in the nutrient solution increased the proline content (Fig. 5). Similar results were obtained on soluble carbohydrates and TSS content. The highest accumulation of compatible solutes was at the application of potassium Silicate as (dipping and soil drench at 0.4%) compared with the control. The biosynthesis of the osmoregulators solutes is a physiological approach to increase disease tolerance in plants. Proline and sucrose usually accumulate in response to abiotic stress (Munns and Tester, 2008). Usually, proline and soluble carbohydrate content were significantly increased under stress conditions. Shekari *et al.*, (2015) found that addition of Si increased soluble carbohydrates content in dill plants grown under abiotic stress. Increase in soluble carbohydrates under abiotic stress was considered as an important way for protective mechanism by means of osmotic adjustment (Munns and Tester, 2008). Normally, accumulation of proline occurs in the cytoplasm where it causes cytosolic osmotic adaptation (Heuer, 2010).

#### 4.4. Effect of potassium silicate treatments on enzyme activities of onion plants:

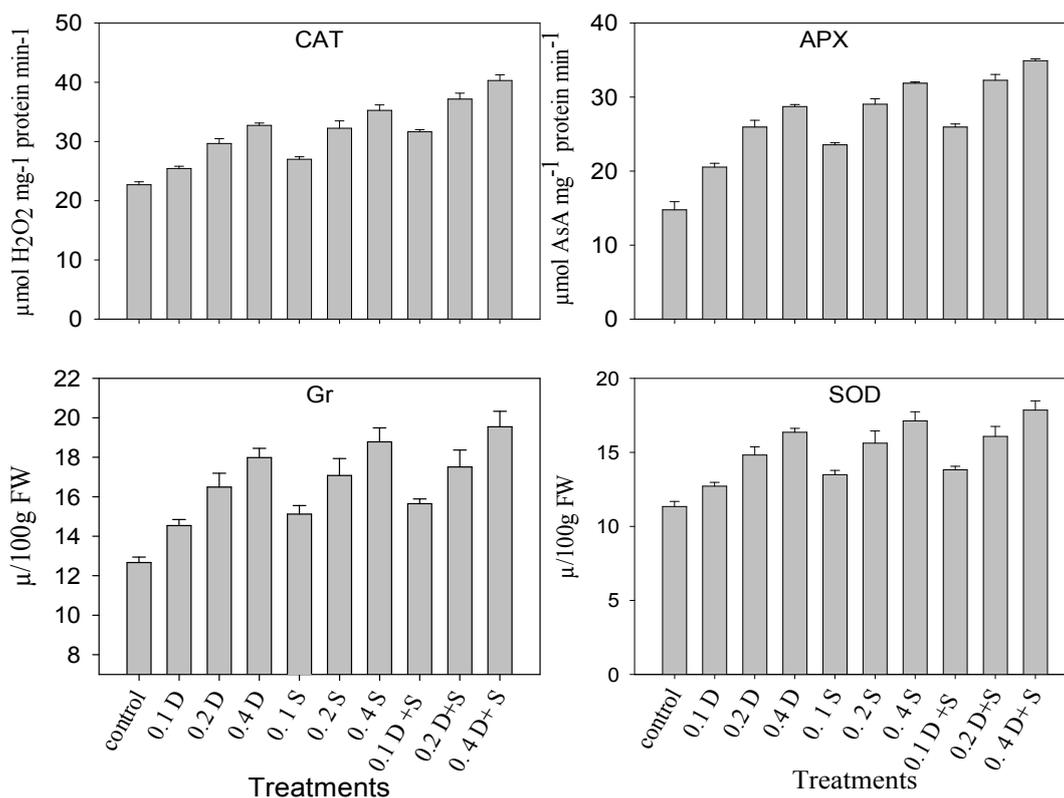
The Super Oxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX) and Glutathione Reductase (Gr) activity in the onion plants under white rot infection stress were increased after application of potassium silicate as dipping, soil drench and both dipping and soil drench. The greatest effect of our applications was dipping and soil drench at 0.4% concentration. According to the results of the present study, there are 136.6%, 54% and 68.3% increase in APX, SOD and Gr activity



**Fig. 5:** Effect of potassium silicate treatments on compatible solutes. Dipping only (D), Soil drenching (S) and Dipping + Soil drenching (D+S).

compared to the control, respectively. Adaptation to disease infection may depend on different mechanisms, including the capacity to maintain high levels of antioxidants and/or through the induction of antioxidant enzymes. In the present study, the activity of CAT, SOD, GR and APX in onion was increased in the leaves under white rot infection stress, while such an increase was more significant and consistent in potassium silicate 0.2% treated as dipping and soil drench treatment than other treatments. Similar are reported by Gong *et al.* (2005), who found out that under a biotic stress the adding of Si can increase the antioxidant activity in wheat. In metabolic processes plants produce  $H_2O_2$  which causes damage to the cell oxidation function, while CAT can eliminate  $H_2O_2$  and play a

key role in the elimination of O<sub>2</sub>. In this experiment, no significant change in the activity of CAT in plants subjected to white rot infection, when compared to the control. However, CAT activity was significantly elevated by K<sub>2</sub>Si treatment in the same conditions (Figure, 6). These results indicated that, higher constitutive levels of CAT suggest the more effective H<sub>2</sub>O<sub>2</sub> dismutation capacity outside the plant chloroplasts under potassium silicate treatments. CAT, plays an essential role in scavenging the H<sub>2</sub>O<sub>2</sub> toxicity, which is a major product produced by SOD. Together, CAT and SOD converts the toxic superoxide radical (O<sub>2</sub><sup>-</sup>) and H<sub>2</sub>O<sub>2</sub> to water and molecular oxygen (O<sub>2</sub>), thus prevention the cellular damage under unfavorable conditions like water stress (Noctor *et al.* 2000). APX plays a vital function in removing H<sub>2</sub>O<sub>2</sub>, dehydroascorbate reductase and glutathione reductase, can provide substrate for APX by a catalyzing reaction. In the present study, the activities of APX were increased in plants when exposed to disease infections (Liang *et al.*, 2005).



**Fig. 6:** Effect of potassium silicate treatments on enzyme activities of onion plants Dipping only(D), Soil drenching (S) and Dipping + Soil drenching (D+S).

Several studies have reported a link between the silicon supply and an improvement in the antioxidant metabolism of plants when they are infected by plant pathogens. The rapid production of reactive oxygen species (ROS) in the apoplast in response to infections by these pathogens has been proposed as one way in which a plant may orchestrate the establishment of defensive barriers, such as the strengthening of host cell walls via the cross-linking of glycoprotein, to delay host tissue colonization (Torres *et al.*, 2006). However, ROS are known to be toxic and can directly cause lipid peroxidation in the cell membrane, leading to a demand for increased capacity in the antioxidant system to scavenge them (Lamb and Dixon 1997). Lipid peroxidation was dramatically alleviated for the banana- *F. oxysporum* f. sp. *cubense*, cotton- *Ramularia areola*, rice- *P. oryzae*, sorghum- *C. sublineolum* and wheat- *P. oryzae* interactions, as indicated by the lower malonaldehyde concentration in plants that were supplied with silicon (Debona *et al.*, 2014 and Domiciano *et al.*, 2015). An increase in the activities of ROS-scavenging enzymes, such as ascorbate peroxidases, glutathione reductases, superoxide dismutases and catalases in plants receiving silicon restricted the ROS-dependent cellular damage that was indirectly linked to the high concentration of malonaldehyde (Domiciano *et al.*, 2015). In a proteomic analysis, Liu *et al.* (2013) found that the

quantities of ascorbate peroxidase, dehydroascorbate reductase and superoxide dismutase were reduced after *P. oryzae* infection, but they increased for rice plants that were supplied with silicon. Collectively, the findings of these authors clearly suggest the pivotal role that is played by silicon in managing the ROS generated in response to infection by plant pathogens through an efficient activation of the ROS-scavenging systems. By contrast, Debona *et al.* (2014) found that wheat plants that were supplied with silicon and infected by *P. oryzae* showed lower cellular damage and decreased superoxide dismutase, catalase, peroxidase, ascorbate peroxidase and glutathione- S-transferase activities, which was postulated to occur because of the activation of other mechanisms that limited leaf tissue colonization by the fungus, therefore reducing cellular oxidative stress.

## Conclusion

It is evident from this study that potassium silicate is considered one of resistance inducers that induced resistance for white rot disease of onion and enhanced yield productivity. As well as it triggers the growth of PGPR and increases total soil bacterial population, in addition to maintaining soil pH, enhancing soil enzymatic activities and promoting onion. Thus, potassium silicate can be suggested for inducer resistant and fertilizer formulations to control different diseases and make the soil more fertile and to improve beneficial soil bacterial community for better yield of crops. The application of potassium silicate can improve the physiological response of plant leaves as increased the photosynthetic pigments, compatible solutes, and enzyme activity then improve the ability to resist against white rot disease then increase the yield production of the onion plants.

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