INFLUENCE OF SULPHUR AND IRON OXIDIZING BACTERIA ON IMPROVING ENVIRONMENT FRIENDLY NUTRIENT BIO AVAILABILITY FOR STRAWBERRY

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Abstract

Iron is vital for plant growth and development however it accessibility is limited by various factors. The availability of iron in accessible form is crucial which is normally done with chemical chelates. These chelates make iron available but on other hand being highly stable they cause drinking water pollution threats. Sulphur is also another important element needed for plant growth. Recent studies showed positive influence of soil microorganisms on improving the fruit quality features. Thiobacillus thiooxidans are well though as soil amendments for improving the availability of macro and micronutrients such as Sulphur Iron, Zinc and Phosphorus. Thiobacillus ferrooxidans are vital for improving the iron availability in Soil. Objective of this study was to study the impact of bacteria in improving the nutrient availability in soil. The impact of bacteria on was evaluated by measuring the yield, fruit weight and sugars contents of strawberry fruits. The study showed that bacteria are effective soil amendments and the combination of two bacteria facilitated the availability and uptake of nutrient by plant in calcareous soils. The results showed that the solubility of iron is improved by the sulphur and iron oxidizing bacteria. It is also concluded that utilization of soil microorganisms can help facilitate in reducing the chemical fertilizer use maintaining the fruit quality. In addition to that it

can bring environmental, economic and human health benefits leading to increased sustainability.

Keywords: Sulphur oxidizing bacteria, iron oxidizing bacteria, Strawberry

Introduction

Iron being co-factor of many enzymes and metabolic pathways is crucial for plant growth and development and if deficient may cause malfunctioning of processes such as respiration and photosynthesis (Guerinot 2010; Zuo and Zhang 2011). Iron is termed as fourth most ample element in the earth's crust and usually present in excess amount in Soil. Iron in aqueous solution exist in two forms Fe^{2+} and Fe^{3+} however due to formation of insoluble oxides and hydroxides which limit the bioavailability $Fe3^{+}$ forms are not readily utilized by plants and microbes (Desai and Archana 2011; Zuo and Zhang 2011). An estimate predicted that about 33 % Soils are deficient in Iron (Yi et al. 1994).

Poor nutrient availability rather than lower nutrient contents major factor for nutrient deficiency especially in calcareous soils. Reducing soil pH is operative method for stabilization of nutrient availability in alkaline soils. Nutrients such as iron, zinc and phosphorus are pH dependent and their availability can be ensured through pH manipulation (Cifuentes et al., 1993; Deluca et al., 1989; Kaplan et al., 1998; Modaihsh et al., 1989 and Tisdale et al., 1993, Heydarnezhad et al., 2012). Oxidized Sulphur and sulfuric acid produced as result of it possess the ability to reduce the pH of soil to the small extent limited to their surroundings. This facilitates the release of essential elements in rhizosphere through the dissolution of insoluble food components (Besharati and Salehrastin, 1999; Ebadi, 1976, Heydarnezhad et al., 2012).

Rhizosphere acidification and secretion of phytosiderophores are two strategies adopted by plants for acquisition of iron (Altomare and Tringovska 2011; Guerinot 2010). However, these strategies failed to meet the iron needs especially when plants are grown in alkaline or calcareous soils. Therefore, providing plant with available forms is vital when it is unavailable in soils. Iron is vital element not only for plants but also for human health. According to WHO around two billion of world population are anaemic due to deficiency of iron. To cope with this problem consumption of fruits and vegetable rich in iron (www. who.int/nutrition/topics/ida/en/index.html). Microorganisms are commercially used for extraction of copper, zinc, uranium, nickel and cobalt from low grade or difficult to process sulfide ores through bioleaching process. This process is helpful in improving the efficiency of mineral processing industry through reduction in capital and processing cost and reducing environmental hazards like pollution risks from smelting operation. It is cost effective process for treating marginal value ores that are remote or have difficult accessibility. In addition to that they are also involved.

The two most common methods for iron delivery to plant are soil management and crop management. Crop management comprised of root and foliar delivery of iron in inorganic form ($FeSO_4$) or through synthetic and non-synthetic iron chelates (Ferna´ndez et al. 2005; Godsey et al. 2003). It is worth mentioning that as compared to inorganic form application of Fe chelates gives more promising results (Vempati and Loeppert 1988). Soil management on other hand involves fertilizing with ammonium and nitrate for pH alteration thus facilitating the iron uptake (Zuo and Zhang 2011). In addition to that using iron chelates and FeSO4 is another way of soil management. Fe-EDTA or Fe-EDDHA chelates application is effective and cheaper method (Shenker and Chen 2005) but it may pose negative impact on environmental (Adesemoye et al. 2009). In recent times the approach of utilizing environment friendly approach such as inoculants containing beneficial microorganism termed as Plant growth promoting bacteria (PGPB)

Iron is essential element involved in various cellular processes like co-factor of numerous enzymes and electron transport chain (Litwin and Calderwood, 1993). Microorganisms developing under aerobic conditions require iron for wide range of functions including oxygen reduction reaction, ATP synthesis, heme formation and other vital purposes. The aerobic atmosphere condition of planet cause oxidation of surface iron converting it to oxyhydroxide polymer which is insoluble and reduces the intensity of free iron.

Microorganism plays a role in solubilization and precipitation of Iron by growing them on account of energy gained either from oxidizing reduced iron or reducing ferric ions (Cameron et al., 1984). Iron Oxidizing Bacteria increase the Fe Oxidation rate in magnitude upto four orders in various type of environment as compared to Abiotic oxidation (Sogaard et al., 2001). FeOB serve as imperative catalyst for Fe Cycling despite little knowledge about their distribution and diversity in various environments (Wang et al., 2009).

Trick (1989) and Okujo et al. (1994) reports the siderophores secretion a material involved in iron solubilization by marine bacteria for facilitating the uptake of iron into microbial cells. Marine bacteria seize more biomass of iron as compared to major primary producers' phytoplankton.

The present study focused on isolation and characterization of Thiobacillus species to improve the agronomic practices in strawberry production with reference to optimization of strawberry growth, yield and nutritional value. The objective of the present work was to evaluate the effect of two Thiobacillus species Iron and Sulphur Oxidizing bacteria on yield and fruit quality (Sugars, Vitamins and Titrable acidity) to reduce the use of chemical fertilizers.

Materials and Methods

The experiment was carried out in Pots at Green House of Department of Horticulture, PMAS-Arid Agriculture University Rawalpindi Pakistan to examine the effect of iron and sulphur oxidizing bacteria for improving fruit quality characteristics of strawberry plants. Strawberry plant runners were obtained from SWAT, Pakistan. They were grown in medium sized pots containing a mixture of sand and farmyard manure. Two bacterial strains were used in the study. The bacteria strains Thiobacillus thiooxidans commonly known as Sulphur oxidizing bacteria (SOB) and Thiobacillus ferrooxidans were kindly provided by Prof. Dr. Ghulam Jilani (Institute of Soil Science, PMAS-Arid Agriculture University Rawalpindi Pakistan). The treatments were T_1 (Control), T_2 (IOB @ 50 ml per kg of soil), T_3 (SOB @ 50 ml per kg of soil) and T_4 (IOB and SOB @ 25 ml each per kg of soil).

Table 1: Chemical, Physical and nutritional status of soil used for experiments.

ParametersAnalysed	**Results**
Soil texture	Loam
Saturation	45%
pH	7.8
EC	0.80dS m^{-1}
Organic matter	0.76 %
Available P	3.2 mg kg^{-1}
Available K	100 mg kg^{-1}

Vegetative traits include height of plant (cm), Average leaves number and Area of leaf (cm^2) was observed while the reproductive traits such as fruit weight were observed. Fruit quality parameters estimation is vital for accessing the final quality of a commodity therefore parameters such as Total soluble solids (TSS), Sugars Contents (Total, Reducing and Non-Reducing) and Ascorbic acid contents were accessed in postharvest laboratory. The experiment was laid out in completely randomized design with 3 treatments and 3 replication and total 15 plants per treatments. The observations were taken, and results were compiled for treatment comparison. Uniform cultural practices were ensured for better growth and development of runners. The data was analyzed using the Statistic Software and means were compared using LSD (Least Square Difference) test at probability level of 5 % (Steel et al., 1997). The Soil and plant analysis was done at the end of experiment to determine the nutritional status of soil and strawberry plants (Table 2&3)

Table. 2: Soil Analysis for determination of nutritional status after Thiobacillus bacteria application

Sr #	**Sample**	**pH**	**Ec (dS/m)**	**OM (%)**	**K (mg/kg)**	**p (mg/kg)**	**NO3-N (mg/kg)**	**Fe (mg/Kg)**	**Mn (mg/Kg)**
1	SOB	7.17	0.303	1.77	162.21	1.85	5.47	3.75	1.02
2	FeOB	7.39	0.28	1.68	146.97	1.48	6.1	4.79	1.12
3	SOB+FeOB	7.34	0.26	1.83	151.6	1.61	5.98	4.46	1.18

Table. 3: Plant Analysis for determination of nutritional status of after Thiobacillus bacteria application

Sr #	**Sample**	N (%)	P (%)	K (%)	Fe (mg/Kg)	Mn (mg/Kg)	Cu (mg/Kg)
1	SOB	1.32	0.636	0.5	78.15	47.18	8.15
2	FeOB	1.1	0.547	0.411	132.45	54.12	13.18
3	SOB+FeOB	1.43	0.98	0.48	124.71	56.31	16.86

Results and Discussion.

The result pertaining to application of IOB and SOB to strawberry growth and development are discussed below.

Plant Growth Parameters

The vegetative growth parameters like plant height (cm), number of leaves per plant and leaf area were significantly affected by IOB and SOB application (Table 4). The treatment combining the effect of IOB and SOB as compared with the other treatments significantly improves the vegetative growth parameters (Table 4)

Table 4: Effect of Iron and Sulphur oxidizing bacteria on Vegetative/Preharvest parameters of strawberry.

Tr. No	**Foliar spray Iron Nanoparticles**	**Plant Height (cm)**	**Number of leaves/plants**	**Leaf Area (cm^2)**	**Fruit Weight (g)**
To	Control	13.267±0.07 B	4.2667± 0.07 B	67.200±0.69 B	11.133±0.07 C
T_1	IOB @50 ml per kg of soil	13.800±0.12 A	5.1333± 0.41A	71.600± 0.61 A	14.467±0.18 A
T_2	SOB @50 ml per kg of soil	13.667±0.07 A	4.8000±0.23AB	67.867± 0.74 B	13.667±0.13 B
T_3	IOB+SOB@25 ml each per kg of soil	13.667± 0.07 A	4.8667± 0.18AB	68.400± 0.80 B	13.733±0.07 B

*****Means sharing a different letter are significantly different

Chaudhary et al. (2019) found that microorganism residing in soil improve plant growth through provision of nutrients and providing defense against pathogen and stress. A positive response regarding plant growth was shown by mustard crop when inoculated with SOB. Inoculation of mustard seeds with SOB improved length, seed weight chlorophyll and oil content of mustard crops (Chaudhary et al., 2019). Microorganisms need iron owing to its utilization as Co-factor for metabolic enzymes and regulatory protein and they obtain it by reduction of Iron. However, some of them also drive energy through oxidation of iron (Chaudhary et al., 2019).

Iron is vital element for plants cycle however its solubility is conditioned by pH. Iron deficiency can cause economic losses to fruit sector by reducing fruit yield and quality (A´ lvarez-Ferna´ndez et al. 2006). Since Fe fertilization is expensive (Rombola´ and Tagliavini 2006) because its availability is ensured through synthetic chelates such as EDTA and EDDHA

(Orera et al. 2009). The above mentioned chelates are poorly degradable and pose environmental threats (Ferna´ndez et al. 2005), most common among these is ground water (Kaparullina et al. 2011). Regarding human though high concentration of EDTA are not toxic however its ability in binding toxic and heavy metals causes contamination of drinking water (Kaparullina et al. 2011). Moreover, wastewater treatment does not easily remove EDTA (Kari and Giger 1995). The need of finding alternative sources for iron supply is vital to provide iron to iron deficiency sensitive plants. Moreover, the necessity of natural products availability is need of time to ensure safe and sustainable agricultural farming

Post-Harvest Quality Parameters

The post-harvest parameters evaluated in this study were Total Soluble Solids (^{o}Brix), Titratable acidity (%), Ascorbic acid (mg/100ml of Juice), Total Sugars (%), Reducing sugars (%) and Non reducing sugars(%). Statistical analysis showed that the most remarkable difference was observed with iron and sulphur oxidizing bacteria application (Table 5). Minimum values were recorded in control.

Table 5: Effect of Iron and Sulphur oxidizing bacteria on post-harvest parameters of Strawberry.

Tr. No	**Foliar spray of Nanoparticles**	**TA**	**TSS**	**Ascorbic acid (mg/100 ml of Juice)**	**Reducing sugar**	**Non-Reducing Sugar**	**Total Sugars**
To	Control	1.5533±0.01 A	4.5800±0.05 C	11.813±0.12 C	4.5400±0.03 B	1.3767±0.02 B	5.9600±0.03C
T_1	IOB @50 ml per kg of soil	1.5400±0.01 A	5.5467±0.05 A	12.633±0.09 B	4.9100±0.07 A	1.4267±0.03 B	6.4667±0.03AB
T_2	SOB @50 ml per kg of soil	1.5533±0.01 A	5.3467±0.07 B	12.527±0.05 B	4.6200±0.03 B	1.5467±0.03 A	6.3667±0.05B
T_3	IOB+SOB@25 ml each per kg of soil	1.5533±0.01 A	5.2533±0.02 B	12.927±0.02 A	4.8300±0.05 A	1.5133±0.02 A	6.5333±0.01A

According to Wills et al. (1998) TSS refers to acids, protein, sugar soluble salts and supplementary dissolved substances of cell sap. In current study we found that the TSS was maximum at T_1 (IOB) followed by T_2 and T_3 and minimum at control as shown in the figure. The trend is similar in all studied parameters.

According to Besharati (2003) Thiobacillus bacteria are the most important sulfur oxidizing microorganism in the soil. S oxidation produced acidity which increases nutrient availability of nutrients like as Mn, P, Ca, Mg and SO_4 in soils (Lindemann *et al.,* 1991, Heydarnezhad et al., 2012). This might be the possible reason of better fruit quality in the strawberry.

Antioxidant enzymes

According to the statistical point of view, IOB concentration revealed the significant improvement in antioxidant enzymes viz., SOD, POD, and CAT of strawberry compared to control (Figure 1, 2 & 3). The treated plants showed better antioxidant activity as compared to the control. It may be attributed to improved plant growth because of their ability of scavenging the free radicals.

Antioxidant enzymes viz., SOD, POD and CAT are vital for providing protection against early senescence caused by reactive oxygen species (ROS) production. SOD plays role in O2 conversion to H2O2 molecules while the other two enzymes viz" CAT and POD further accelerate the process of converting hydrogen peroxide (H_2O_2) molecules providing a protective mechanism against lipid peroxidation (Farzpourmachiani *et al.,* 2013; Sharifzadeh, et al. 2014).

Sulphur is indispensable part of antioxidants and enzymes and may be helpful in forming a defense line against reactive oxygen and nitrogen species (Zembron-Lacny et al., 2007, Mukwevho et al., 2014). Therefore, availability of sulphur not only facilitates plant growth and quality but also protect plant from any adverse effect thy may occur during plant growth and development.

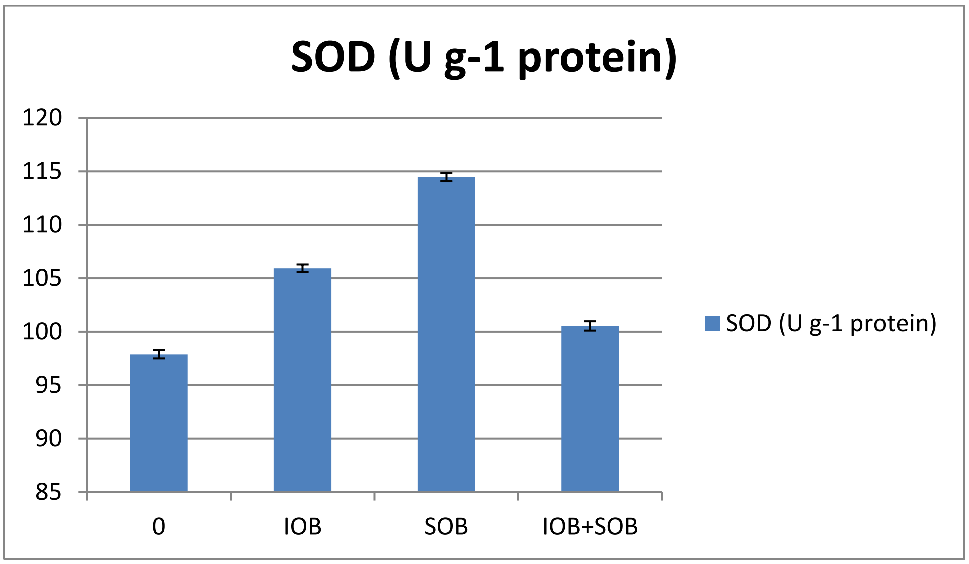

Figure 1: Effect of IOB and SOB on SOD activity

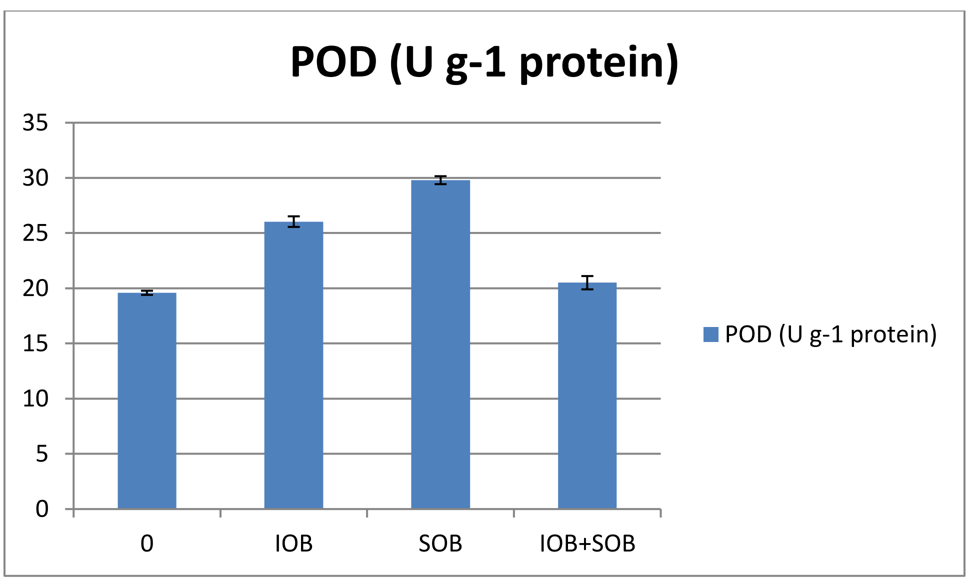

Figure 2: Effect of IOB and SOB on POD activity

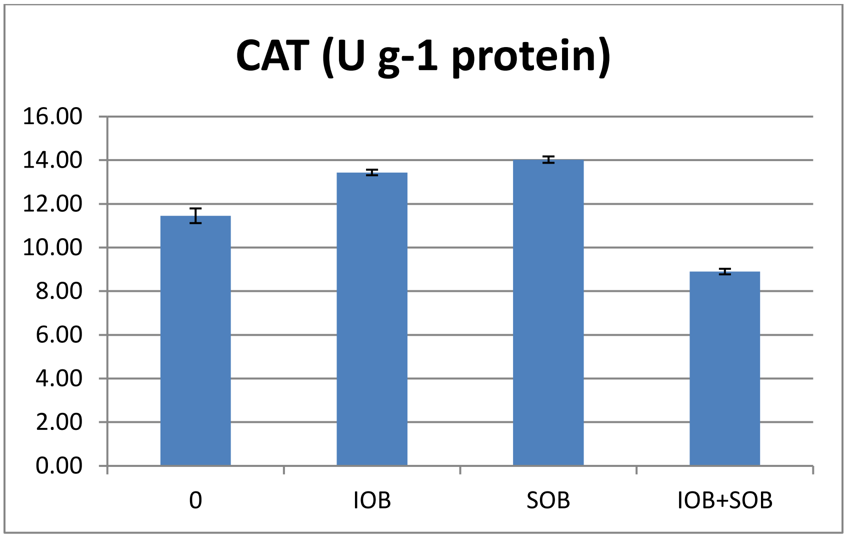

Figure 3: Effect of IOB and SOB on CAT activity

Conclusion

The results of current study show that application of beneficial soil microorganism is helpful in reducing chemical fertilization use and beneficial in maintaining and sometimes improving fruit yield and quality parameters. This activity of microbes provides sustainability and yields environmental, economic and human health benefits. Based on present study we can conclude that bacteria have diverse effects on plants if used alone or in combination however the mixed application (IOB and SOB) seems to be more effective on increasing production and improving the nutritional quality of fruit. Moreover, it is vital to test these bacteria on different plants to assess effect of microbes in more predictable manner.

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