PRINT: ISSN 0970-9274 ONLINE: ISSN 2456-6608
J Hum Ecol, 56(1,2): 42-54 (2016)
DOI: 10.31901/24566608.2016/56.1-2.05

RAPD Profiling of *Bacillus* spp with PGPR Potential and Their Effects on Mineral Composition of Tomatoes

Caroline F. Ajilogba and Olubukola O. Babalola*

Food Security and Safety Niche Area, North-West University, Mafikeng Campus, Private Bag X2046 Mmabatho 2735, South Africa

KEYWORDS *Bacillus* spp. Biocontrol. *Fusarium* Wilt. Minerals. PGPR. RAPD. Tomatoes

ABSTRACT *Bacillus* species have been known to antagonize and inhibit the growth of various plant pathogens but quality of crop from such plants is yet to be investigated. The aim of this study is to analyze the mineral composition of tomato to which *Bacillus* isolates were applied as a biocontrol agents whose relatedness and diversity has been profiled using the Random Amplification of Polymorphic Deoxyribonucleic acid (RAPD). Eleven *Bacillus* isolates were characterized by using RAPD primers and four of them, that is, *B. amyloliquefaciens, B. cereus, B. pumilus* and *B. subtilis* were randomly selected as antagonists against *Fusarium* wilt pathogen in tomatoes. Harvested tomatoes were analyzed for mineral composition using Energy Dispersive X-ray 720. Diversity of the eleven *Bacillus* isolates were profiled using primers S4, A9B7 and OPH 19 and 76 bands were produced. Bands from primer A9B7, OPH 19 and S4 were 90.9, 87.5 and 92.5 percent polymorphic, respectively. Plants treated with *B. cereus* had highest fresh and dry mass of tomato plant as compared to control, whereas, B. *amyloliquefaciens* treated plants had significantly bigger tomatoes with highest quantity of potassium, copper and rubidium. Tomatoes from treatment with *B. subtilis* had highest quantity of calcium while those from *B. pumilus* had highest quantity of manganese and rubidium. These results suggested that these *Bacillus* isolates can be used to promote plant growth and quality of tomato while their consortium can be researched.

INTRODUCTION

Tomato fruits are rich sources of vitamins, macro-minerals like iron, calcium, sulphur and especially potassium. They are rich in sugars (fructose and glucose), essential amino acids, organic acids and dietary fibers (Ajilogba and Babalola 2013; Tepic et al. 2006). Lycopene is found in red tomatoes (Calvo and Santa-Maria 2008) and it is an important antioxidant found to be effective against common cancers like breast cancer, coronary cancer, cancer of the lungs and prostate cancer (Sabio et al. 2003). Yellow tomatoes contain vitamin A, which is necessary for good eyesight (Miller et al. 2002).

Tomato plants suffer from a lot of soil-borne pathogens causing tomato diseases. The diseases could be bacterial like bacterial wilt, bacterial specks and bacterial canker, viral like the tomato spotted wilt virus (TSWV) and tomato yellow leaf curl virus, nematodal like root knot, and fungal like early blight, late blight and the *Fusarium* wilt.

Fusarium wilt destroys the vascular tissues of the plant and causes yellowing and final wilting of the plant, which later dies (Babalola 2010). It destroys plants very fast because its growth within the vascular tissues is swift. Before the outward symptoms begin to appear, internal destruction had gone far, so if it is not quickly treated the plant dies (Babalola 2008). Every tomato grower is faced with the danger of losses due to *Fusarium* wilt. Losses in tomato production caused by plant pathogens especially *Fusarium* spp fungus can reach fifty percent worldwide and cause severely limited production of food (Nirmaladevi and Srinivas 2012).

After the ban of methyl bromide from use in agriculture because of its threat to health and the environment, the need for other control measures of plant diseases has become inevitable. Initially other control measures like cultural methods and physical methods were used but they have been ineffective in controlling *Fusarium* spp under conditions conducive for the disease. Under such conditions, biocontrol is excellent, ecologically sound and a viable alternative to overcome the limitations of the other methods.

Biocontrol method using *Bacillus* spp has been effective in the inhibition of the growth of

*Address for correspondence:
Olubukola.Oluranti Babalola
Food Security and Safety, Niche Area,
North West University, Mafikeng Campus,
Private Bag X2046,
Mmabatho 2735 South Africa
Telephone: (+27) 18 389 2568,
Fax: (+27) 18 389 2134,
E-mail: Olubukola.Babalola@nwu.ac.za

tomato plant pathogens (Jetiyanon and Kloepper 2002; Jacobsen et al. 2004). Different *Bacillus* spp have been shown to suppress tomato diseases in various field trials (Choudhary and Johri 2009; Kloepper et al. 2004). Disease incidence and severity affects the yield of the tomato plant in quantity and quality.

Besides protecting the crop plants from microbial infection, these biocontrol agents have been reported to influence the mineral contents in the crop, which increases its nutritional quality. For example, Esitken et al. (2010), observed that the mineral content in strawberry increased after they were treated with three PGPB strains (*Pseudomonas* BA-8, *Bacillus* OSU-142 and *Bacillus* M-3) especially in terms of phosphorus and zinc content. Research has also shown that organically produced fruits contain more carbon-containing compounds such as sugars, vitamins and minerals (Lombardi-Boccia et al. 2004; Hallmann and Rembialkowska 2007). Since these biocontrol agents, that is, *Bacillus* spp are rhizospheric bacteria, it is assumed that they should also initiate the production of these compounds in plants treated with them. In spite of this, there is scarce data on the effect and impact of biocontrol agents on the mineral composition of tomatoes.

The mineral content of the tomato fruits from each treatment in this study will be analyzed by using the Energy Dispersive X-ray (EDX) 720. It uses the Scanning Electron Microscopy (SEM) technique. The beam from the electron using uniform energy activates the atoms in the sample, which automatically send out X-rays of specific energies for each element. The radiation from this X-ray is shown as spectra with different peaks, which give information about the elemental composition of the sample. For a particular energy (wavelength) of fluorescent light emitted by an element, the number of photons per unit time (generally referred to as peak intensity or count rate) is related to the amount of that analyzed in the sample. Therefore, by determining the energy of the X-ray peaks in a sample's spectrum, and by calculating the count rate of the various elemental peaks, the elemental composition and concentration of elements of the samples is established.

Objectives

In view of this, the present study was a follow-up on the biocontrol effect of different *Bacillus* spp on *Fusarium* wilt of tomato and seeks to,

- Determine the relatedness of these *Bacillus* spp species molecularly.
- Determine effect of these *Bacillus* spp species on the quality of the mineral composition of the tomato fruit.

METHODOLOGY

Preparation of Inoculum of *Bacillus* spp *In Vitro*

Inoculum of *Bacillus* isolates that is, *B. subtilis* (ATCC11774), *B. cereus* (ATCC11778), *B. amyloliquefaciens* (LIOBac179) and *B. pumilus* (LIOBac269) was prepared as described by Cavaglieri et al. (2005). These *Bacillus* strains were isolated in a previous study.

Greenhouse Experiment

Transplanted tomato seedlings (5 weeks old) were planted in pots that contained a combination of sterile peat, perlite and vermiculite. Seedlings were infected with *Fusarium solani* ATCC 36031 (Davies Diagnostic, South Africa) after inoculum was prepared as described in previous study by Ajilogba et al. (2013). Seedling infection was carried out after the roots of 5 weeks old seedlings were dipped in already prepared *Bacillus* inoculum. Samples were harvested at 10 weeks old and growth parameters were recorded. The growth parameters assessed include number of leaves and branches, fresh and dry weight of harvested tomato, fresh and dry weight of tomato shoot and root. Other parameters taken include the physical analysis of the tomato and moisture content. There were four trials comprising 120 plants in each trial of which 4 plants were assessed to determine each parameter per treatment (4 treatments). This experiment was carried out twice.

Determination of Number of Leaves and Branches

Number of leaves and branches were determined manually by counting. The leaves and branches on each plant were counted while taking the weekly readings and were recorded. The mean of the reading from each treatment were taken.

Determination of Fresh and Dry Weight of Plant Shoots and Roots

The fresh weight of seedlings of different treatments was determined. This was after recording the symptom development and percentage of infection. They were removed, washed with sterile distilled water, blotted with tissue paper, and weighed. Seedlings were then dried at 28°C for three weeks until the dry weights were stable and the dry weights taken.

Determination of Fresh and Dry Weight of Tomato Fruits

The samples were harvested at the end of 10 weeks to analyze the quality and mineral composition of the fruits. Freshly plucked tomatoes from each treatment were weighed and recorded. The colors were also noted. Tomato fruits were sliced transversely with a sterile scalpel and dried at 28°C for three weeks until the dry weights were stable and then recorded.

Determination of Mineral Composition of Tomato Fruits

Harvested tomato fruits were washed and dried with tissue paper. Each was sliced using a sterile scalpel. Each tomato sample was kept on sterile plates and air dried in the oven for 5 weeks and pounded separately in a clean sterile mortar. The pounded samples were taken into the EDX 720 to determine their mineral composition. The EDX 720 (Shimadzu Scientific Instruments, USA) is an analytical tool for the chemical characterization of a sample. The data generated by EDX analysis consists of spectra showing peaks corresponding to the mineral elements making up the true composition of the sample being analyzed. It measures a range of atomic elements from sodium (Na) (11) to uranium (U) (92) (Na-U) (potassium, calcium, sulphur, copper, manganese, iron and rubidium).

Statistical Analysis

Analysis of variance (ANOVA) was performed to find out if there was any difference between groups on specific treatments and the degree of significance of the differences among the variables (treatments) was determined using mean values considering the standard error of mean (SEM). Differences were calculated with Least Significant Difference (LSD) analysis (P=0.05) with the Statistical Package for the Social Sciences (SPSS) 11.0 between treatments. Duncan's LSD test at p=0.05 was used to compare the means. Data is reported in the text, tables and figures as mean values for each treatment per test or for all tests ± standard error of the mean (SEM), which is a statistical parameter measuring the accuracy with which a sample represents a population. The smaller the standard error, the more representative the sample will be of the overall population.

Physical Analysis

The physical colors of tomatoes were analyzed by grouping them into colors visually as red, light red, orange and light orange.

Random Amplification of Polymorphic Deoxyribonucleic acid (RAPD) Profiling of *Bacillus* isolates

DNA Extraction from Bacterial Isolates

Genomic DNA of all isolates (Table 1) was extracted using the ZR soil Microbe DNA Mini-Prep™ (Zymo Research, USA) extraction kit. This was carried out according to the manufacturer's manual.

Table 1: Molecular description of *Bacillus* isolates

Organis	*Description*	*Isolate accession/ mlegend assigned accession number*
Bac A	*B. amyloliquefaciens*	KF433086
Bac C	*B. cereus*	KF433085
Bac P	*B. pumilus*	KF433087
Bac S	*B. subtilis*	KF433084
Bac 282	*B. subtilis*	KF433088
Bac Bb	*Brevibacillus* sp	JX971521
Bac M5	*B. mojavensis*	JX971525
Bac M8	*B. methylotrophicus*	JX971526
Bac S12	*B. subtilis*	JX971518
Bac S35	*B. safensis*	KC113510
Bac T51	*B. cereus*	KC113513

RAPD-PCR Analysis and Agarose Gel Electrophoresis

Three primers were used in this study, namely, OPH-19 (5'-CTGACCAGCC-3'), S4 (5'-GTCGC-

CGTCA-3') and A9B7 (5'-GGTGACGCAGGGG-TAACGCC-3') (Inqaba biotechnology SA). DNA amplifications were carried out in a total volume of 25 µl (1.5mM, PCR Buffer, 2.5mMMgCl$_2$,, 0.2mM (each) dNTP, 50 ng primer, 5 U Taq DNA polymerase (Fermentas), 20 mM Tris-HCl, pH 8.4containing 50 mM KCl), 50 ng template DNA, and ddH_2O according to Zhao (2006). The cycling conditions for RAPD carried out using a C1000 Touch™ thermal cycler (Bio-Rad, USA) were as follows: an initial denaturing at 94°C for one minute, 40 cycles of denaturation at 94°C for one minute, annealing at 36°C for one minute, and 72°C for two minutes. A final extension of 10 minutes at 72°C was included.

Agarose Gel Electrophoresis

PCR products were separated on the agarose gel electrophoresis. The outcome of running the gel was recorded/captured using the Chemidoc™ MP imaging system (Bio-RAD USA).

Data Analysis of RAPD-PCR

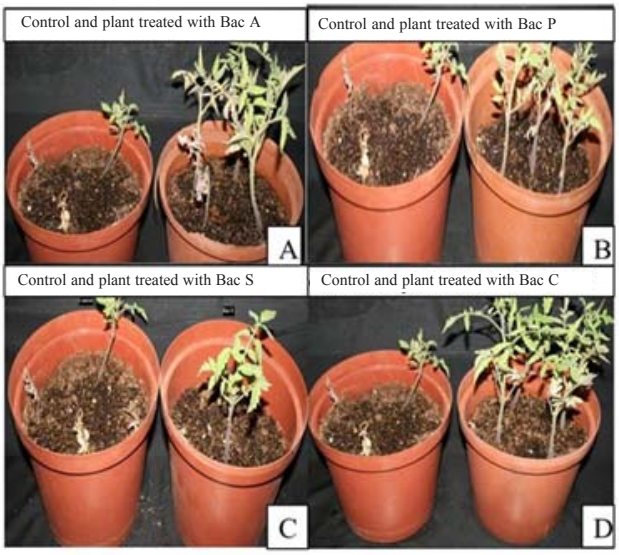
Derivation of Dendrograms from the Gel Pictures

In order to derive dendrograms from gel pictures, a scoring matrix was created for the gel. Then the bands were named by their primers and isolate number, for example, a1 means primer A9B7 was used and 1 is isolate 1. On the columns, presence of bands is represented as 1 while absence of band is represented as 0. Once this is done, the site below is opened and the value in column 1 is put in as 1, column 2 in as 2 and this is done until the entire column values are exhausted. When this is submitted, the similarity matrix is automatically constructed and phenogram is selected, which draws the dendrograms (http://genomes.urv.cat/UPGMA/index.php? entrada= Example 2).

RESULTS

Effect of *Bacillus* Isolates as a Biocontrol Agent on *Fusarium* Wilt

This is evidenced in the growth of the plants treated with the *Bacillus* isolates and the control that is those not treated with *Bacillus* spp but only infected with *Fusarium solani.* In treatment with *B. amyloliquefaciens*, out of four plants, one was dead, one was not fully wilted while the remaining two plants were still green. In treatment with *B. pumilus*, one of the four plants was dead while in treatment with *B. subtilis*, two out of four plants were dead and in treatment with *B. cereus*, four plants were all green compared to the control, which had all dead plants except 1 (Fig. 1).



Each treatment is compared with the control where there is no treatment. Bac A=*B. amyloliquefaciens*, Bc C=*B. cereus.* Bac P=*B. pumilus,* Bac S=*B. subtilis* in 'A' above, our of the four plants, I was dead, I was not fully wilted while the remaining 2 plants were still green. In B, one of the four plants was dead while in 'C', 2 out of the 4 plants were dead and in 'D'. the four plants wee all green comared to the control whih had all dead plants except 1.

Fig. 1. Biocontrol of Fusarium wilt in which the treatments (A, B, C, D) and *F. solani* were simultaneously inoculated into the tomato plant roots

The Number of Branches of Tomato Plants Treated with *Bacillus* Isolates

Tomato plants treated with *B. subtilis* had 15 branches, which was the highest number of branches compared with the control having six branches. It was significantly different from the number of branches in the control treatments with *B. amyloliquefaciens*, *B. cereus* and *B. pumilus*, which are 11, 8 and 5, respectively.

The Number of Leaves on Tomato Plants Treated with *Bacillus* Isolates

The number of leaves was not necessarily determined by the number of branches. This was seen in treatment with *B. amyloliquefaciens* having 77 leaves as the highest number of leaves but with 11 branches compared to *B. subtilis* with the highest number of branches but 65 leaves. Both of them are significantly different from the control with 45 leaves and treatment with *B. pumilus* and *B. cereus* with 26 leaves and 56 leaves, respectively (Table 2).

Fresh Mass of Shoot of Tomato Plants Treated with Bacillus Isolates

The fresh mass of shoots of plants treated with *B. cereus* was 5.2 g and significantly higher than the control having 1.5 g and the other treatments, *B. pumilus* with 2.1 g, *B. subtilis* with 2.3 g and *B. amyloliquefaciens* with 2.8 g. There was no significant difference between treatments with *B. amyloliquefaciens*, *B. cereus* and *B. Subtilis* but there was significant difference between *B. amyloliquefaciens* and control (Table 2).

Fresh Mass of Root of Tomato Plants Treated with *Bacillus* Isolates

There was a significant difference between the root fresh mass of control and those treated with *B. cereus* having 1.4 g and the control having 1.2 g. There was no significant difference between the other treatments and the control (Table 2).

Dry Mass of Shoot of Tomato Plants Treated with *Bacillus* Isolates

The dry mass of shoots treated with *B. cereus* with 0.9 g was significantly higher than the oth-

Table 2: Effect of various treatments on plant growth parameters in tomato plants treated with *Bacillus* isolates and infected with *F. solani*.

Treatments	*Number of branches*	*Number of leaves*	*Shoot fresh mass (g)*	*Shoot dry mass (g)*	*Root fresh mass (g)*	*Root dry mass (g)*	*% dry weight*	*% moisture content*
Bac AF	11± 0.81c	77± 4.70d	2.8± 0.38b	0.5± 0.03b	1.2± 0.01a	0.1± 0.00d	12.6± 1.42a	87.3± 1.42a
Bac CF	8± 0.40b	56± 3.2c	5.2± 0.15c	0.9± 0.01c	1.4± 0.04b	0.2± 0.01e	9.9± 1.42ab	90.1± 1.42ab
Bac PF	5± 0.00a	26± 3.65a	2.1± 0.10ab	0.2± 0.07a	1.2± 0.00a	0.1± 0.00c	9.3± 1.42b	90.1± 1.42b
Bac SF	15± 0.81d	65± 4.02c	2.3± 0.46ab	0.3± 0.11a	1.2± 0.33a	0.1± 0.00b	9.2± 1.42b	90.7± 1.42b
Control	6± 0.00a	45± 0.91b	1.5± 0.21a	0.1± 0.00a	1.2± 0.01a	0.1± 0.00a	7.4± 1.42b	92.5± 1.42b

Percent dry weight and wet weight of tomato plants treated with the four *Bacillus* isolates are mean values of 4 replicates ± SE. values with different letters are significantly different at $P = 0.05$ by LSD. treatment of *F. solani* with Bac A (BAC AF), *F. solani* with Bac C (BAC CF), *F. solani* with Bac P (Bac PF), *F. solani* with Bac S (BAC SF), only infection with *F. solani* (FSP)

er treatments and significantly different from them. The shoot dry mass of plants treated with *B. amyloliquefaciens* of 0.5 g was also significantly higher than that of the control, which was 0.1 g, *B. pumilus*, which was 0.2 g and *B. subtilis*, which was 0.3 g. Treatments with *B. pumilus* and *B. subtilis* were not significantly different from each other and the control (Table 2).

Dry Mass of Root of Tomato Plants Treated with Bacillus Isolates

Treatment with *B. cereus* had the highest root dry mass of 0.2 g and was significantly different from all the other treatments and the control. The control had the smallest root dry mass with 0.1 g and all the other treatments, *B. cereus* with 0.1 g, *B. pumilus* with 0.1 g and *B. subtilis* with 0.1 g were all significantly higher than the control (Table 2).

Physical Analysis

Fruits from the different samples showed variation in their physical and chemical/mineral compositions. Physical analysis of the tomato fruits such as the color of the tomato fruits ranged from light orange to orange to light red and red. The red color shows the presence of lycopene while in yellow fruits, lycopene is in smaller amount but there is the presence of vitamin A (Table 3 and Fig. 2). Tomatoes from plants treated with *B. amyloliquefaciens* were significantly bigger with dry weight of 12.6 percent of the wet weight compared to the other treatments and the control whose value was 7.4 percent. Plants treated with *B. cereus* had tomatoes whose percent dry weight was 9.9 percent of the wet weight

Table 3: Effect of treatments on tomato fruit color

Treatment	*Description of fruit*
Bac A + *F. solani*	Orange
Bac C + *F. solani*	Light orange
Bac P + *F. solani*	Light red
Bac S + *F. solani*	Light red
Negative control (NBNF)	Red
Positive control (*F. solani*)	Light orange

Tomato plants treated with the four *Bacillus* isolates have variation in colour of their fruits. tomato colour ranged from orange to red based on the different *Bacillus* isolates used for the treatment. treatment of *F. solani* with Bac A (BAC AF), *F. solani* with Bac C (BAC CF), *F. solani* with Bac P (Bac PF), *F. solani* with Bac S (BAC SF), only infection with *F. solani* (FSP) and no treatment with *Bacillus* isolates and no infection with *F. solani* (NBNF).

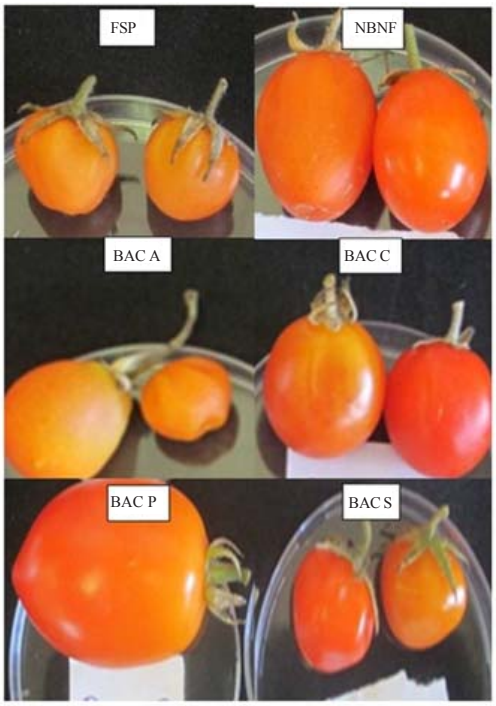


Fig. 2. Effect of various treatments on the yield of the tomato fruits.
The different Bacillus isolates had efect on the colour of the tomato ranging from orange to red. Bac A= *B. amyloliquefaciens*, Bac C=*B. cereus*, Bac P= *B. pumilus*, Bac = *B. subtilis*, FSP= Plants only infected with *F. solani*, and NBNF=No treatment with *Bacillus* isolates and no infection with *F. solani*
Source: Author

and significantly different from and bigger than those of *B. pumilus* and *B. subtilis* whose percent dry weight were 9.3 percent and 9.2 percent, respectively of the wet weights (Table 2). The percentage moisture content of tomato fruit also ranged from 92.5 percent in control to 87.3 percent in treatment with *B. amyloliquefaciens*. This was significantly different compared with the control and the other treatments except for plants treated with *B. cereus* having 90.1 percent (Table 2).

RAPD Analysis of *Bacillus* Isolates used for Biocontrol of Tomato *Fusarium* Wilt

Three oligonucleotide primers used showed clear and sharp bands for the 11 *Bacillus* isolates. Primers S4 and OPH-19 are decamers while primer A9B7 is a 20-mer primer. The total number of bands or fragments scored from the three prim-

ers was 76 and they were used for the estimation of genetic diversity from the eleven *Bacillus* isolates (Tables 4 and 5).

Table 4: Number of bands and percentage of DNA polymorphic bands in eleven *Bacillus* isolates amplified with three primers

Primer	*Primer sequence*	*Number of fragments scored*	*Percentage of polymorphic loci (%)*
S4	5'-GTCGCCGTCA-3'	27	92.5
OPH-19	5'-CTGACCAGCC-3'	16	87.5
A9B7	5'-GGTGACGCAGG GGTAACGCC-3'	33	90.9

Table 5: Number of bands per isolate from each of the primers

Isolates	*Number of fragments scored*			
	Primer A (A9B7)	*Primer S4*	*Primer OPH (OPH 19)*	*Total*
Bac A	5	2	4	11
Bac C	3	3	2	8
Bac P	0	3	1	4
Bac S	3	3	0	6
Bac 282	3	0	2	5
Bac Bb	3	3	2	8
Bac M5	4	2	2	8
Bac M8	3	3	1	7
Bac S12	3	2	0	5
Bac S35	3	3	2	8
Bac T51	3	3	0	6
Total	33	27	16	76

Molecular Characterization using RAPD-PCR Primer A9B7

A 20-mer primer of arbitrary sequence 5'-GGTGACGCAGGGGTAACGCC-3' was used. Products of the RAPD-PCR yielded varying sizes and they were separated by gel electrophoresis. The dendrogram obtained from the RAPD marker showed 6 classes of clusters, which showed relatedness (Fig. 3). Cluster analysis revealed that out of the eleven *Bacillus* isolates, ten of them paired in twos with each other, *B. amyloliquefaciens* paired with *B. subtilis*, *B. cereus* paired with *Brevibacillus* sp, *B subtilis* 282 paired with *B. safensis* S35, while *B. mojavensis* M5 and *B. methylotrophicus* M8 also formed a pair. *B. subtilis* S12 and *B. cereus* T51 showed close similarity and also formed a pair. *B. pumilus* did not cluster with the others, which show it is either from the same strain or a different species.

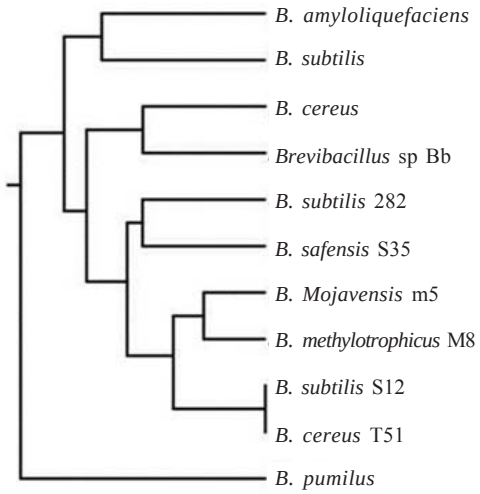


Fig. 3. Dendrogram from unweighted pair group method with arithmetic averages showing relationships among the 11 *Bacillus* isolates
Source: Author

Molecular Characterization using RAPD-PCR Primer OPH 19

OPH 19 is a decamer primer with arbitrary sequence of 5'-CTGACCAGCC-3'. The dendrogram clearly depicts 5 major clusters (Figure. 4).

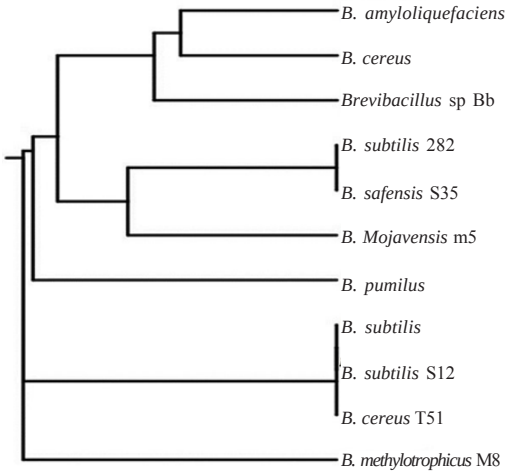


Fig. 4. Dendrogram from Unweighted pair group method with arithmetic averages showing relationships among the 11 *Bacillus* isolates
Source: Author

Only *B. pumilus* and *B. methylotrophicus* M8 formed individual clusters. *B. amyloliquefaciens*, *B. cereus* and *Brevibacillus* sp formed a cluster, *B. subtilis*, *B. safensis* S35 and *B. mojavensis* M5 formed another cluster while *B. subtilis*, *B. subtilis* S12 and *B. cereus* T51 also formed a different cluster.

Molecular Characterization using RAPD-PCR Primer S4

The primer S4 is a decamer with the arbitrary sequence of 5'-GTCGCCGTCA-3'. The dendrogram revealed that there were 2 basic clusters while the other five groups had only one isolates each (Fig. 5). Primer did not display very sharp reproducible bands but it also reveals that though the isolates were from the same root, they are different strains. Primers A9B7, OPH19, S4 were able to complement one another in revealing the relationship between the eleven isolates. This is seen in isolate *B. pumilus*, which was not shown to produce any bands using primer A9B7 but produced sharp bands using primers S4 and OPH 19.

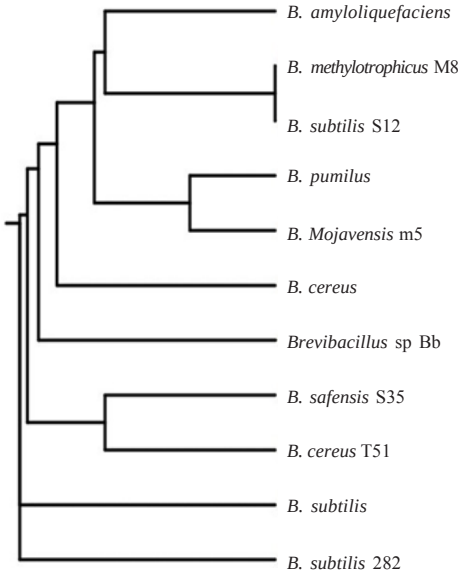


Fig. 5. Dendrogram from Unweighted pair group method with arithmetic averages showing relationships among the 11 *Bacillus* isolates

Quantitative Results for Mineral Composition of Tomato Fruits from Each Treatment

The minerals of interest in the tomato samples analyzed included potassium (K), calcium (Ca), sulphur (S), iron (Fe), manganese (Mn), rubidium (Rb) and copper (Cu) (Table 6). The results were converted to mg/g. The analysis shows that the mineral that was mostly abundant in all the fruits is K (Table 6). Plants treated with *B. amyloliquefaciens* had tomatoes with the highest composition of K, which is 3.8 mg/g while the control and plants treated with *B. cereus* had the least with 1.8 mg/g and 1.0 mg/g, respectively. Plants treated with *B. subtilis* had the highest composition of Ca, which is 0.7 mg/g compared with the control of 0.1 mg/g, which is the least. Plants treated with *B. pumilus* had the highest composition of S and Fe of 0.1 mg/g and 0.03 mg/g, which is not significantly different from the control and others having 0.10 mg/g and 0.02 mg/g, respectively. Cu (0.06 mg/g) and Mn (0.01 mg/) were found only in tomatoes whose plants were treated with *B. amyloliquefaciens* and *B. pumilus* respectively while both treatments has tomatoes composed of 0.01 mg/g of Rb.

DISCUSSION

In the screen house experiment, it was observed from this study that disease incidence

Table 6: Mineral analysis of tomato fruits from the different treatments

Treatments	*K (mg/g)*	*Ca (mg/g)*	*S (mg/g)*	*Fe (mg/g)*	*Cu (mg/g)*	*Rb (mg/g)*	*Mn (mg/g)*
Bac AF	3.8±0.08	0.4±0.08	0.1 ± 0.03	0.02±0.00	0.06±0.0	0.01±0.00	-
Bac CF	1.0±0.07	0.2±0.07	0.03 ± 0.02	-	-	-	-
Bac PF	2.7±0.08	0.4±0.08	0.1 ± 0.03	0.03±0.00	-	0.01±0.00	0.01±0.00
Bac SF	3.5±0.11	0.7±0.11	0.1 ± 0.055	0.02±0.00	-	-	-
FSP	1.8±0.08	0.1±0.09	0.1 ± 0.04	0.01±0.06	-		-

Four minerals were common to all samples i.e. K, Ca, S and Fe. Values are mean of 4 replicates ± SE. Values with different letters are significantly different at *P*= 0.05 by LSD. Treatment of *F. solani* with Bac A (BAC AF), *F. solani* with Bac C (BAC CF), *F. solani* with Bac P (Bac PF), *F. solani* with Bac S (BAC SF), only infection with *F. solani*(FSP) andno treatment with *Bacillus* isolates and no infection with *F. solani* (NBNF).

was reduced and growth parameters were better compared to the control. The four treatments effectively controlled disease and improved plant growth, which was evident in the growth parameters compared to the control. This evidence is in agreement with Gardener (2004) who emphasized that diseases control is observed whenever *Bacillus* spp by products and *Bacillus* spp are used as inoculum that are applied to plants.

Disease incidence was highest in treatment with *B. subtilis* and *B. pumilus* and disease control was least in both treatments. Plant growth promoting activities in terms of number of leaves and branches, percentage dry weight, fresh and dry shoot mass were also the least. Disease incidence was least in treatment with *B. cereus* while disease control and plant growth promoting activities were highest. In treatment with *B. amyloliquefaciens*, disease incidence was higher than treatment with *B. cereus* but lower than treatment with *B. subtilis* and *B. pumilus.* Plant growth promoting activities of *B. amyloliquefaciens* were lower than treatment with *B. cereus* but higher than treatments with *B. subtilis* and *B. pumilus*. The dry weight of treatment with *B. amyloliquefaciens* was the highest compared with the other treatments and the control. This shows that the higher the plant growth promoting activities of the treatments the lower the disease incidence and higher disease control.

Dry Matter Content of Tomatoes from Different Treatments

The dry matter of tomatoes in this study ranged from 12.6 percent in treatment with *B. amyloliquefaciens* to 7.4 percent in the control. This is slightly higher compared to those reported by Majkowska-Godomska et al. (2008) and Turhan and Sentz (2009) (7%-3.8%) but similar to those reported by Babu et al. (2012) whose dry matter content ranged from 10.2 percent to 2.89 percent. In order to improve the quality of tomato both processed and fresh, the dry content matter must be at least five percent (DePascale et al. 2001). In tomatoes, eight percent of the dry matter content makes up the mineral composition especially K and P. These minerals have a way of affecting the taste of tomatoes by affecting the buffering capacity, titratable acidity and pH of the tomatoes (Petro-Turza 1987; Yilmaz 2001). The high dry matter could be as a result of the interaction between the microorganisms since all of the treatments produced higher percent of dry matter including the plants that were infected and not treated (control). The nutritional quality of harvested crops can be linked to the biological activities of microorganisms in the soil and the soil organic matter. This is because microorganisms are responsible for production of vital biochemical compounds that are the basis of the nutritional values in crops (Marler and Wallin 2006). When there are increased activities of microorganisms in the soil, soil fertility is increased, which leads to higher levels of dry matter in harvested crops and invariably an increase in mineral composition leading to production of healthy nutrient-rich foods.

Moisture Content of Tomatoes from Different Treatments

Moisture content also ranged between 92.5 percent in control to 87.3 percent in *B. amyloliquefaciens* treatment. This result is similar to the moisture content range of tomato fruits planted using organic or conventional system with range from 94.8 percent to 92.0 percent (Pieper and Barrett 2009). They also observed that moisture content of tomatoes from conventional system was more compared to the organic system, and this can be important in this study as those plants treated with *Bacillus* spp had less moisture content compared to the control. It is also similar to those reported by (Gupta et al. 2011) whose moisture content ranged from 94.4 percent to 92.2 percent.

Mineral Analysis of Tomatoes using EDX 720

Mineral content of tomatoes is affected by various factors and they include maturity level of the plant at the time of harvest, soil type, and types of organic and inorganic matter in the soil. Tomatoes were analyzed to know the effect of the various treatments on the mineral content. A total of eleven different minerals were detected. The spectrometers of the EDX 720 enable non-destructive, rapid analysis of solids and liquids at the ppm level without much pre-treatment. This is quite important as other analysis can be carried out on the samples after they have been used for analysis in EDX 720.

EDX 720 allowed the detection of various minerals in the tomato samples. The minerals

detected were potassium, calcium and sulphur (macro elements) manganese, zinc, copper and iron (micro elements), cobalt, bromine and rubidium and aluminum (trace elements). Those not reported in this study had very minute quantity.

Potassium Content

The concentration of K in the treatments ranged from 3.8 mg/g to 1.0 mg/g. Treatment with *B. amyloliquefaciens* had the highest concentration of K with 3.8 mg/g while treatment with *B. cereus* had the least with 1.0 mg/g treatment with *B. pumilus*, *B. subtilis* and *F. solani* had 2.7, 3.5 and 1.8 mg/g, respectively. These results are similar to the concentration range from 4.29 to 0.28 g/kg reported by Adotey et al. (2009), 1.5 g/kg reported by Zaichick (2002), 4114.77 to 2391.08 mg/kg reported by Shar et al. (2012) and 1.56 g/kg in medicinal plants reported by Serfor-Armah et al. (2001). But it is lower than the concentration that ranged from 7.31 to 3.26 g/kg reported by Quartey et al. (2012). Fresh tomatoes are rich in K, which is an important cell and body fluid component that helps in controlling blood pressure and as a result the heart beat (Freebern 2012). Potassium also helps transmit nerve impulses. It is also important in coagulating ATP with sodium. Deficiency of K in the human body can lead to muscular weakness and paralysis, cardiac and mental disorder and nerve irritability (Adotey et al. 2009).

Calcium Content

Calcium content of tomatoes from the various treatments ranged from 0.1 to 0.7 mg/g with treatment with *B. subtilis* having the highest value and the control having the lowest. *B. cereus*, *B. amyloliquefaciens* and *B. pumilus* had concentrations of 0.2, 0.4 and 0.4 mg/g, respectively. This is similar to the range of 2.47 to 3.83 g/kg reported by Olaniyi et al. (2010). Calcium is an essential component that is needed for the formation of fibrinogen, which is very important and needed for the clotting of blood. It is also an important component of teeth and bones. Deficiency can lead to osteoporosis and rickets in children (Adotey et al. 2009). Calcium can be provided by vegetables even though in low quantity but can add up to complement the daily intake needed by the body for proper functioning.

Sulphur Content

The concentration of sulphur in the various treatments ranged from 0.1 mg/g in the treatment with *B. pumilus* to 0.03 mg/g in the treatment with *B. cereus*. Treatments with *B. amyloliquefaciens*, *B. subtilis* and control had 0.1, 0.1 and 0.1 mg/g of sulphur, respectively. It is important and used for production of amino acids and is an important and fundamental building block. It is important for the skin, hair and nails. As a component of insulin, it helps in reducing blood sugar. It is also important in the digestion and absorption of fat.

Iron Content

The iron content ranged from 0.00 mg/g in treatments with *B. cereus* to 0.03 mg/g in treatment with *B. pumilus*. Treatments with *B. amyloliquefaciens*, *B. subtilis* and control had 0.02, 0.02 and 0.01 mg/g of Fe, respectively. Christian and West (1998) had similar results with millet (range from 39.75 to 50.37 mg/kg). The result from this research was lower than the range of 11.61 to 12.29 mg/100g recorded by Gupta et al. (2011). Fe is needed in small quantity to help prevent anemia.

Copper Content

The copper content of treated tomatoes ranged from 0.01 mg/g in treatment with *B. subtilis* to 0.01 mg/g in the *B. cereus*. The control had no copper content while *B. amyloliquefaciens* and *B. pumilus* had 0.01 and 0.01 mg/g, respectively. This result shows that these *Bacillus* isolates can increase the Cu content of tomatoes since the control, which was not treated with any *Bacillus* isolate, was deficient in it.

Rubidium Content

The rubidium content of tomatoes whose plants were treated is quite small and limited to only two of the treatment that is, *B. amyloliquefaciens* having concentration of 0.01 mg/g and *B. pumilus* having concentration of 0.01 mg/g and the control having the least concentration of 0.001 mg/g. Rubidium behaves like potassium and it is important in helping patients suffering from depression as a result of dialysis by supplementing their rubidium level (Canavese et al.

2001). The presence of rubidium in plants treated with *Bacillus* isolates is worthy of note. As such, the relationship between the amount of inoculum of *Bacillus* isolates and the concentration of rubidium can be observed. So far, sources of rubidium have not been researched extensively, and therefore the use of *Bacillus*-treated tomato could also be considered a potent source.

Manganese Content

Manganese content of tomatoes was only available to plants that have been treated with *B. cereus* and *B. pumilus* and they are 0.002 and 0.12 mg/g, respectively. It is important in urea formation and energy metabolism. Also it is a key component of the antioxidant enzyme superoxide dismutase (Holley et al. 2011).

Molecular Characterization and Biocontrol of Tomato *Fusarium* Wilt

Results obtained using the three primers revealed a high similarity matrix among the *Bacillus* isolates was not unexpected because they are of the same species while their diversity was also not unexpected because of the differences in their genetic level. It was also observed that even in isolation of the same species polymorphism exist but not as high because of the fact that they are from the same species.

In this study, out of 10-mer primers, S4 and OPH 19, OPH 19 produced the lowest number of bands that is 16. The dendrogram showed that the *Bacillus* isolate *B. cereus* that had the best growth is distant from *B. pumilus* and *B. subtilis* but closest to *B. amyloliquefaciens* and *Brevibacillus*. This relatedness is also observed as biocontrol activities in their growth parameters. While using primer A9B7, even though *B. amyloliquefaciens* was closer to *B. subtilis* than *B. pumilus*, the effect can also be likened to the effect from the use of primer OPH 19. This also means that the relationship between primer OPH 19 and biocontrol activities of the *Bacillus* isolates is the same with primer A9B7. Primer S4 showed the diversity among all isolates. This is reflected in the variations in their different growth parameters.

In this study, all the primers were quite informative since they all had high level of polymorphism 90.9 percent for primer A9B7, 92.5 percent for primer S4 and 87.5 percent for primer OPH 19. Baysal et al. (2008) while using primer OPH 19 in characterizing 2 strains of *Bacillus subtilis*, EU 07 and QST 713 had 6 observed bands, which included 2 polymorphic bands, which is 33.3 percent polymorphism. This is also in agreement with Kozyrenko et al. (2001), who observed that the banding patterns were marked differently from one another with each primer. Also, the common amplicons in the agarose profile differ only in the intensity of their bands in the RAPD spectra.

Using primer A9B7, the number of bands from this study is in agreement with the observation recorded by El-Mokadem and Hadia (2008), whose level of polymorphism was higher. In their study, Primer A9B7 produced bands ranging from 2 to 6 bands with only 1 common band in 5 isolates including the control showing 77.27 percent polymorphism.

According to Abdel Ghany and Zaki (2003) while using primer A9B7, RAPD products from four *Gossypium barbadense* cultivars generated bands between 300 and 1500bp each cultivar having a minimum of 2 bands and a maximum of 4 bands, which is also similar in terms of number of bands observed in this study with minimum of 1 and a maximum of 5 visible bands, which ranged in size from 75 to 600 bp. Even though *G. Barbadense* is an angiosperm, this result shows the ability of the primer to bind randomly to genes on its genomes.

Using primer A9B7 was quite informative and was able to distinguish six isolates of rhizobacteria from rhizosphere of water hyacinth out of which one of the isolates produced no bands and number of bands ranged between 2 and 4 bands among the other five (Abou-Shanab et al. 2007), which is also quite similar to the result of this study.

Based on the different primers used, the 11 *Bacillus* isolates of this study were grouped into 2 basic clusters using primer A9B7 that is, the cluster with only *B. pumilus* and the cluster including the 5 sub clusters. Primer OPH 19 was able to group the 11 isolates into 5 basic clusters and in this case only *B. pumilus* and *B. methylotrophicus* M8 had individual clusters. Primer S4 showed greater diversity among the *Bacillus* isolate by grouping them into 8 clusters. *B. cereus, B. subtilis, B. subtilis 282, Brevibacillus* sp formed individual clusters, isolates while the others formed 2 main clusters.

This diversity experienced based on each of the three primers used might account for the differences in the growth parameters observed in the tomato plants as a result of the different *Bacillus* isolates. Using the example of *B. amyloliquefaciens* and *B. cereus*, primers A9B7 and S4 help show that they are diverse and distant but a converging point from primer OPH 19 helps reveal their similarity in being involved in biocontrol but at different levels.

CONCLUSION

In conclusion, the present study results showed that tomato plants treated with *Bacillus* spp protect the tomato plants from *Fusarium* wilt also treated plants contained vital micro and macronutrients as compared to control. The similarity and diversity of the *Bacillus* isolates as seen in the RAPD-PCR put into perspective the variation in the biocontrol activities of the different isolates in the greenhouse as observed in their growth parameters. The variation in the nutritive values of the different tomatoes could be as a result of the different treatments on the plants and the microbial interactions that took place. All the tomatoes are good sources of qualitative mineral elements. Even though treatment with *B. cereus* supported growth and controlled *Fusarium* wilt, the mineral elements were the least. More research would be carried out to understand this and also to put into perspective the issues of particular and specific genes in each *Bacillus* isolate of interest that improved growth and reduced disease such that they can all be cloned into one organism or better still having a consortium of these organisms to be formulated into microbial products. This will help improve individual treatments so that they are not just increasing vegetative growth but can also be involved in reproductive aspect of the plants, mineral composition inclusive.

RECOMMENDATIONS

The benefit of biocontrol is being brought to the limelight so that the use of chemicals as pesticides, insecticides, herbicides, bactericides and fungicides can be reduced. This will in turn reduce the effect of environmental pollution and increase food safety. The *Bacillus* isolates used in this study can be formulated into biocontrol products that can be used in place of fertilizers. Other studies can also be carried out to know the impact of biocontrol on flavonoids and other phytochemicals of tomato.

ACKNOWLEDGEMENTS

The researchers gratefully acknowledge the North West University for bursary to the first researchers and the OOB would like to thank the National Research Foundation, South Africa for grant (UID:81192) that have supported research in her laboratory.

REFERENCES

Abdel Ghany AGA, Zaki EA 2003. DNA sequences of RAPD fragments in the Egyptian cotton *Gossypium barbadense*. *Afri J Biotechnol,* 2: 129-132.

Abou-Shanab RAI, Angle JS, Berkum PV 2007. Chromate-tolerant bacteria for enhanced metal uptake by *Eichhornia crassipes* (mart.). *Int J Phytorem,* 9: 91-105.

Adotey DK, Serfor-Armah Y, Fianko JR, Yeboah PO 2009. Essential elements content in core vegetables grown and consumed in Ghana by instrumental neutron activation analysis. *Afr J Food Sci,* 3(9): 243-249.

Ajilogba CF, Babalola OO 2013. Integrated management for tomato *Fusarium* wilt. *Biocontrol Sci J,* 18(3): 117-127.

Ajilogba CF, Babalola OO, Ahmad F 2013. Antagonistic effects of *Bacillus* species in biocontrol of tomato *Fusarium* wilt. *Studies on Ethno Medicine,* 7(3): 205-216.

Babalola OO 2008. Pectinase and cellulase enhance Mycoherbicidal activity of *Fusarium Arthrosporoides* on *Orobanche aegyptiaca*. In: D Thangadurai, W Tang, A Ramachandran, IO Moraes, MAA Pinheirode Carvalho (Eds.): *Biotechnology for Food, Environment and Agriculture,* Volume 1. India: Agrobios, P. 291.

Babalola OO 2010. Pectinolytic and cellulolytic enzymes enhance *Fusarium compactum* virulence on tubercles infection of Egyptian broomrape. *Int J Microbiol,* 7. doi:10.1155/2010/273264

Babu MA, Singh D, Gothandam KM 2012. The effect of salinity on growth, hormones and mineral elements in leaf and fruit of tomato cultivar pkm1. *J Animal Plant Sci,* 22(1):159-164.

Baysal Ö, Çaliskan M, Yesilova O 2008. An inhibitory effect of a new *Bacillus subtilis* strain (EU07) against *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Physiol Mol Plant Pathol,* 73: 25-32.

Calvo MM, Santa-Maria G 2008. Effect of illumination and chlorophylls on stability of tomato carotenoids. *Food Chem,* 107: 1365-1370.

Canavese C, Decostanzi E, Branciforte L, Caropreso A, Nonnato A, Sabbioni E 2001. Depression in dialysis patients: Rubidium supplementation before other drugs and encouragement? *Kidney Int,* 60(3): 1201.

Cavaglieri L, Orlando J, Rodriquez MI, Chulze S, Etcheverry M 2005. Biocontrol of *Bacillus subtilis* against

Fusarium verticilliodes in vitro and at maize root level. *Res Microbiol,* 156: 748-754.
Choudhary DK, Johri BN 2009. Interactions of *Bacillus* sp. and plants–With special reference to induced systemic resistance (ISR). *Microbiol Res,* 164: 493-513.
Christian P, West KP 1998. Interactions between zinc and vitamin A: An update. *Ame J Clinical Nutr,* 68(2): 435.
DePascale S, Maggio A, Fogliano V, Ambrosino P, Retieni A 2001. Irrigation with saline water improves carotenoids content and antioxidant activity of tomato. *J Hortic Sci Biotechnol,* 76: 447-453.
El-Mokadem HE, Hadia HA 2008. Induction of dwarfism in *Encelia farinosa* by cycocel and evaluation of regenerants using RAPD and ISSR Markers. *Aust J Basic Appl Sci,* 2: 331-342.
Esitken A, Yildiz HE, Ercisli S, Donmez MF, Turan M, Gunes A 2010. Effects of plant growth promoting bacteria (PGPB) on yield, growth and nutrient contents of organically grown strawberry. *Scientia Horticulturae,* 124: 62–66.
Freebern K 2012. Tomatoes. *Ingredients of the Month,* 1-3.
Gardener BBM 2004. Ecology of Bacillus and Paenibacillus sp in agricultural systems. *Phytopathol,* 94: 1252-1258.
Gupta A, Kawatra A, Sehgal S 2011. Physical-chemical properties and nutritional evaluation of newly developed tomato genotypes. *Afr J Food Sci Technol,* 2(7): 167-172.
Hallmann E, Rembialkowska E 2007. Estimation of fruits quality of selected tomato cultivars *(Lycopersicon esculentum Mill*) from organic and conventional cultivation with special consideration of bioactive compounds content. *J Res Appl Agric Engin,* 52: 55.
Holley AK, Bakthavatchalu V, Velez-Roman JM, Clair DKS 2011. Manganese superoxide dismutase: Guardian of the powerhouse. *Int J Mol Sci,* 12(10): 7114–7162. doi:10.3390/ijms12107114
Jacobsen BJ, Zidack NK, Larson BJ 2004. The role of *Bacillus*-based biological control agents in integrated pest management systems: Plant diseases. In: Symposium- The nature and application of biocontrol microbes. *Bacillus* sp. *Phytopathol,* 94: 1272-1275.
Jetiyanon K, Kloepper JW 2002. Mixture of plant growth-promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. *Biol Control,* 24: 285-291.
Kloepper JW, Ryu CM, Zhang S 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathol,* 94: 1259-1266.
Kozyrenko MM, Artiukova EV, Lauve LS, Zhuravlev YN, Reunova GD 2001. The genetic variability of Panax ginseng callus lines. *Biotechnologiya,* 1: 19-26.
Lombardi-Boccia G, Lucarini M, Lanzi S, Aguzzi A, Cappelloni M 2004. Nutrients and antioxidant molecules in yellow plums *(Prumus domestica L.*) from conventional and organic production: A comparative study. *J Agric Food Chem,* 51: 90.
Majkowska-Godomska J, Francke A, Wierzbicka B 2008. Effect of soil substrate on the chemical composition of fruit of some tomato cultivars grown in a unheated plastic tunnel. *J Elementol,* 13(2): 261-268.
Marler JB, Wallin JR 2006. *Human Health, the Nutritional Quality of Harvested Food and Sustainable Farming Systems*. WA, USA: Nutrition Security Institute.
Miller EC, Hardley CW, Schwartz SJ, Erdman JW, Boileau TMW, Clinton SK 2002. Lycopene, tomato products and prostate cancer prevention. Have we established casualty? *Pure Appl Chem,* 74(8): 1435-1441.
Nirmaladevi D, Srinivas C 2012. Cultural, morphological, and pathogenicity variation in *Fusarium oxysporum* f. sp. *lycopersici* causing wilt of tomato. *Batman University Journal of Life Sciences,* 2(1): 1-16.
Olaniyi JO, Akanbi WB, Adejumo TA, Akande OG 2010. Growth, fruit yield and nutritional quality of tomato varieties. *Afr J Food Sci,* 4(6): 398 - 402.
Petro-Turza M 1987. Flavor of tomato and tomato products. *Food Rev Int,* 2(3): 309-351.
Pieper JR, Barrett DM 2009. Effects of organic and conventional production systems on quality and nutritional parameters of processing tomatoes. *J Sci Food Agric,* 89: 177–194. doi:DOI 10.1002/jsfa.3437.
Quartey EK, Opata NS, Amoatey HM, Klu GYP 2012. Elemental composition in fruits of gamma-radiation induced variant lines of *Solanum pimpinellifolium* L. *Asian J Agric Sci,* 4(3): 198-204.
Sabio E, Lozano M, Espinosa V, Mendes RL, Pereira AP, Palavra AF, Coelho JA 2003. Lycopene and β-carotene extraction from tomato processing waste using supercritical CO_2. *Ind Eng Chem Res,* 42: 6641-6646.
Serfor-Armah Y, Nyarko BJB, Akaho EHK, Kyere AWK, Osae S, Oppong-Boachie K, Osae EKJ 2001. Activation analysis of some essential elements in five medicinal plants used in Ghana. *Nucl Chem,* 250(1): 173-176.
Shar GQ, Shar LA, Makhija PM, Sahito SB 2012 Evaluation of eleven macro and micro elements present in various hybrids of millet *(Pennisetum glaucum, or P. Americanum*). *Pak J Anal Environ Chem,* 13(1): 78-84.
Tepic AN, Vujicic BL, Takac AJ, Krstic BJ, Calic LJ 2006. Chemical heterogeneity of tomato inbred lines. *Acta Periodica Technologica,* 37(apteff 37): 45-50.
Turhan A, Sentz V 2009. Estimation of certain chemical constituents of fruits of selected tomato genotypes grown in Turkey. *Afr J Agric Res,* 4(10): 1086-1092.
Yilmaz E 2001. The chemistry of fresh tomato flavor. *Turk J Agric For,* 25: 149-155.
Zaichick V 2002. Instrumental neutron activation analysis of minor and trace elements in food in the Russian region that suffered from the Chernobyl disaster. *Food Nutr Bull,* 23(3): 191-194.