Journal of Applied Botany and Food Quality 90, 288 - 297 (2017), DOI:10.5073/JABFQ.2017.090.036

[1]Postharvest and Food Biochemistry Laboratory, Department of Food Science and Technology, University of Karachi, Karachi, Pakistan
[2]Agricultural Biotechnology and Phytopathology Laboratory, Department of Botany, University of Karachi, Karachi, Pakistan
[3]HEJ Research Institute of Chemistry and ICCBS, University of Karachi, Karachi, Pakistan
[4]Biotechnology and Drug Development Laboratory, Department of Biochemistry, University of Karachi, Karachi, Pakistan

Protective role of epiphytic fluorescent *Pseudomonas* on natural postharvest decay of tomato at room temperature

Shah Habiba[1], Rubina Noreen[2], Syed Abid Ali[3], Khwaja Ali Hasan[3], Jehan Ara[1], Viqar Sultana[4], Syed Ehteshamul-Haque[2]

(Received February 18, 2017; Accepted May 20, 2017)

Summary

Following harvest, tomato fruits are susceptible to an attack by many fungal pathogens, but healthy fruits may also harbor beneficial microflora, which can delay the spoilage of fruit. In this study, 30 isolates of epiphytic fluorescent *Pseudomonas* were isolated from healthy fruits of lemon, melon, grapefruit, tomato and orange. Twelve isolates were identified on molecular basis by amplifying 16S rDNA using a genus-specific primer set PA-GS-F 5'-GACGGGTGAGTAATGCCTA-3' and PA-GS-R F 5'-CACTGGTGTTCCTTCCTATA-3' on a conserved sequence of the genus *Pseudomonas* with a product size of ~618 bp. Three potential isolates were examined for their ability to delay the postharvest natural spoilage and maintained the physiochemical properties during storage for fifteen days in season 2013 and 2014. All three isolates showed promising control of postharvest diseases of tomato in comparison with control in both seasons up to fifteen days of storage at room temperature (23±4 ºC, Rh 25-70%). The epiphytic bacterial isolates have delayed fruit weight loss and maintained fruit firmness, total solids, pH and titratable acidity. Isolates also slowed the accumulation of lycopene indicating their potential in controlling the major changes in physiochemical properties. In both seasons *Pseudomonas* treated tomatoes showed no or negligible infestation of common postharvest fungi and bacteria as compared to control and positive control (1% K-sorbate).

Key words: Postharvest infections, tomato epiphytes, fluorescent *Pseudomonas*, physiochemical properties, disease control.

Introduction

Tomato is considered as one of the most widespread and commonly grown vegetable/fruit in the world. It is the second abundant source of vitamins and minerals in human diet (Bombelli and Wright, 2006). In developing countries, more than 30% of the crop yield is lost due to postharvest diseases (Kader, 2002; Tirupathi et al., 2006; Fatima et al., 2009; Etebu et al., 2013; Gomes et al., 2015). Tomato handling at postharvest stage, packaging, storage and transportation affects its quality. These factors can alter the physiology of fruit due to microorganism's production and decay (Wilson et al., 1991; Buonassisi et al., 2013). After harvest the succulent nature of the epicarp of fruit makes it susceptible to fungal attack. Species of *Penicillium, Fusarium, Geotrichum, Aspergillus, Alternaria, Botrytis* and *Phytophthora,* are common fungi attacking tomatoes after harvest (Etebu et al., 2013; Abu Baker et al., 2013). The infected fruits become unmarketable (Nurulhuda et al., 2009).

For the control of postharvest diseases of fruits and vegetables two main methods have been suggested (Sharma et al., 2009). A first approach suggests the management and promotion of antagonistic microflora present on the fruit surface, and a second approach suggests the artificial introduction of antagonistic organisms against postharvest pathogens (Abano and Sam-Amoah, 2012). The development of disease is controlled by natural microbial antagonists present on the surface of fruits (Wilson and Wisniewski, 1989; Talibi et al., 2014). Several microorganisms have been identified, which have demonstrated antagonistic activity against multiple postharvest pathogens on various fruits and vegetables (Wilson and Wisniewski, 1994; El-Ghaouth, 1997; Janisiewicz and Korsten, 2002; Mari et al., 2003; Talibi et al., 2014). The use of bacteria, yeasts and fungi as microbial antagonists is well documented (Janisiewicz and Korsten, 2002; Zhang et al., 2005; Droby, 2006; Korsten, 2006; Sharma et al., 2009), although the mechanisms by which microbial antagonists suppress postharvest diseases is still to be explained and explored. Postharvest application of microbial antagonists is more effective than preharvest application (Sharma et al., 2009). However, very few studies suggest the successful use of bacteria as biocontrol agents of postharvest pathogens.

Fluorescent *Pseudomonas* species have been reported to possess strong biocontrol activity against multiple plant pathogens, associated with the rhizosphere and rhizoplane, as endophytes and also as epiphytes (Siddiqui et al., 2000; Siddiqui and Ehteshamul-Haque, 2001; Tariq et al., 2009; Afzal et al., 2013; Ehteshamul-Haque et al., 2013; Noreen et al., 2015; Habiba et al., 2016). However, the role of fluorescent *Pseudomonas* species associated with the fruit surface and their role in suppressing postharvest diseases has received little attention. The current study describes the isolation and identification of epiphytic fluorescent *Pseudomonas* species from healthy fruits and vegetables and their role in the management of disease severity of tomato along with the study of its physiochemical properties up to 15 d storage.

Materials and methods

Sample collection

Fresh healthy fruits and vegetables (tomato, lemon, melon, grape fruit and orange) were collected from the field and supermarkets in Karachi, Pakistan. Samples were brought to the laboratory and isolation of fluorescent *Pseudomonas* species was performed within 24 h.

Isolation and identification of epiphytic fluorescent *Pseudomonas* species

Fruit samples were washed with sterilized water and 2 g of the samples were taken from the fruit surface, placed in 20 mL of 0.05 M phosphate buffer (pH 6.5), and ground using a sterile thistle mortar. One hundred µL of each sample was transferred to a Petri dish containing Gould's S1 medium (Gould et al., 1985) supplemented with the antibiotic trimethoprim (Bashan et al., 1993) and incubated at

* Corresponding author

28 ºC. Bacterial colonies showing fluorescence under UV light after 3 d were purified on King's B agar medium (KING'S et al., 1954). *Pseudomonas* spp. were initially identified according to the Bergey's manual (GARRITY et al., 2005). Identification of selected isolates was further confirmed by using established molecular biology techniques recently described by us (NOREEN et al., 2015).

Briefly, a genus-specific primer set PA-GS-F 5'-GACGGGTGAGT AATGCCTA-3' and PA-GS-R F 5'-CACTGGTGTTCCTTCCTATA -3' was used to amplify 16S rDNA on a conserved sequence of the genus *Pseudomonas* with a product size of ~618 bp. Amplification was performed by using a Master cycler ProS (Eppendorf, Germany) and the product lengths were estimated by 1.5% agarose gel electrophoresis. Initially, heterogeneity between the *Pseudomonas* isolates was established by RFLP analysis of the 16S rDNA product. The restriction enzyme digest was prepared using the restriction endonuclease fast digest HaeIII (Fermentas, USA). Each restriction reaction was performed at a final volume of 30 µL and consisted of 17 µL PCR grade nuclease free water, 2 µL of 10x fast digest green buffer, 10 µL of PCR product and 1 µL of fast digest HaeIII. Incubation was done at 37 ºC for 5 min. The restriction digest was subjected to 2% agarose gel for analysis. A theoretical restriction enzyme digest of the 16S rRNA gene of *Pseudomonas aeruginosa* PA96 (GenBank: CP007224.1) was prepared using SnapGene Viewer 2.2.2 for restriction endonuclease HaeIII (BsuRI) and used as a reference for restriction analysis.

Intra-strain variations were also established by performing the BOX PCR, using the BOXA1R 5'-CTACGGCAAGGCGACGCTGACG-3' primer as described by us (NOREEN et al., 2015). Indigenous *P. aeruginosa* (F2, F3) and *P. monteilii* (F1), which were previously identified by 16S rDNA sequencing, were used as positive controls. To establish intra-strain heterogeneity and phylogenetics of *Pseudomonas* isolates, an image of BOX PCR was analyzed using PyElph1.4 software (PAVEL and VASILE, 2012). A phylogenetic tree was computed based on the information extracted from the gel image. Clustering and distance matrices were computed using band matching with reference to standard markers. The phylogenetic tree was constructed based on the Neighbor-Joining clustering method applied on the distance matrix as established by PAVEL and VASILE (2012).

Application of epiphytic *Pseudomonas* species on tomato

Three isolates HAB-10, HAB-15 and HAB-25 were selected based on their strong *in vitro* inhibitory activity against *Penicillium* spp., and *Fusarium solani* (data not presented). Fresh, uniform sized and disease-free tomato fruits collected during 2013-2014 at ripened stage were surface sterilized and air dried at ambient conditions before giving treatments. The fruits were dipped for 5 min in the aqueous suspensions of epiphytic fluorescent *Pseudomon*as HAB-10, HAB-25, HAB-15 containing 10^7 CFU/mL (BARKAI-GOLAN, 2001; SHARMA et al., 2009). The fruits were further air dried and placed in baskets (4 fruits per basket). Fruits treated with sterile water served as control and fruits dipped in 1% aqueous suspension of K-sorbate served as positive control. Room temperature recorded was 23±4 ºC with relative humidity in the range of 25%-70%. The physiological parameters were recorded at five days interval under ambient room storage conditions.

Effect of epiphytic fluorescent *Pseudomonas* species on physiochemical properties of tomato

Weight loss

The weight loss of tomato fruits was calculated by a standard procedure as described in AOAC (1994).

Weight loss = W_1-W_2/W_1 × 100

Where:

W_1 = Initial weight of tomato

W_2 = final weight of the tomato fruit on subsequent days of the study.

Firmness of fruit

Hand-held penetrometer (PCE-PTR 200) with a cross head of 8 mm was used to measure firmness of tomato fruit and measurements were taken at two points on its cheeks at two opposite sides (ABBASI et al., 2009).

Total soluble solids (TSS)

Hand refractometer (Atago Co., Tokyo, Japan) was used to measure the total soluble solid content of tomato fruits (AOAC, 1994).

pH

The standard method as described in AOAC (1994) was used to determine the pH of tomato fruit.

Titratable acidity (TA)

Five mL of tomato juice was titrated against 0.1 N sodium hydroxide, using phenolphthalein as an indicator. The data were expressed in % citric acid according to a standard method (AOAC, 1994).

% citric acid = V × N × Wmeq × 100/Y

Where: V = mL of NaOH solution used for titration,

N = Normality of NaOH solution,

Wmeq = Milliequivalent of citric acid (0.064),

Y = sample weight in g or mL.

Quantitative analysis of lycopene

A spectrophotometric method described by FISH et al. (2002) was used for lycopene estimation. A 0.6 g sample was weighed from each puree into two 40 mL screw-capped vials that contained 5 mL of 0.05% (w/v) BHT in acetone, 5 mL of 95% ethanol and 10 mL of hexane (cold). The mixture was centrifuged at 180 rpm for 15 min at 0 ºC. Distilled water (0.3 mL) was added and the mixture was centrifuged for another 5 min at 0 ºC. The tubes were kept at room temperature for phase separation for 15-20 min. The upper solvent layer was collected and lycopene was measured at 503 nm, using hexane as a blank and expressed as mg/g of tissue.

Decay/rotting percent

Visual observations were used to determine the decay percent of stored tomato fruits. Following formula was used for calculation:

Decay percent = Number of decayed fruits/Total number of fruits × 100.

Disease Severity

Calculations of disease severity were made on every fifth day of storage in season 2013 and 2014 by using a severity scale of 1-5 for *Alternaria*, *Fusarium, Penicillium* and bacterial rots (CORIKIDI et al., 2006) as shown below (Tab. 1).

Tab. 1: Disease severity scale for postharvest rot.

Scale	Percent Severity
1	0-1%
2	2-5%
3	6-10%
4	11-49%
5	50-100%

Data analysis

Treatment means and time intervals were compared by subjecting the data to two way analysis of variance (ANOVA). The follow-up of ANOVA included the least significant difference (LSD) test at *p=0.05* to compare treatment means (GOMEZ and GOMEZ, 1984).

Results and discussion

Isolation and identification of epiphytic fluorescent *Pseudomonas* species

Thirty epiphytic isolates of fluorescent *Pseudomonas* were isolated from healthy fruits and vegetables, including lemon, melon, tomato, grapefruit and orange (Tab. 2). They were initially identified accor--ding to Bergey's manual. Selected isolates ie, PS1 (HAB-1), PS2 (HAB-2), PS4(HAB-14), PS7 (HAB-15), PS8 (HAB-5), PS9 (HAB-21), PS10 (HAB-25), PS11 (HAB-8), PS12 (HAB-9), PS13 (HAB-29), PS14 (HAB-30) and PS15 (HAB-12) were further subjected to molecular identification and genetic variation analysis by PCR amplification and restriction pattern analysis of the 16S rDNA gene product as a molecular marker (Fig. 1a-b). Results revealed that all selected isolates belonged to the genus *Pseudomonas* as demonstrated by the presence of a single expected gene product of ~620 bp. The restriction pattern of the gene products generated by HaeIII showed very close relatedness demonstrated by two major and identical DNA fragments of 175 and 500 bp. More variation was observed only in isolates PS7, PS12 and PS13 where some additional bands appeared (Fig. 1b). Results were further complemented by BOX PCR and dendrogram analysis (Fig. 1c-d), suggesting the high intra-strain variation within *Pseudomonas* spp.

Tab. 2: List of different isolates of epiphytic fluorescent *Pseudomonas* isolated from healthy fruits and vegetables.

Pseudomonas isolates	Source	Collection area
HAB-1*	lemon	Metro cash & carry
HAB-2*	lemon	"
HAB-3	lemon	"
HAB-4	lemon	"
HAB-5*	melon	"
HAB-6	tomato	Fruits and Vegetable Market, Karachi
HAB-7	tomato	"
HAB-8*	grape fruit	Metro cash & carry
HAB-9*	tomato	Fruits and Vegetable Market, Karachi
HAB-10	orange	Metro cash & carry
HAB-11	orange	"
HAB-12*	Lemon	"
HAB-13	Lemon	"
HAB-14*	Lemon	"
HAB-15*	Melon	Fruits and Vegetable Market, Karachi
HAB-16	Lemon	Metro cash & carry
HAB-17	Melon	Agricultural field of Malir, Karachi
HAB-18	Melon	Fruits and Vegetable Market, Karachi
HAB-19	Melon	"
HAB-20	Melon	"
HAB-21*	Melon	"
HAB-22	Tomato	"
HAB-23	Tomato	"
HAB-24	Orange	Metro cash & carry
HAB-25*	Tomato	Fruits and Vegetable Market, Karachi
HAB-26	grape fruit	Metro cash & carry
HAB-27	Lemon	"
HAB-28	grape fruit	"
HAB-29*	Melon	Agricultural field of Malir, Karachi
HAB-30*	Lemon	Fruits and Vegetable Market, Karachi

* = identification and genetic variation between the selected isolates of fluorescent *Pseudomonas* was confirmed by using PCR amplification.

Effect of epiphytic fluorescent *Pseudomonas* on physiochemical properties of tomato

Weight loss

The weight loss of tomato fruit increased with time during storage. However, reduction in weight of fruits was found to be less in fruits treated with suspensions of epiphytic fluorescent *Pseudomonas* species (HAB-10, HAB-25, HAB-15) in comparison with untreated fruits stored up to 15 d (Tab. 3). Fruits treated with HAB-25 recorded the lowest significant value of weight loss (9.37%) in the first season and 10.3% in the second one on the 15th day of study. The fruits in the control treatment exhibited greater weight loss on the 10th day (13.69% and 12.33%) and the 15th day (13.78% and 13.47%) in both seasons as compared to epiphytic *Pseudomonas* (HAB-25), which showed 8.70% and 8.33% weight loss at the 10th day and 9.37% and 10.3% at the 15th day. However, K-sorbate was found to be effective in reducing weight loss at the 10th day (12.33% and 10.70%) in both seasons as compared to untreated control. These findings are in agreement with previous findings in which the weight of fruits decreased with time due to water loss via transpiration (ABD-ALLAH et al., 2011; NEI et al., 2005; HOLCROFT, 2015) and treatment with biocontrol bacteria reduced weight loss and maintained tomato quality during storage (ABD-ALLAH et al., 2011).

Firmness of fruit

Fruit firmness is often used as a criterion to determine the effects of storage and shelf-life (SINGH and REDDY, 2006). Firmness of fruit decreased with time in all treatments in storage, but some *Pseudomonas* treatments were effective in maintaining firmness in comparison with the control and positive control. Control of ripening is one way to increase shelf life. Firmness is related to the stage of maturity. Usually, firmness decreases with advancement of ripening. Solublization and depolymerisation of pectin during ripening results in loosening and breakdown of the cell wall structure (ADEDEJI et al., 2006; POZRL et al., 2010). VAN DIJK et al. (2006) explained changes that occur during the ripening process by stating that polygalacturonase is the prime and responsible factor over β-galactosidase for the decrease of fruit firmness. Textural changes in tomato might be due to changes in the metabolism of primary cell wall (HARKER et al., 1997) and the decrease in cell wall turgor (SHACKEL et al., 1991). Usually, at the initial stages of fruit development, firmness remains almost constant whereas it decreases as the fruit ripens, apparently due to changes in the assembly of pectin polymers in the cell wall (KALRA et al., 1995). TEKA (2013) correlated decrease in firmness with advancement in maturity stage (1.57 to 0.78). In this study, the firmness of tomato fruits decreased with increase in storage time but firmness of *Pseudomonas* treated fruits was greater than that in untreated fruits. Best retention of fruit firmness in epiphytic fluorescent *Pseudomonas* treated fruits in both seasons as compared to untreated ones can be explained by delayed degradation of non-soluble protopectins to the more soluble pectins and pectic acid (HUBER et al., 2001) as shown in Tab. 4.

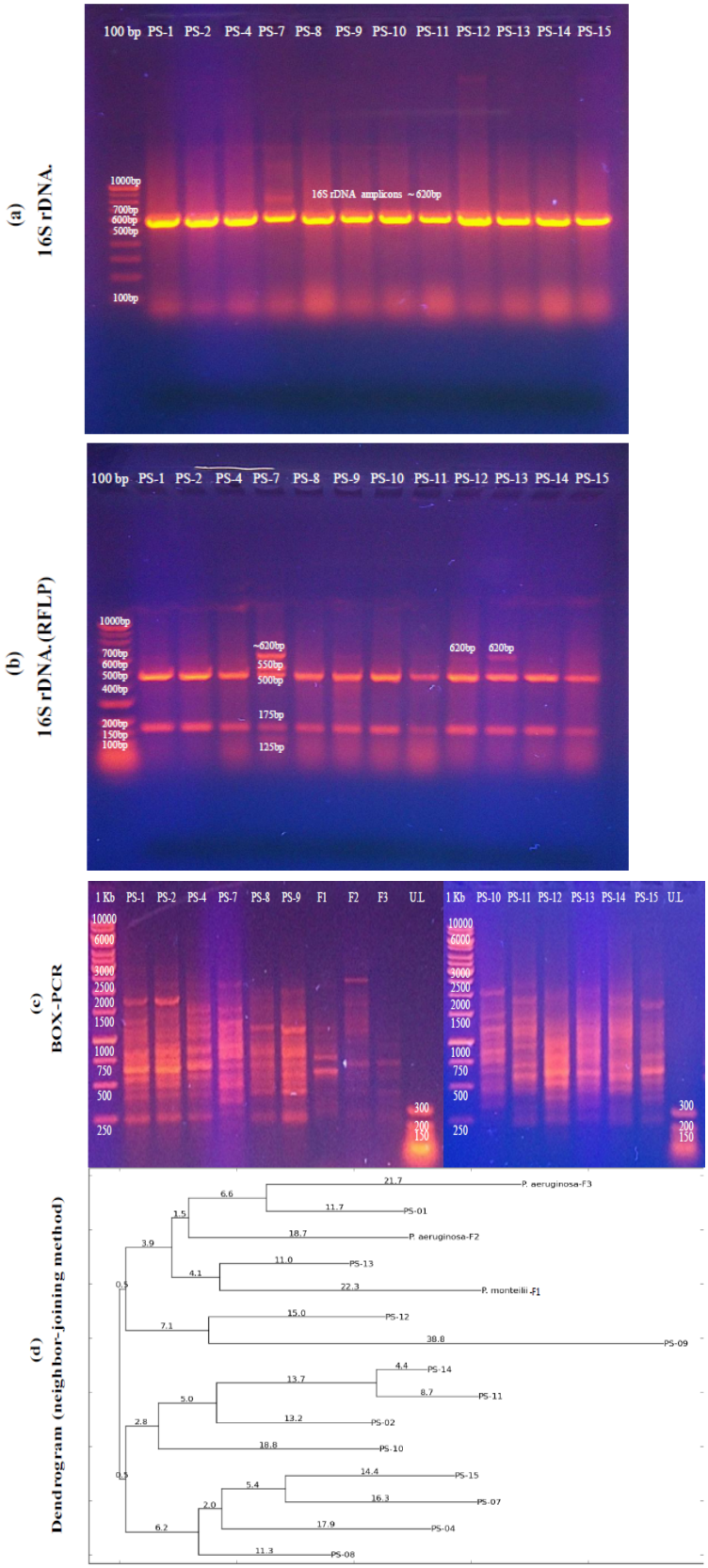

Fig. 1: Molecular basis of identification of *Pseudomonas* spp. (a) PCR amplification of 16S rDNA, (b) RFLP analysis of 16S rDNA gene PCR products obtained by the restriction enzyme *Hae*III, (c) BOX PCR analysis for establishing intra-species variation and (d) phylogenetic tree constructed using the neighbor-joining method by PyElph1.4 software. The reaction products were analyzed on 1.5% (a and c) and 2% (b) agarose gels and visualized by staining in ethidium bromide. F1 and F2-F3 are the indigenous control strains of *P. monteilii* and *P. aeruginosa,* respectively, while PS numbers represent *Pseudomonas* isolates in this study.
1 Kb, 100 bp and, UL, ultra-low range DNA ladders

Tab. 3: Effect of fluorescent *Pseudomonas* species on the weight loss of tomato stored at 23±4 ºC with relative humidity in the range of 25% - 70%.

Treatments	Season 2013				Season 2014			
	Storage Period/Days							
	0D	5D	10D	15D	0D	5D	10D	15D
Control	0±0	5.12±0.14	13.69±0.16	13.78±1.37	0±0	4.81±0.39	12.33±3.74	13.47±1.12
1% K-sorbate	0±0	4.60±0.28	12.33±3.74	13.16±1.53	0±0	4.80±0.43	10.70±1.60	13.46±1.79
HAB-10	0±0	3.77±0.91	10.70±1.60	12.68±0.93	0±0	4.67±0.67	10.37±1.33	12.68±0.93
HAB-25	0±0	4.01±0.61	8.70±1.65	9.37±1.02	0±0	4.13±0.87	8.33±1.78	10.3±0.12
HAB-15	0±0	3.69±0.16	9.54±0.61	10.14±0.75	0±0	4.58±0.46	9.76±1.42	10.76±1.49
$LSD_{0.05}$	Treatment1 = 0.859		Days[2] = 0.7690		Treatment[1] = 0.908		Days[2] = 0.812	

[1]Mean values in a column showing a difference greater than the LSD value are significantly different at p=0.05.
[2]Mean values in a row showing a difference greater than the LSD value are significantly different at p=0.05.

Tab. 4: Effect of fluorescent *Pseudomonas* species on firmness (N) of tomato stored at 23±4 ºC with relative humidity in the range of 25% - 70%.

Treatments	Season 2013				Season 2014			
	Storage Period/Days							
	0D	5D	10D	15D	0D	5D	10D	15D
Control	2.12±0.79	0.83±0.10	0.82±0.15	0.67±0.46	1.72±0.58	1.25±0.1	1.17±0.26	0.97±0.05
1% K-sorbate	2.12±0.79	1.07±0.25	1.05±0.05	0.98±0.08	1.72±0.58	1.27±0.28	1.15±0.12	1±0.08
HAB-10	2.12±0.79	1.08±0.34	1.01±0.10	0.93±0.04	1.72±0.58	1.25±0.20	1.2±0.24	1.1±0.29
HAB-25	2.12±0.79	1.02±0.05	0.97±0.17	0.9±0.08	1.72±0.58	1.37±0.28	1.17±0.17	1.12±0.28
HAB-15	2.12±0.79	1.12±0.12	1.07±0.125	1±0.09	1.72±0.58	1.45±0.05	1.35±0.36	1.2±0.14
LSD0.05	Treatment[1] = 0.304		Days[2] = 0.272		Treatment[1] = 0.250		Days[2] = 0.223	

[1]Mean values in a column showing a difference greater than the LSD value are significantly different at p=0.05.
[2]Mean values in a row showing a difference greater than the LSD value are significantly different at p=0.05.

Total soluble solids (TSS) / Brix

The total soluble solids of tomato fruits predominantly consist of sugar. However, pectin, amino acids, ascorbic acid, organic acids (citric and malic acids) etc., are also present, but the sugar content is used as a quality parameter to evaluate texture and composition of fruits and vegetables (Kamiloglu, 2011). In this study, the values obtained for soluble solids in different treatments of tomato fruit ranged from 3.95% (on day zero) to 6.3% (on day fifteen) (Tab. 5). Borji and Jafarpour (2012) reported an increase in the value of total soluble solids in tomato from 5.1% at the mature green stage to 6.2% at the full ripe stage. Large portion of total solids are total soluble solids, which indicates the sweetness level (Magwaza and Opara, 2015). It has been suggested that high levels of total soluble solids increase tomato paste competency and these values ranged from 5% to 6.5% (Garcia and Barett, 2005). In our study total soluble solids increased with ripening and treated fruits showed a variable percentage of solids among treatments with different isolates of *Pseudomonas* on day fifteen. HAB-10 showed a significantly higher content of soluble solids on the 15th day in comparison with the control and positive control in season one. However, in season two treatments have showed gradual and minimum increase in total soluble content after fifteen days of storage. Various reports suggest an increase in total soluble solids with color and maturity (Salunkhe et al., 1974), which is in agreement with our results. This increment of total soluble solids in tomato fruits could be due to breakdown of carbohydrates to soluble sugars and uncontrolled moisture loss (Nath et al., 2012; Siddiqui et al., 2015). The gradual increase of total soluble solids in treatments might be the reason for the least disease severity observed in treated tomatoes over the non-treated tomatoes.

Titratable acidity (TA) and pH

The titratable acidity and pH are two important quality parameters of tomatoes that are linked. Acid content of the fruit is used as the indicator of pH (Anthon et al., 2011). In this study, tomatoes stored for 15 d at room temperature after treatment with isolates of fluorescent *Pseudomonas* (HAB-15, HAB-10 and HAB-25) showed significantly ($p \leq 0.05$) less increase in pH up to ten days as compared to the control in season 2013 and 2014. Tomatoes treated with HAB-15 and HAB-25 showed less increase in pH up to the 15th day in both seasons (Tab. 6). It has been reported that pH values increase as the fruit progress towards the ripening stage (Anthon et al., 2011).

The most important attribute to determine the quality and acceptability of fruit is its titratable acidity (Alemu et al., 2014). There are reports that citric acid is the most abundant acid in tomatoes and the largest contributor to the total TA (Paulson and Stevens, 1974; Stevens, 1972). The decrease in TA with maturity and over-maturity is generally assumed to be due to a loss of citric acid (Anthon et al., 2011). Due to higher respiration rate as ripening advances and organic acids are being utilized as a substrate during this process (Lurie and Klein, 1990). In this study, titratable acidity decreased with time in all treatments in season one and season two; however the decrease in titratable acidity was found to be significantly ($p<0.05$) less in bacteria-treated tomatoes compared to untreated controls

Tab. 5: Effect of fluorescent *Pseudomonas* species on total soluble solids (%) of tomato stored at 23±4 ºC with relative humidity in the range of 25% - 70%.

Treatments	Season 2013				Season 2014			
	Storage Period/Days							
	0D	5D	10D	15D	0D	5D	10D	15D
Control	5.1±0.34	5.1±0.34	5.35±0.47	6±0	5.2±0.28	5.4±0.48	5.95±0.8	6.15±0.66
1% K-sorbate	5.1±0.34	5.25±0.66	5.35±0.44	5.75±0.5	5.2±0.28	5.5±0.70	5.85±0.59	6±0
HAB-10	5.1±0.34	5.3±0.47	5.85±0.59	6.15±0.66	5.2±0.28	5.35±0.47	5.5±0.70	5.75±0.5
HAB-25	5.1±0.34	5.4±0.48	5.5±0.70	5.5±0.70	5.2±0.28	5.3±0.47	5.45±0.57	5.5±0.57
HAB-15	5.1±0.34	5.1±0.34	5.25±0.597	5.4±0.43	5.2±0.28	5.25±0.59	5.6±0.58	5.4±0.43
LSD0.05	Treatment[1] = 0.347		Days[2] = 0.310		Treatment[1] = 0.365		Days[2] = 0.327	

[1]Mean values in a column showing a difference greater than the LSD value are significantly different at p=0.05.
[2]Mean values in a row showing a difference greater than the LSD value are significantly different at p=0.05.

Tab. 6: Effect of fluorescent *Pseudomonas* species on pH of tomato stored at 23±4 ºC with relative humidity in the range of 25% - 70%.

Treatments	Season 2013				Season 2014			
	Storage Period/Days							
	0D	5D	10D	15D	0D	5D	10D	15D
Control	4.26±0.04	4.32±0.06	4.51±0.09	4.75±0.06	4.24±0.60	4.42±0.05	4.51±0.09	4.73±0.07
1% K-sorbate	4.26±0.04	4.38±0.06	4.41±0.17	4.69±0.06	4.24±0.60	4.38±0.06	4.41±0.17	4.70±0.07
HAB-10	4.26±0.04	4.39±0.05	4.42±0.08	4.63±0.11	4.24±0.60	4.39±0.05	4.63±0.11	4.63±0.11
HAB-25	4.26±0.04	4.27±0.04	4.48±0.03	4.59±0.10	4.24±0.60	4.29±0.03	4.48±0.03	4.58±0.10
HAB-15	4.26±0.04	4.42±0.05	4.44±0.03	4.51±0.12	4.24±0.60	4.32±0.06	4.44±0.03	4.51±0.12
$LSD_{0.05}$	Treatment[1] = 0.055		Days[2] = 0.0495		Treatment[1] = 0.058		Days[2] = 0.050	

[1]Mean values in a column showing a difference greater than the LSD value are significantly different at p=0.05.
[2]Mean values in a row showing a difference greater than the LSD value are significantly different at p=0.05.

Tab. 7: Effect of fluorescent *Pseudomonas* species on total titratable acidity (% citric acid) of tomato stored at 23±4 ºC with relative humidity in the range of 25% - 70%.

Treatments	Season 2013				Season 2014			
	Storage Period/Days							
	0D	5D	10D	15D	0D	5D	10D	15D
Control	1.22±0.37	0.43±0.08	0.41±0.05	0.31±0.04	1.18±0.38	0.36±0.04	0.35±0.06	0.31±0.04
1% K-sorbate	1.22±0.37	0.36±0.04	0.35±0.06	0.33±0.05	1.18±0.38	0.42±0.08	0.40±0.05	0.32±0.05
HAB-10	1.22±0.37	0.49±0.06	0.41±0.08	0.41±0.08	1.18±0.38	0.42±0.04	0.36±0.05	0.35±0.05
HAB-25	1.22±0.37	0.42±0.04	0.36±0.05	0.35±0.05	1.18±0.38	0.48±0.06	0.41±0.08	0.37±0.05
HAB-15	1.22±0.37	0.55±0.06	0.40±0.09	0.38±0.05	1.18±0.38	0.54±0.06	0.41±0.08	0.41±0.08
$LSD_{0.05}$	Treatment[1] = 0.137		Days[2] = 0.122		Treatment[1] = 0.140		Days[2] = 0.125	

[1]Mean values in a column showing a difference greater than the LSD value are significantly different at p=0.05.
[2]Mean values in a row showing a difference greater than the LSD value are significantly different at p=0.05.

after 15 d (Tab. 7). Minimum reduction in titratable acidity in *Pseudomonas* treated tomatoes indicates that treatments had an effect on respiration rate during ripening, hence, on citric acid content and had the potential to extend storage life. The increased pH and decreased acidity with longer storage time detected in the present study are in agreement with the conclusions of TIGIST et al. (2013).

Quantitative analysis of lycopene

Color is an important quality indicator in tomatoes. The change in tomato fruits leads to chlorophyll loss and quick accumulation of carotenoids (lycopene) during ripening process (GRIERSON and KADER, 1986; IBITOYE et al., 2009). In our study, the lycopene content was found to increase with time. A significant difference was

observed among treated and untreated fruits on every fifth day of storage in season one and two (5, 10, and 15 d) (Tab. 8). The lycopene content in *Pseudomonas* treated tomato fruits was less than the respective content in control and positive control sets indicating a delay in ripening. The ripening process of tomatoes is characterized by color change (Hertog et al., 2007) due to breakdown of chlorophyll and accumulation of lycopene (Brandt et al., 2006; Pek and Helyes, 2010).

Decay rotting percent

Treatment with epiphytic fluorescent *Pseudomonas* species (HAB-15, HAB-25 and HAB-10) completely protected the tomato fruits from decay up to fifteen days in season two, whereas HAB-10 has shown only 8.32% decay during storage in season one. The untreated tomatoes showed highest percent of decay (41.65% and 49.97%) as compared to *Pseudomonas* treated fruits (0%) and K-sorbate (24.97%) treated fruits in season one and two, respectively (Fig. 2a, Fig. 2b). The potential of *Pseudomonas* spp. as biological control agents against postharvest diseases has been reported (Bull et al., 1997; Demirci, 2011). The mode of action of these isolates through which the epiphytic fluorescent *Pseudomonas* species reduce decay at postharvest phase is not yet clearly stated. Antibiosis, resistance induction and antifungal metabolite production might be the possible mode of actions for the biocontrol activity of *Pseudomonas* spp. against fungal deterioration and better retention of tomato fruit quality in this study.

Disease Severity

Aspergillus, Alternaria, Penicillium, Geotrichum, Fusarium, Phytophthora species are reported as common postharvest pathogens responsible for 10% - 30% yield losses of tomato crop (Etebu et al., 2013). In this study, all three bacterial isolates have successfully controlled and minimized the prevalence of *Alternaria, Fusarium* and bacterial rot during storage of fifteen days in season 2013 and 2014. Control and positive control (1% K-sorbate) failed to control the prevalence and severity of *Fusarium* and bacterial rot. In 2013 (season one), untreated fruits (control) showed 11% - 49% bacterial rot and 50% - 100% *Fusarium* rot. However, positive control (1% K-sorbate) has shown least *Fusarium* and bacterial disease severity of 11% - 49% in 2013 (season one) (Fig. 3a). In 2014 (season two), scale 5 (50% - 100%), and scale 4 (11% - 49%) bacterial disease severity has been observed in control set and positive control set, respectively. However, symptoms of the disease were not observed in *Pseudomonas* treated fruits in 2014 (Fig. 3b). The use of biological control agents at postharvest stage has been considered a successful environment for the control and minimization of postharvest losses in fruits and vegetables (Di Francesco et al., 2016).

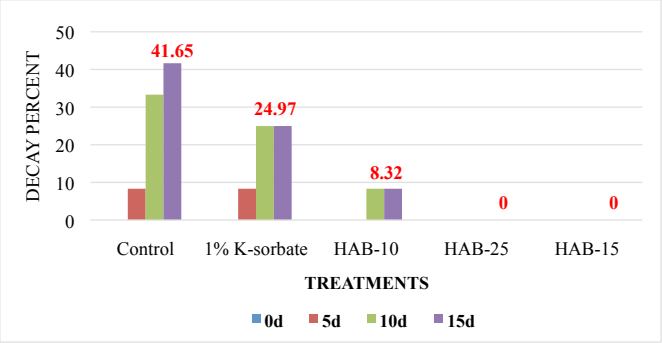

Fig. 2A: Effect of fluorescent *Pseudomonas* species on decay percent of tomato stored at 23±4 ºC with relative humidity in the range of 25-70%
2A = Season 2013, $LSD_{0.05}$ = Treatment[1] = 13.3, Days[2] = 11.9

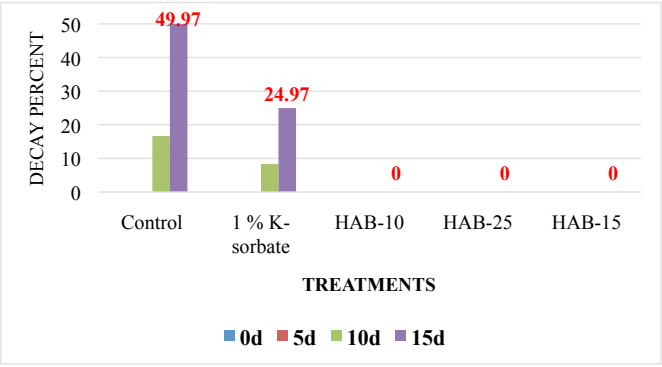

Fig. 2B: Effect of fluorescent *Pseudomonas* species on decay percent of tomato stored at 23±4 ºC with relative humidity in the range of 25%-70%.
2B = Season 2014, $LSD_{0.05}$ = Treatment[1] = 8.7, Days[2] = 7.8
[1]Mean values of bar in graph for treatment showing differences greater than LSD values are significantly different at p<0.05
[2]Mean values of bar at different days in graph showing differences greater than LSD values are significantly different at p<0.05.

Tab. 8: Effect of fluorescent *Pseudomonas* species on the lycopene content (mg/g) of tomato stored at 23±4 ºC with relative humidity in the range of 25% - 70%.

Treatments	Season 2013			Season 2014				
	Storage Period/Days							
	0D	5D10D	15D	0D	5D	10D	15D	
Control	6.98±0.33	11.61±2.24	12.74±1.80	17.32±2.63	6.67±1.71	11.60±.4	12.74±1.81	16.05±.67
1% K-sorbate	6.98±0.33	8.98±1.55	12.65±0.97	18.12±1.71	6.67±1.71	9.11±1.61	12.20±1.17	15.58±1.58
HAB-10	6.98±0.33	9.11±1.60	10.06±0.38	14.31±2.82	6.67±1.71	8.98±1.55	10.05±0.38	14.30±2.83
HAB-25	6.98±0.33	7.65± 0.89	9.32±0.57	13.34±1.73	6.67±1.71	7.31±0.97	8.54±1.25	13.44±2.70
HAB-15	6.98±0.33	6.67±1.71	8.55±1.25	12.65±1.55	6.67±1.71	7.07±0.46	8.44±1.27	12.65±1.50
LSD0.05	Treatment[1] = 1.048		Days[2] = 0.937		Treatment[1] = 1.239		Days[2] = 1.108	

[1]Mean values in a column showing a difference greater than the LSD value are significantly different at p=0.05.
[2]Mean values in a row showing a difference greater than the LSD value are significantly different atp=0.05.

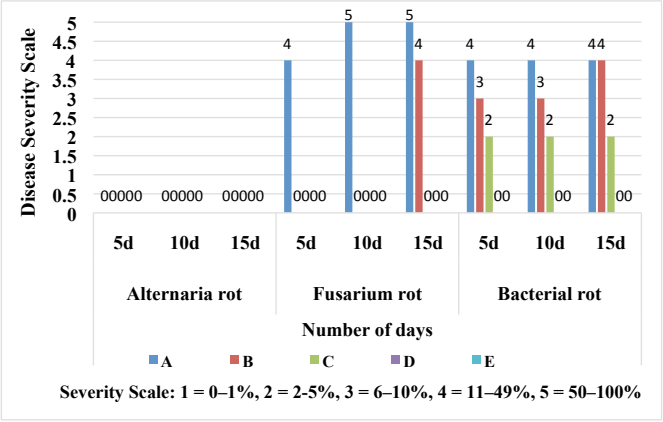

Fig. 3A: Effect of fluorescent *Pseudomonas* species on disease severity of tomato stored at 23±4 ºC with relative humidity in the range of 25-70% in season 2013.
A = Control, B = 1% K-sorbate, C = HAB-10, D = HAB-25, E = HAB-15

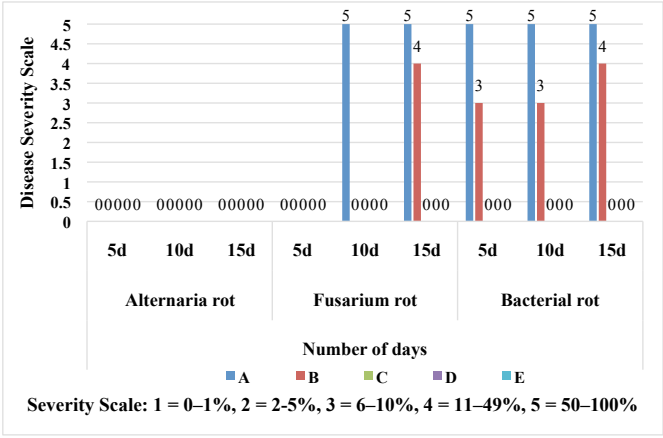

Fig. 3B: Effect of fluorescent *Pseudomonas* species on disease severity of tomato stored at 23±4 ºC with relative humidity in the range of 25-70% in season 2014.
A = Control, B = 1% K-sorbate, C = HAB-10, D = HAB-25, E = HAB-15

Conclusions

The present study has revealed that the surface of healthy fruits and vegetables harbors beneficial microflora, specifically fluorescent *Pseudomonas* species, which can exhibit biocontrol potential against post-harvest fungal diseases. Epiphytic fluorescent *Pseudomonas* species can play a vital role in controlling the compositional changes of tomato, including total soluble solids, pH, total titratable acidity and lycopene content with minimum weight loss in tomato stored at room temperature. Treatments resulted in delayed fungal spoilage of tomato fruits with less negative changes in fruit quality as compared to the controls, which showed greater compositional changes with higher loss of quality during storage in season 2013 and 2014. This study also concluded that tomato postharvest rot caused by *Alternaria* spp., *Penicillium* sp., *Fusarium* spp., and bacteria can be managed by using epiphytic fluorescent *Pseudomonas* suspensions as alternative and ecofriendly methods. This biologically based strategy deserves further development and application in commercial production of tomatoes especially in developing countries.

Acknowledgements

Financial support was provided by the Dean, Faculty of Science, University of Karachi and is sincerely acknowledged. We are also thankful to Dr. Stephen Wegulo, Professor, Department of Plant Pathology, University of Nebraska, Lincoln, USA for his comments, criticism and suggestions on manuscript.

References

A.O.A.C., 1994: Official Methods of Analysis, 16th edition, Association of Official Analytical Chemists. Virginia, U.S.A.

Abano, E.E., Sam-Amoah, L.K., 2012: Application of antagonistic microorganisms for the control of postharvest decays in fruits and vegetables. Int. J. Adv. Biol. Res. 2, 1-8. www.scienceandnature.org/IJABR_Vol2(1)2012/IJABR_V2(1)1R.pdf

Abbasi, N.A., Iqbal, Z., Maqbool, M., Hafiz, I.A., 2009: Postharvest quality of mango (*Mangifera indica* L.) fruit as affected by chitosan coating. Pak. J. Bot. 41, 343-357. www.pakbs.org/pjbot/PDFs/41(1)/PJB41(1)343.pdf

Abd Allah, E.F., Hashem, A., Al-Huqail, A., 2011: Biologically-based strategies to reduced postharvest losses of tomato. Afr. J. Biotechnol. 10, 6040-6044. DOI: 10.5897/AJB11.387

Abu Bakar, A.I., Zainuddin, M., Azzati, N.A., Kalsom, Y.U., 2013: Diversity of *Fusarium* species associated with potharvest fruit rot of tomato. Saina Malaysiana, 42,911-920. http://journalarticle.ukm.my/6297/1/04_A.I._Abu_Bakar.pdf

Adedeji, O.R., Ajani, R.T., Akanbi, C.C., Taiwo, K.A., 2006: Physiochemical properties of four tomato cultivars grown in Nigeria. Food Process. Preserv. 30, 79-86. DOI: 10.1111/j.1745-4549.2005.00049.x

Afzal, S., Tariq, S., Sultana, V., Ara, J., Ehteshamul-Haque, S., 2013: Managing the root diseases of okra with endo-root plant growth promoting *Pseudomonas* and *Trichoderma viride* associated with healthy okra roots. Pak. J. Bot. 45, 1455-1460. www.pakbs.org/pjbot/PDFs/45(4)/49.pdf

Anthon, G.E., LeStrange, M., Barrett, D.M., 2011: Changes in pH, acids, sugar and other quality parameters during extended vine holding of ripe processing tomatoes. J. Sci. Food Agric. 91, 1175-1181. DOI: 10.1002/jsfa.4312. Epub 2011 Mar 7

Barkai-Golan, R., 2001: Postharvest Diseases of Fruit and Vegetables: Development and Control. Elsevier Sciences, Amsterdam, Netherlands.

Bashan, Y., Holguin, G., Lifshitz, R., 1993: Isolation and characterization of plant growth promoting rhizobacteria. In: Glick, B.R., Thompson, J.E. (eds.), methods in plant molecular biology and biotechnology, 331-344. CRC Press, London.

Bombelli, E.C., Wright, E.R., 2006: Tomato fruit quality conservation during post-harvest by application of potassium bicarbonate and its effect on *Botrytis cinerea*. Cien. Inv. Agr. 33, 167-172. (In English) DOI: 10.7764/rcia.v33i3.346

Borji, H., Jafarpour, G.H., 2012: A comparison between tomato quality of mature-green and red- ripe stages in soilless culture. Afr. J. Agric. Res. 7, 1601-1603. DOI: 10.5897/AJAR11.1148

Brandt, S., Pék, Z., Barna, E., Lugasi, A., Helyes, L., 2006: Lycopene content and colour of ripening tomatoes as affected by environmental conditions. J. Sci. Food Agr. 86, 568-572. DOI: 10.1002/jsfa.2390

Bull, C.T., Stack, J.P., Smianick J.L., 1997: *Pseudomonas syringae* strains ESC-10 and ESC-11 survive in wounds on citrus and control green and blue mould of citrus. Biol Control 8, 81-88. DOI: 10.1006/bcon.1996.0476

Buonassisi, A.J., Sabaratnam, S., Bitterlich, I., 2013: Biosecurity Guidelines for Post-harvest Greenhouse Tomatoes: Prevention of Postharvest and Storage Rot. Research Report. Submitted to B.C. Ministry of Agriculture & B.C. Greenhouse Growers' Association, Canada. www2.gov.bc.ca/assets/gov/farming...and.../agriculture.../biosecurity-tomato.pdf

Corkidi, G., Balderas-Ruiz, K.A., Taboada, B., Serrano-Carreon, L.,

Galindo, E., 2006: Assessing mango anthracnose using a new three-dimensional image analysis technique to quantify lesions on fruit. Plant Path. 55, 250-257. DOI: 10.1111/j.1365-3059.2005.01321.x

Demireci, F., 2011: Effect of *Pseudomonas fluorescens* and *Candida famata* on blue mould of citrus caused by *Penicillium italumicum*. Aust. J. Crop Sci. 5, 344-349. DOI: 10.21475/ajcs.2016.10.06

Di Francesco, A., Martini, C., Mari, M., 2016: Biological control of post-harvest diseases by microbial antagonists: how many mechanisms of action? Eur. J. Plant Pathol. 145, 1-7. DOI: 10.1007/s10658-016-0867-0

Droby, S., 2006: Improving quality and safety of fresh fruit and vegetables after harvest by the use of biocontrol agents and natural materials. Acta Hortic. 709, 45-51. DOI: 10.17660/ActaHortic.2006.709.5

Ehteshamul-Haque, S., Baloch, G.N., Sultana, V., Ara, J., Tariq, M.R., Athar, M., 2013: Impact of seaweeds on fluorescent *Pseudomonas* and their role in suppressing the root diseases of soybean and pepper. J. Appl. Bot. Food Qual. 86, 126-132. DOI: 10.5073/JABFQ.2017.090.006

El Ghaouth, A., 1997: Biologically based alternatives to synthetic fungicides for the control of postharvest diseases. J. Indus. Microbiol. Biotech. 19, 160-162. DOI: 10.1038/sj.jim.2900428

Etebu, E., Nwauzoma, A.B., Bawo, D.D.S., 2013: Postharvest spoilage of tomato (*Lycopersicon esculentum* Mill.) and control strategies in Nigeria. J. Biol. Agric. HealthCare 3, 51-60.
www.iiste.org › Home › Vol 3, No 10 (2013) › Etebu

Fatima, N., Batool, H., Sultana, Ara, J., Ehteshamul-Haque, S., 2009: Prevalence of postharvest rot of vegetables and fruits in Karachi, Pakistan. Pak. J. Bot. 41, 3185-3190.
www.pakbs.org/pjbot/PDFs/41(6)/PJB41(6)3185.pdf.

Fish, W.W., Perkins-Veazie, P., Collins, J.K., 2002: A quantitative assay for lycopene that utilizes reduced volume of organic solvents. J. Food Comp. Anal. 15, 309-317. DOI: 10.1006/jfca.2002.1069

Garcia, E., Barrett, D.M., 2005: Evaluation of processing tomatoes from two consecutive growing seasons: quality attributes, peelability and yield. University of California, Davis.

Garrity, G., Brenner, D.J., Krieg, N.R., Staley, J.R., 2005: Bergey's Manual® of Systematic Bacteriology. Volume 2: The Proteobacteria, Part B: The Gamma-proteobacteria. Springer-Verlag US.

Gomes, A.A.M., Machado, A.R., Ferreira, A.P.S., Durta, D.C., Pereira, O.L., 2015: Postharvest fruit rot of tomato in Brazil caused by *Fusarium boothii*. Pl. Disease 99, 55.
DOI: 10.1094/PDIS-09-14-0903-PDN

Gomez, K.A., Gomez, A.A., 1984: Statistical procedures for agricultural research. 2nd ed. Wiley, New York.

Gould, W.D., Hagedorn, C., Bardinelli, T.R., Zablotswicz, R.M., 1985: New selective media for enumeration and recovery of fluorescent *Pseudomonas* from various habitats. Appl. Environ. Microbiol. 49, 28-32. 0099-2240/85/010028-05$02.00/0.

Grierson, D., Kader, A.A., 1986: Fruit ripening and quality. In: Atherton, J.G., Rudich, J. (eds.), The tomato crop: A scientific basis for improvement, 241-280. London, Chapman and Hall.

Habiba, Noreen, R., Ali, S.A., Sultana, V., Ara, J., Ehteshamul-Haque, S., 2016: Evaluation of biocontrol potential of epiphytic fluorescent *Pseudomonas* associated with healthy fruits and vegetables against root rot and root knot pathogens of mungbean. Pak. J. Bot. 48, 1299-1303. https://www.pakbs.org/pjbot/PDFs/48(3)/53.pdf

Harker, F.R., Redgwell, R.J., Hallett, I.C., Murray, S.H., Carter, G., 1997: Texture of fresh fruits. Hortic. Rev. 20, 121-224.
DOI: 10.1002/9780470650646.ch2

Hertog, M.L.A.T.M., Lammertyn, J., Scheerlinck, N., Nicolai, B.M., 2007: The impact of biological variation on postharvest behaviour: The case of dynamic temperature conditions. Postharvest Biol. Technol. 43, 183-192. DOI: 10.1016/j.postharvbio. 2006.07.007

Holcroft, D., 2015: Water Relations in Harvested Fresh Produce. The Postharvest Education Foundation, La Pine, Oregon, USA.

Huber, D.J., Karakurt, Y., Jeong, J., 2001: Pectin degradation in ripening and wounded fruits. R. Bras. Fisiol. Veg. 13, 224-241.
DOI: 10.1590/S0103-31312001000200009

Ibitoye, D.O., Akin-Idowu, P.E., Ademoyegun, O.T., 2009: Agronomic and lycopene evaluation in tomato (*Lycopersicon esculentum* Mill.) as a function of genotype. World. J. Agric. Sci. 5, 892-895.
https://www.idosi.org/wjas/wjas5(S)/13.pdf

Janisiewicz, W.J., Korstenl, L., 2002: Biological control of postharvest diseases of fruit. Ann. Rev. Phytopath. 40, 411-441.
DOI: 10.1146/annurev.phyto.40.120401.130158

Kader, A.A., 2002: Post-harvest Technology of Horticultural crops. University of California, Agriculture and Natural Resources.

Kalra, S.K., Tandon, D.K., Singh, B.P., 1995: Handbook of fruit science and technology. In: Salunkhe, D.K., Kadam, S.S. (eds.), Production, composition, storage and processing, 123-170. Marcel Dekker Inc., New York, USA.

Kamiloglu, O., 2011: Influence of some cultural practices on yield, fruit quality and individual anthocyanins of table grape cv. 'HorozKarasi'. J. Anim. Plant Sci. 21, 240-245.
www.thejaps.org.pk/docs/21-2/10-148_Revise-proof.pdf

King, E.O., Ward, M.K., Raney, D.E., 1954: Two simple media for the demonstration of pycocyanin and fluorescin. J. Lab. Clin. Med. 44, 301-307. PMID: 13184240

Korsten, L., 2006: Advances in control of postharvest diseases in tropical fresh produce. Int. Postharvest Technol. Innov. 1, 48-61.
DOI: 10.1504/IJPTI.2006.009181

Lurie, S., Klein, J.D., 1990: Heat treatment on ripening apples: differential effect on physiology and biochemistry. Physiol. Plant 78, 181-186.
DOI: 10.1111/j.1399-3054.1990.tb02078.x

Magwaza, L.S., Opara, U.L., 2015: Analytical methods for determination of sugars and sweetness of horticultural products – A review. Sci. Hortic. 184, 179-192. DOI: 10.1016/j.scienta.2015.01.001

Mari, M., Bertolini, P., Pratella, G.C., 2003: Non-conventional methods for the control of post-harvest pear diseases. J. Appl. Microbiol. 94, 761-766. DOI: 10.1046/j.1365-2672.2003.01920.x

Nath, A., Deka, B.C., Singh, A., Patel, R.K., Paul, D., Misra, L.K., Ojha, H., 2012: Extension of shelf life of pear fruits using different packaging materials. J. Food Sci. Technol. 49, 556-563.
DOI: 10.1007/s13197-011-0305-4

Nei, D., Uchino, T., Sakai, N., Tanaka, S., 2005: The effect of temperature on the quality of tomato and eggplant fruit during distribution. J. Fac. Agri. 50, 213-221. Kyushu Univ., citeseerx.ist.psu.edu/viewdoc/summary? DOI: 10.1.1.659.6769

Noreen, R., Ali, S.A., Hasan, K.A., Sultana, V., Ara, J., Ehteshamul-Haque, S., 2015: Evaluation of biocontrol potential of fluorescent *Pseudomonas* associated with root nodules of mungbean. Crop Protect. 75, 18-24. DOI: 10.1016/j.cropro.2015.04.018

Nurulhuda, M.S., Latiffah, Z., Baharuddin, S., Maziah, Z., 2009: Diversity of *Fusarium* species from vegetables fruits. J. Malaysian Appl. Biol. 38, 43-47. www.mabjournal.com/index.php?option=com.

Paulson, K.N., Stevens, M.A., 1974: Relationship among titrable acidity, pH and buffer composition of tomato fruits. J. Food Sci. 39, 354-357.
DOI: 10.1111/j.1365-2621.1974.tb02893.x

Pavel, A.B., Vasile, C.I., 2012: PyElph – a software tool for gel images analysis and phylogenetics. BMC Bioinformatics 13, 9.
DOI: 10.1186/1471-2105-13-9.

Pek, Z., Helyes, L., 2010: Color changes and antioxidant content of vine and postharvest ripened tomato fruits. HortSci. 45, 466-468.
hortsci.ashspublications.org/content/45/3/466.full

Požrl, T., Znidarčič, D., Kopjar, M., Hirbar, J., Simčič, M., 2010: Change of textural properties of tomatoes due to storage and storage temperatures. J. Food. Agric. Envir. 8, 292-296.
world-food.net/download/journals/2010-issue_2/f58.pdf

Salunkhe, D.K., Jadhav, S.J., Yu, M.H., 1974: Quality and nutritional composition of tomato fruit as influenced by certain biochemical and physiological changes. Qual. Plant. – H. Fds. Hum. Nutr. 24, 85-113.
ucanr.edu/datastoreFiles/608-310.pdf

SHACKEL, K.A., GREVE, L.C., LABAVITCH, J.M., AHMADI, H., 1991: Cell turgor changes associated with ripening in tomato pericarp tissue. Plant Physiol. 97, 814-815. 0032-0889/91/97/081 4/03/$01 .00/0

SHARMA, R.R., SINGH, D., SINGH, R., 2009: Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review, Biol. Control 50, 205-221. DOI: 10.1016/j.biocontrol.2009.05.001

SIDDIQUI, I.A., EHTESHAMUL-HAQUE, S., 2001: Suppression of the root rot-root knot disease complex by *Pseudomonas aeruginosa* in tomato: The influence of inoculum density, nematode population, moisture and other plant associated bacteria. Plant Soil 237, 81-89. DOI: 10.1023/A:1013313103032

SIDDIQUI, I.A., QURESHI, S.A., SULTANA, V., EHTESHAMUL-HAQUE, S., GHAFFAR, A., 2000: Biological control of root rot-root knot disease complex of tomato. Plant Soil 227, 163-169. DOI:10.1023/A:1026599532684

SIDDIQUI, M.W., CHAKRABORTY, I., MISHRA, P., HAZRA, P., AYALA-ZAVALA, J.F., 2015: Postharvest physicochemical changes in mutant (dg, ogc, and rin) and non-mutant tomatoes. Acta Physiol. Plant. 37, 1728. DOI: 10.1007/s11738-014-1728-0

SINGH, K.K., REDDY, B.S., 2006: Post-harvest physico-mechanical properties of orange peel and fruit. J. Food Eng. 73, 112-120. DOI: 10.1016/j.jfoodeng.2005.01.010

STEVENS, M.A., 1972: Citrate and malate concentrations in tomato fruits: genetic control and muturational effects. J. Am. Soc. Hortic. Sci. 97, 655-658.

TALIBI, I., BOUBAKER, H., BOUDYACH, E.H., AIT BEN AOUMAR, A., 2014: Alternative methods for the control of postharvest citrus diseases. J. Appl. Microbiol. 117, 1-17. DOI: 10.1111/jam.12495. Epub 2014 Apr 3

TARIQ, S., KHAN, R., SULTANA, V., ARA, J., EHTESHAMUL-HAQUE, S., 2009: Utilization of endo-root fluorescent Pseudomonas of chili for the management of root diseases of chili. Pak. J. Bot. 41, 3191-3198. www.pakbs.org/pjbot/PDFs/41(6)/PJB41(6)3191.pdf

TIGIST, M., WORKNEH, T. S.,WOLDETSADIK, K., 2013: Effects of variety on the quality of tomato stored under ambient conditions. J. Food Sci. Technol. 50, 477-486. DOI: 10.1007/s13197-011-0378-0

TEKA, T.A., 2013: Analysis of effect of maturity stage on the postharvest biochemical quality characteristics of tomato (*Lycopersicon esculentum* MILL.) Fruit. Int. Res. J. Pharm. App. Sci. 3, 180-186. www.irjpas.com/File_Folder/IRJPAS%203(5)180-186.pdf

TIRUPATHI, V.S., SASIKALA, KENNEDY, Z.J., 2006: Preservation of fruits and vegetables by wax coating. In: Mittal, H.K. et al. (eds.), Science Technology Entrepreneur, 1-10. NSTEDS, DST. Delhi.

VAN DIJIK, C., BOERIU, C., STOLLE-SMITS, T., TIJSKEN, L.M.M., 2006: The firmness of stored tomatoes (cv. Tradiro). 2: Kinetic and Near infrared model to describe pectin degrading enzymes and firmness loss. J. Food. Engn. 77, 585-593. DOI: 10.1016/ j.jfoodeng.2005.07.017

WILSON, C.L., WISNIEWSKI, M.E., 1989: Biological control of postharvest diseases of fruits and vegetables: an emerging technology. Annu. Rev. Phytopathol. 27, 425-441. DOI: 10.1146/annurev.py.27.090189.002233

WILSON, C.L., WISINIEWSKI, M.E., BILES, C.L., MCLAUGHLIN, R., CHALUTZ, E., DROBY, S., 1991: Biological control of post-harvest diseases of fruits and vegetables: alternative to synthetic fungicides. Crop Protect. 10, 172-177. DOI: 10.1016/0261-2194(91)90039-T

WILSON, L.C.,WISNIEWSKI, M.E., 1994: Biological control of postharvest disease of fruits and vegetables-Theory and practice. CRC Press, Boca Raton, Florida.

ZHANG, H., ZHENG, X., FU, C., XI, Y., 2005: Postharvest biological control of gray mold rot of pear with *Cryptococcus laurentii*. Postharvest Biol. Technol. 35, 79-86. DOI: 10.1016/j.postharvbio.2004.03.011

Address of the corresponding authors:
E-mail: sehaq@uok.edu.pk