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ENDOPHYTIC FLUORESCENT *PSEUDOMONAS AERUGINOSA* COMPETENCY AGAINST POST-HARVEST DECAY TO EXACERBATE SHELF LIFE AND QUALITY OF APPLE (*MALUS DOMESTICA* L.) FRUIT

Eesha Wasim[1], Sumara Shaheen[1], Noureen Rehan[1], Tayyab Raza Fraz[3] and Hafza Asma Shafique[1,2*]

[1]*Agricultural Biotechnology and Phytopathology Laboratory, Department of Botany University of Karachi, Karachi-75270, Pakistan*
[2]*Department of Botany, University of Karachi, Karachi-75270, Pakistan*
[3]*Department of Statistics, University of Karachi, Karachi--75270, Sindh, Pakistan*
*Corresponding author's email: h.a.shafique@uok.edu.pk

ABSTRACT

The aim of this study was the determination of the promising approach of using Bio-control agent (Bacteria) among the other various innovative technologies as an alternative of chemical fungicides and pesticides which have peril impact on human health consciousness and environment both while extolling the fresh fruits and vegetables shelf life and their quality. Pakistan is endowed with the production of Apple fruit, many cultivars are grown (Red delicious, golden delicious, royal gala, kulu etc.), but a significant amount of apples are destroyed due to post-harvest rotting. Present experiment was conducted at Agricultural Biotechnology and phytopathology Laboratory, Department of Botany, University of Karachi. In this study apples were treated with the application of bacterial isolates (RDMD-02, RDMD-03, RDMD-04 and RDMD-02 + RDMD-04) of *Pseudomonas aeruginosa,* stored at room temperature for one week to observe the shelf life and post-harvest decay. The findings of our experiment were that shelf life of apples were maintained better when treated with bacterial isolates as compared to untreated apples (control). Hence, it is concluded that use of bio-control as a microbial antagonist works efficient in maintaining the quality and shelf life of apple fruit.

Keywords: Bio-control agent, Post-harvest rot, *Pseudomonas aeruginosa,* Shelf life, Microbial antagonist

INTRODUCTION

Apple (*Malus domestica* L.) belongs to family Rosaceae which is widely cultivated in Murree Hills (Rawalpindi), Northern areas, Kashmir and Quetta (Muqeet *et al.,* 2020). The average yield of apple being 6.6 thousand tones/ha in Pakistan during 2011 year, along with the total area of 47.7 thousand tons/ha of Pakistan under apple cultivation with 315.4 thousand tons of apple annual production of Pakistan. The famous varieties of apple are Tur-kulu (Red delicious) and Shin-kulu (Golden Delicious) due to of their exalted antioxidant, poly phenolic properties and vitamins those are eminently contributing to human health. The desideratum of organic fruits and vegetables have been raised world widely because of their essential nutrients and vitamins indispensable for human's diet, along with an alliance to mitigate the affliction of peril from many chronic degenerative illnesses (WHO, 2003). Post-harvest decay is a threat to the economic sustainability of a country (Droby, 2006). Many temperate region fruits including apples has shown greater rate of susceptibility towards anthracnose disease, in Korea this disease is caused by *Colletotrichum gloeosporioides* and *Colletotrichum acutatum* (Lee *et al.,* 2007). Phytopthogens assailment becomes the reason of post-harvest losses of fresh fruits and vegetables which is due to the mechanical injuries during transportation and of novice handling etc.

The Post-harvest decay of fresh fruits and vegetables have been overcoming by the efforts of international market while in search of flexible, human and environment friendly alternative to fungicide and chemical pesticides species of antagonistic bacteria play vital role (Droby *et al*., 2009). Bio-control has proved as an excellent and effective alternative to fungicide while having healthy relationship with an environment with no hazardous effects on human health (Palmieri *et al*., 2022). Bacteria, fungi and yeast were considered as most prominent antagonist organisms at the stage of post-harvest (Shah *et al.,* 2017). It has been reported that bacteria such as *Pseudomonas syringae, Pseudomonas cepacia, Bacillus subtilis,* also yeast like *Debaryomyces hansenii, Candida sake, Rhodotorula glutinis* have been using for both in vitro and in vivo testing in preventing post- harvest fruit losses caused by specific fungal pathogenic attack, such as *Penicillium italicum, Penicillum digitatum, Botrytis, Fusarium, Alternaria, Monilinia, Penicillium, Mucor, Aspergillus, Colletotrichum, Gloeosporium, Rhizopus* (Medina-Cordova *et al*., 2018, Shafique *et al*., 2015). As, organic fruits have pre-eminent requisition due to their essential nutrients and antioxidant properties to human's health. Application of Biological control is a propitious substitute to many opulent and precarious methods to ameliorate post-harvest decay of fruits while maintaining country's economy (Wasim *et*

al., 2022). The outline of this research experiment was to observe the effectuation of *Pseudomonas aeruginosa* as bio-control on post-harvest decay of red delicious apples due to of phytopathogen assailment.

MATERIALS AND METHODS

Sample collection

Healthy and fresh Red delicious *Malus domestica* (RDMD) were collected from the North Nazimabad, Karachi, Pakistan fruit market. *Pseudomonas* spp. was isolated within 24h, when samples were brought to the laboratory.

Isolation of endophytic fluorescent *Pseudomonas*

Samples of apple were washed with sterilized water and apple peal of 2 g from the fruit was taken, added in five mL buffer (pH 6.5) of 0.05 M phosphate, and sterile thistle mortar is used to pulverize it. In a petri plate containing Gould's S1 medium, 100 uL of each sample was transferred (Gould *et al.,* 1985) and plates were incubated for 2 days. Under UV light, bacterial colonies showing fluorescence and are purified on King's B agar medium after two days (King's *et al.,* 1954). According to Bergey's manual, *Pseudomonas* spp. was initially identified (Garrity *et al.,* 2005).

Gram staining

Bacterial culture's tiny amount was thinly spread over the slide, air-dried, and heat-fixed over a flame. A primary stain (crystal violet) is added for 1 minute and washed with a gentle stream of water, then blotted dry and Lugol's iodine is then added to the slide for 1 minute to fix the primary stain, then washed under running water. The decolorizer (ethyl alcohol) was then added for about 30 seconds and washed. The secondary stain (safranin) is added to the bacterial smear for 1 minute, washed, dried, and under the microscope its observation was done. Purple to blue-black color was shown by Gram-positive bacteria whereas, red color rendered by Gram-negative bacteria under a microscope.

Test for differentiation among fluorescent *Pseudomonas* species

Levan formation test

Nutrient agar media supplemented with 5% sucrose (w/v) was used to differentiate among the *Pseudomonas* species i.e. *Pseudomonas fluorescence, P. putida* and *P. aeruginosa,* when test bacterium was grown on it. *Pseudomonas fluorescence* showed positive results, exhibiting convex, mucoid colonies at room temperature after three to five days of incubation which indicated Levan formation (Kreig and Holt, 1984), whereas negative results were shown by *P. aeruginosa* and *P. putida*.

Growth at 41°C

Saprophytic fluorescent *Pseudomonas* species were differentiated when test bacterium was grown at 41°C. This test was done to observe the growth of *Pseudomonas aeruginosa,* if not grown then the test is negative, (Krieg and Holt, 1984).

Biochemical testing of bacteria

Starch hydrolysis

In a former culture suspension, the filter paper was souse and placed on petri plate seated with starch agar and then left for incubation of two days. Then 1% iodine solution was flooded on the plates. The utilization of starch by the microorganisms was divulged by the formation of colorless halo near the growth and by the occurrence of blue color in the reaming plates.

Gelatin liquefaction

In a suspension of one day old culture the discs of filter paper was dipped and placed on petri plates immersed with the media of gelatin nutrient agar. An incubation time was two days for petri plates at room temperature and then $HgCl_2$ solution was flooded on petri plates. The utilization of gelatin was indicated by the formation of yellow halo around the growth.

Catalase test

In a 10ml beaker of 3% H_2O_2 capillary tubes (1mm outer isolated and 67mm in length) were kept. The rapid evolution of oxygen by bubbling is a positive result. Capillary tubes (1mm outer isolated by 67mm in length were

placed in a 50-mL beaker containing about 10 ml of 3% H_2O_2. This appearance results because hydrogenperoxide splitted into H_2O and O_2.

***Fusarium* spp. isolation by soil dilution technique**

In 9 mL of 0.1% agar suspension was suspended with one gram sample of soil and dilution series were contrived. One mL of aliquot was taken from the final dilution of0.1% suspension of agar and transferred on petri plates containing PCNB (Nash and Synder, 1962), and by dishes rotation the suspension was spread on the surface of agar. Incubation period for plates were five days at 28°C. Identification *Fusarium* species were done after reference to Booth (1971) and Nelson *et al.,* (1983).

***Rhizoctonia solani* isolation by baiting technique**

Sorghum seeds were sterilized and used as baits and stationed on the moist surface of soil. The baits were removed after twenty four hours, under the tap water they were washed and relocated on PDA. The pH of 5.5 is requisite for growth and identification of *Rhizoctoni solani* (Whilem, 1955). The Population of *Rhizoctonia solani* in the soil was considered by the seeds colonization percentage.

***Macrophomina phaseolina* isolation by wet sieving and dilution technique**

Isolation technique of *Macrophomina phaseolina* through wet sieving was exhorted by Sheikh and Ghaffar, (1975). By 100-mesh (150-µm) 20 g of soil sample was wet sieved and settled on the screen of 300-mesh (53 µm). Under the running tap water the residue obtained 53-µm mesh screen was washed for one min and shifted into a solution of 0.5 % $Ca(OCL)_2$ in a beaker and set level up to 100mL to make dilution ratio of 1:5 . On the magnetic stirrer, the sclerotial suspension was placed and an aliquot of one mL was finely spread on the PDA containing petri plates surface, inoculated with streptomycin (0.2 g/L), penicillin (100,000 units/L), rose bengal (0.1 g/L) and demosan (0.3 g/L). The incubation time for plates were five days at 28° C and grayish to black color *Macrophomina phaseolina* colonies were recognized.

Dual plate culture method (Anti-fungal Activity)

Antifungal activity of bacterial strains were determined by using this technique explained by Drapeau *et al.* (1973), on one side of the petri dish was streaked with bacterial strain containing Czapek's Dox Agar media at 7.2 pH, and a disc of 5mm diameter disc of root-rooting test fungus was inoculated on the other side of the petri plate. The time period of incubation for petri plates was three to seven days at 28°C and the record of zone of inhibition was estimated from three to seven days (Rehan. N. *et al*., 2024)

Application of Endophytic *Pseudomonas* species on Apples

Three isolates and one combination of two isolates were selected and cultures were prepared in broth of King's B media and left for three days of incubation. The collection of fresh, diseased free, healthy, and of equal sized apples were done from fruit market in 2022 and before the application of treatments, were surface sterilized and air dried. In an aqueous suspension of Endophytic *Pseudomonas* isolates, the fruits were dipped for about five minutes. Later fruits were air-dried and kept at room temperature (three fruits in each basket). Sterile water act as negative control and fruits are treated with it and fruits immersed in positive control i.e. Topsin-M. The estimation of room temperature was 25±1°C with the range of 25-70% of relative humidity. On the day first, fourth and seventh, the physiological parameters were estimated.

Effect of Endophytic fluorescent *Pseudomonas* species on physio-chemical properties of Apples

Weight loss

The Apple fruits weight loss was determined by a standard procedure explained in AOAC (1994).

Weight loss = W1- W2/W1 × 100

Where:

W1 = initial weight of an apple

W2 = final weight of apple fruit on subsequent days of the study

Fruit Diameter

Apple fruit diameter was measured using Vernier caliper.

Total soluble solids (TSS)

The total soluble solid content of tomato fruits (AOAC, 1994) was measured by a hand refractometer (Atago Co., Tokyo, Japan)

pH

The pH of apple fruit was determined by the standard method as explained in AOAC (1994).

Titratable acidity (TA)

0.1 N sodium hydroxide was being titrated by 2.5mL of apple juice, using phenolphthalein as an indicator. The data were represented in % citric acid according to a standard method (AOAC, 1994).

% Citric acid = V× N × Wmeq100/Y

Where:

V = mL of NaOH solution used for titration,

N = Normality of NaOH solution,

Wmeq = Miliequivalent of citric acid (0.064),

Y = sample weight in g or mL.

Ascorbic acid

2,6-dichlorophenol-indolphenol (DCIP) photometric method was used to conduct the ascorbic acid assay. One gram fresh weight of Primary leaves, are uniform in size were rapidly parted and a Sorvall Omni mixer in 10 mL of ice-cold 0.0005 M disodium EDTA solution containing 3Va tri-chloroacetic acid (TCA) about one to two minutes was used to pulverized the leaves. The Whatman no. 4 paper was used to rapidly filter the homogenate and marked up to 20 mL with EDA-TCA extracting solution. The 2.0 mL of filtered leaf extract, 1 mL of distilled water and 2.0 mL of DCIP reagent were incorporated to each test tube and at the range of 600nm their optical densities were estimated.

Total phenols

The determination of total phenolic content was carried out by the method of Folin-Ciocalteu proposed by Singleton and Rossi (1965).

Data analysis

The performance of all the statistical analysis was conducted by using IBM SPSS Statistics. 21 for windows. The grouped column figures were plotted using Origin 2019 b 64 Bit. The principal component analysis (PCA) was also carried out.

RESULTS

Isolation of endophytic fluorescent *Pseudomonas*

Thirteen species of endophytic fluorescent *Pseudomonas spp.* were isolated form the collected apple samples and King's B agar medium was used for their purification (Table 1).

Gram staining

The bacteria was found to be gram-negative in nature due to the pink-red color appearance of bacteria as they persisted in the color of secondary dye.

Differentiation test for among the fluorescent *Pseudomonas* species

Levan formation test

The results showed a negative levan formation test (Table 2), as there was no exhibition of convex, mucoid colonies after three to five days of incubation.

Growth at 41°C

The positive results were shown by all the strains at 41°C, as they grow on King's B media (Table 2).

Biochemical testing of bacteria

Starch hydrolysis

All the strains showed negative results on starch hydrolysis test (Table 2).

Gelatin liquefaction

The positive results were shown by all the thirteen strains on gelatin liquefaction test (Table 2).

Catalase test

The rapid evolution of oxygen by bubbling was recorded as a positive result (Table 2). Bacteria's gram-negative nature , negative levan formation, grew on 41°C, positive catalase test, liquefies the gelatin, positive catalase test and negative starch hydrolysis test established the results that all the bacterial strains are of *Pseudomonas aeruginosa.*

Dual culture plate method (Anti-Fungal Activity)

The bacterial strains anti-fungal activity was recorded against the major root-rotting fungi. The majority of the strains showed a zone of inhibition while lysis and overlapping of fungal mycelium around bacteria were also observed. Amongst all 13 strains, 3 strains (RDMD-02, RDMD-03, RDMD-04) were highly efficient and showed complete suppression of root-infecting fungi by eminent zone of inhibition formation against *F. solani,* (17mm, 23mm, 10mm) *F. oxysporum* (24mm, 35mm, 31mm)*, M. phaseolina* (25mm, 15mm, 22mm)*,* and *R. solani* (19mm, 23mm, 20mm) respectively (Fig. 1).

Effect of Endophytic fluorescent Pseudomonas species on onphysio-chemical properties of Apple

Weight loss

Four various treatments namely negative control, Topsin-M, individual bacteria, and integrated bacterial treatments were given to the apples. Later, the difference was shown by all the three treatments on the weight loss percent of the stored apples in comparison of negative control. The minimum weight loss was rendered on the day seventh by RDMD-02+RDMD-04 (8.65%) and RDMD-02 (8.67%) at room temperature in comparison of treatment control and other treatments (Fig. 2).

Fruit Diameter

An increase in diameter of all the apple fruits was observed in all the treatments, whereas it was observed a slighter difference on the seventh day in RDMD-02+RDMD-04 (0.06) and RDMD-04 (0.07) in comparison of treatment control (Fig. 3).

Total soluble solids (TSS)

In this experiment, there was increase in TSS in apple fruit was observed both in treated and untreated fruit. But in the fruit treated with bacterial strains, this increasing pattern was least to be found in comparison of control (Fig. 4).

pH

A rising trend in pH of stored apple fruit was observes in Fig. 5. In both treated and untreated fruit, this increase is found to be tenacious. Whereas, a lesser increasing pattern was seen in the bacterial treatments comparatively to the control.

Titratable acidity (TA)

Total titratable acidity in the apples was deceased till the 7th day of storage. The fruits treated with bacteria has shown the minimum decreased total titratable acidity at room temperature among in all the treatments (Fig. 6).

Ascorbic acid

The amount of ascorbic acid or Vitamin-C was increased in all treatments. However, the increase was observed greatly among the apples treated with bacterial strains RDMD-02 (29.69) as compared to the control (17.24) (Fig. 7).

Total phenols

The amount of total phenol in apple fruits was increased in all treatments. However, the increase was observed greatly among the apples treated with bacterial strains RDMD-02+RDMD-04 (47.14) and RDMD-04 (45.94) as compared to the control (33.96) (Fig. 8).

Table 1. Table showing the source of RDMD strains, locality, and species of *Pseudomonas.*

Serial no.	**Culture**	**Fruit sources**	**Species**	**Locality**
1	RDMD-01	Red delicious	*Pseudomonas aeruginosa*	North Nazimabad fruit market
2	RDMD-02	Red delicious	*Pseudomonas aeruginosa*	North Nazimabad fruit market
3	RDMD-03	Red delicious	*Pseudomonas aeruginosa*	North Nazimabad fruit market
4	RDMD-04	Red delicious	*Pseudomonas aeruginosa*	North Nazimabad fruit market
5	RDMD-05	Red delicious	*Pseudomonas aeruginosa*	North Nazimabad fruit market
6	RDMD-06	Red delicious	*Pseudomonas aeruginosa*	North Nazimabad fruit market
7	RDMD-07	Red delicious	*Pseudomonas aeruginosa*	North Nazimabad fruit market
8	RDMD-08	Red delicious	*Pseudomonas aeruginosa*	North Nazimabad fruit market
9	RDMD-9	Red delicious	*Pseudomonas aeruginosa*	North Nazimabad fruit market
10	RDMD-10	Red delicious	*Pseudomonas aeruginosa*	North Nazimabad fruit market
11	RDMD-11	Red delicious	*Pseudomonas aeruginosa*	North Nazimabad fruit market
12	RDMD-12	Red delicious	*Pseudomonas aeruginosa*	North Nazimabad fruit market
13	RDMD-13	Red delicious	*Pseudomonas aeruginosa*	North Nazimabad fruit market

Table 2. Tests for differentiation among the species and biochemical tests of fluorescent *Pseudomonas.*

Bacterial strains	**Levan formation test**	**Growth at 41°C**	**Starch hydrolysis**	**Gelatin liquefaction**	**Catalase test**
RDMD-01	-	+	-	+	+
RDMD-02	-	+	-	+	+
RDMD-03	-	+	-	+	+
RDMD-04	-	+	-	+	+
RDMD-05	-	+	-	+	+
RDMD-06	-	+	-	+	+
RDMD-07	-	+	-	+	+
RDMD-08	-	+	-	+	+
RDMD-9	-	+	-	+	+
RDMD-10	-	+	-	+	+
RDMD-11	-	+	-	+	+
RDMD-12	-	+	-	+	+
RDMD-13	-	+	-	+	+

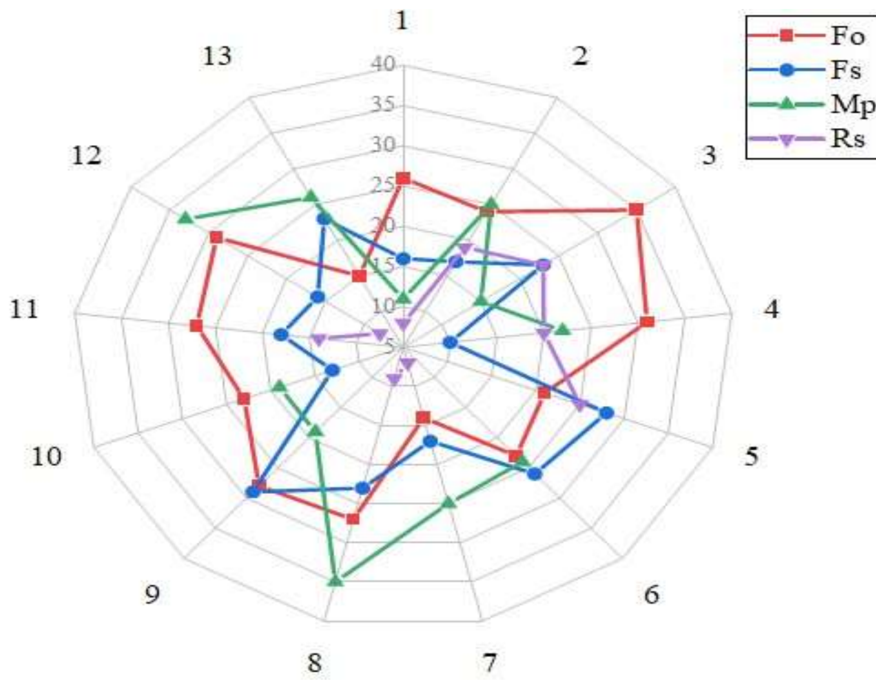

Fig. 1. The Spider plot shows the zone of inhibition by *Pseudomonas aeruginosa* against the major root-rot fungi namely *F. solani, F. oxysporum, M. phaseolina*, and *R. solani*in dual culture plate method.

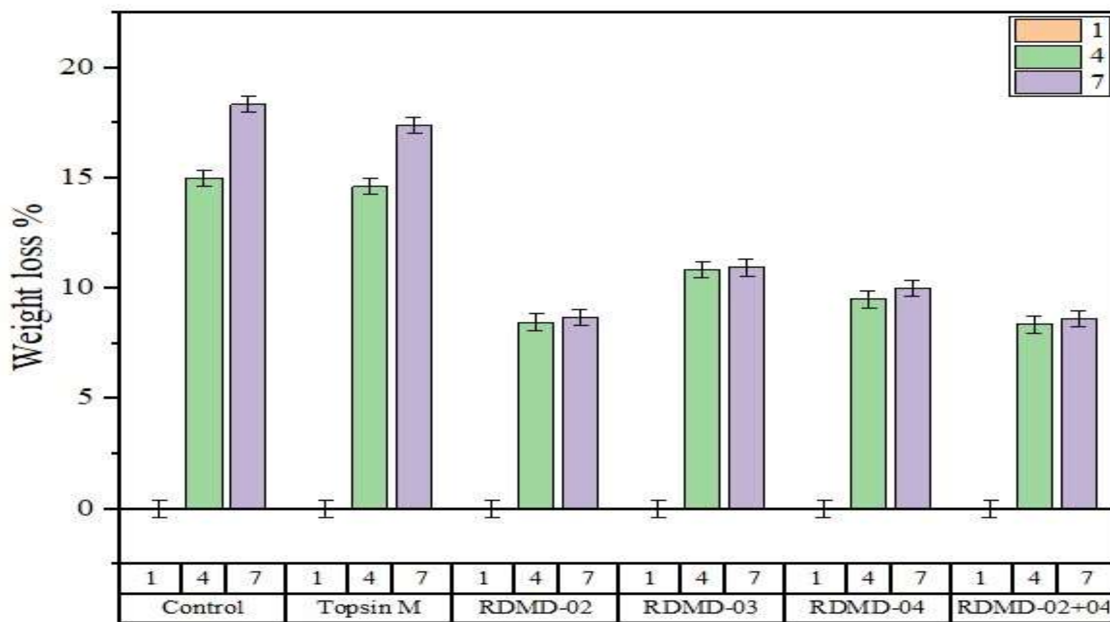

Fig. 2. Effect of fluorescent *Pseudomonas* species on the Weight loss of apples stored 23 ± 4 ºC.
The $LSD_{(0.05)}$ for treatments is 6.81 and time is 4.83. Difference higher than LSD values among means in the graph is significant at $p<0.05$

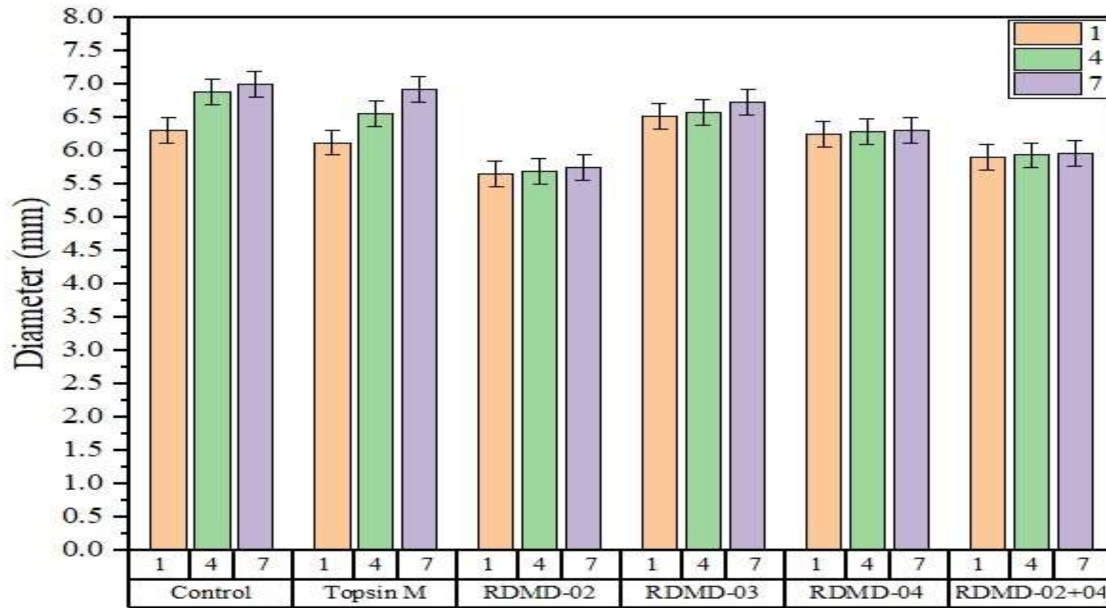

Fig. 3. Effect of fluorescent *Pseudomonas* species on the Diameter (mm) of apples stored 23 ± 4 ºC.
The $LSD_{(0.05)}$ for treatments is 0.40 and time is 0.28. Difference higher than LSD values among means in the graph is significant at $p<0.05$

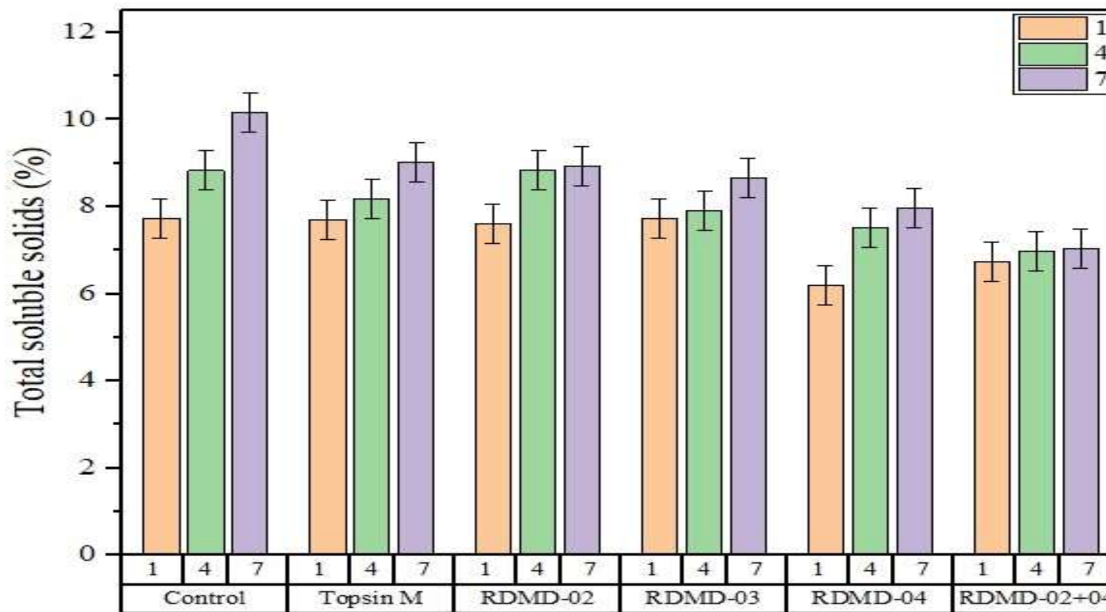

Fig. 4. Effect of fluorescent *Pseudomonas* species on the Total soluble solids (Brix %) of apples stored 23 ± 4 ºC.
The $LSD_{(0.05)}$ for treatments is 0.92 and time is 0.32. Difference higher than LSD values among means in the graph is significant at $p<0.05$

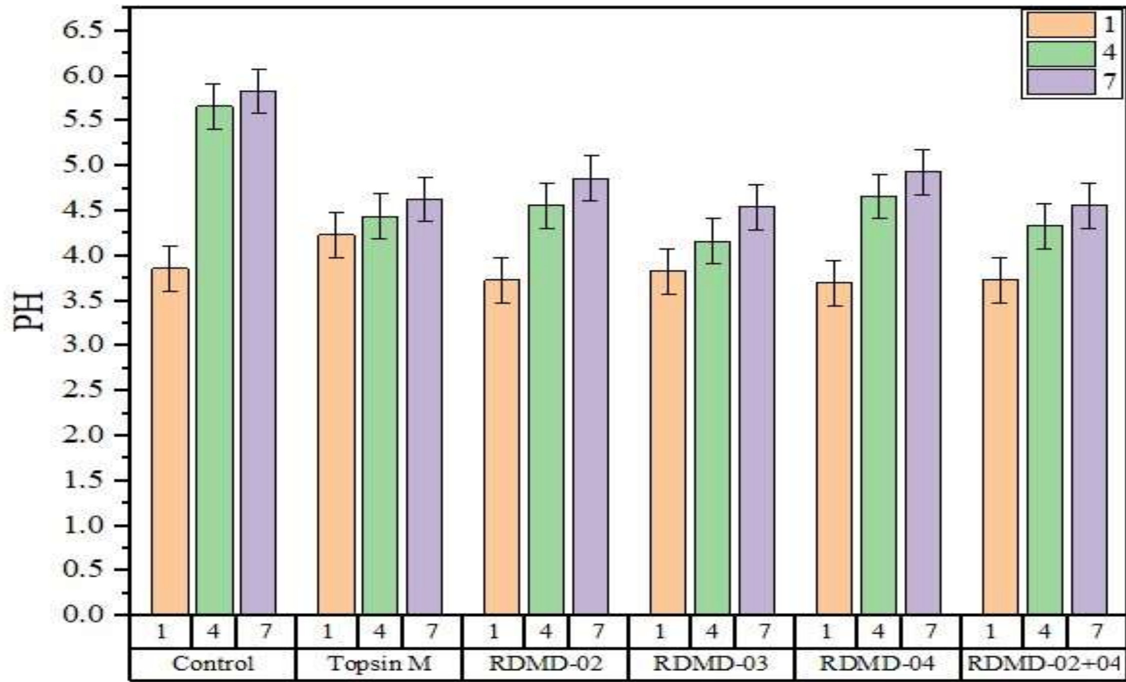

Fig. 5. Effect of fluorescent *Pseudomonas* species on the pH of apples stored 23 ± 4 ºC.
The $LSD_{(0.05)}$ for treatments is 0.51 and time is 0.37. Difference higher than LSD values among means in the graph is significant at $p<0.05$

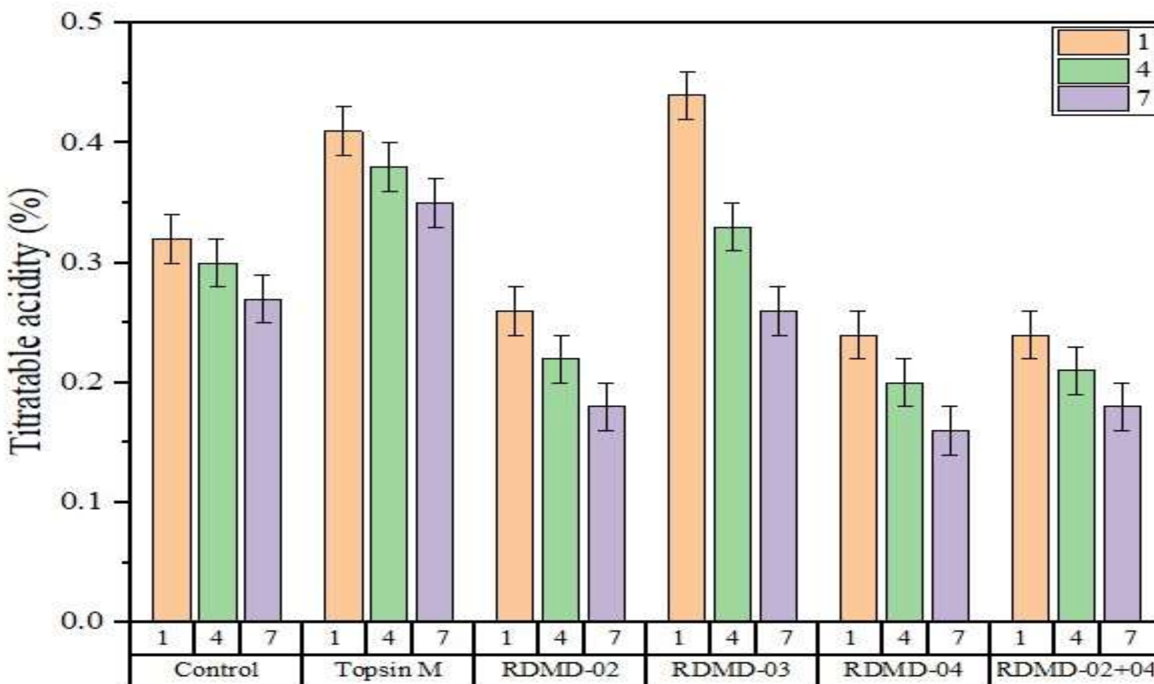

Fig. 6. Effect of fluorescent *Pseudomonas* species on the Titratable acidity of apples stored 23±4 ºC.
The $LSD_{(0.05)}$ for treatments is 0.05 and time is 0.04. Difference higher than LSD values among means in the graph is significant at $p<0.05$

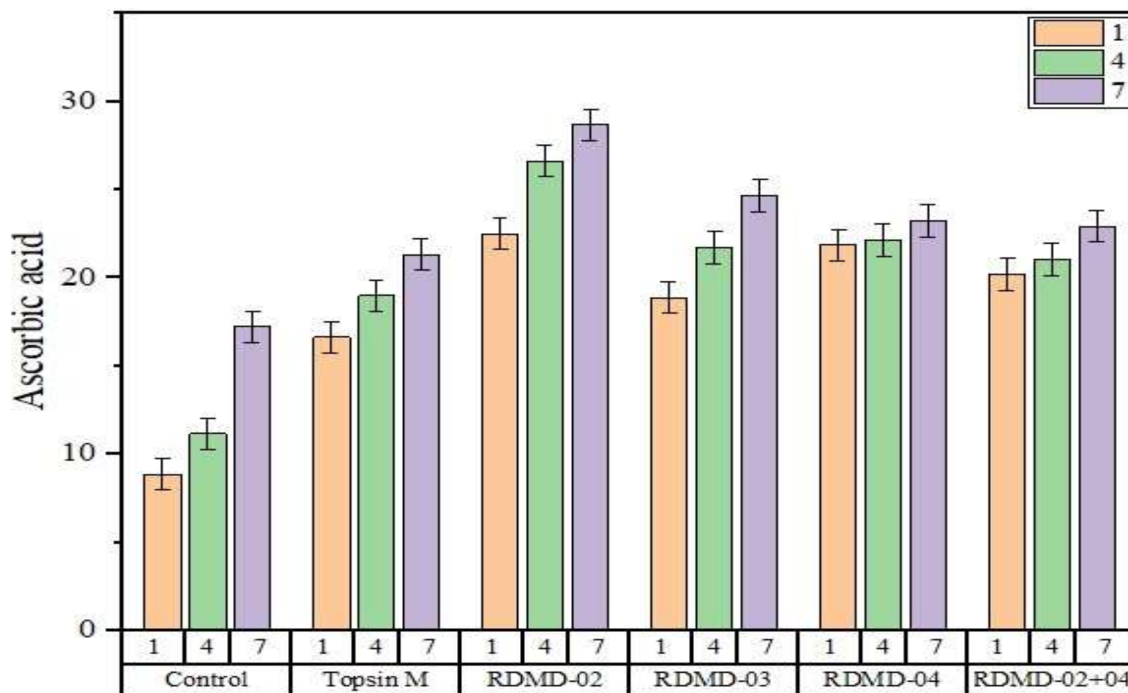

Fig. 7. Effect of fluorescent *Pseudomonas* species on the Ascorbic acid of apples stored 23 ± 4 ºC.
The $LSD_{(0.05)}$ for treatments is 10.09 and time is 7.13. Difference higher than LSD values among means in the graph is significant at $p<0.05$

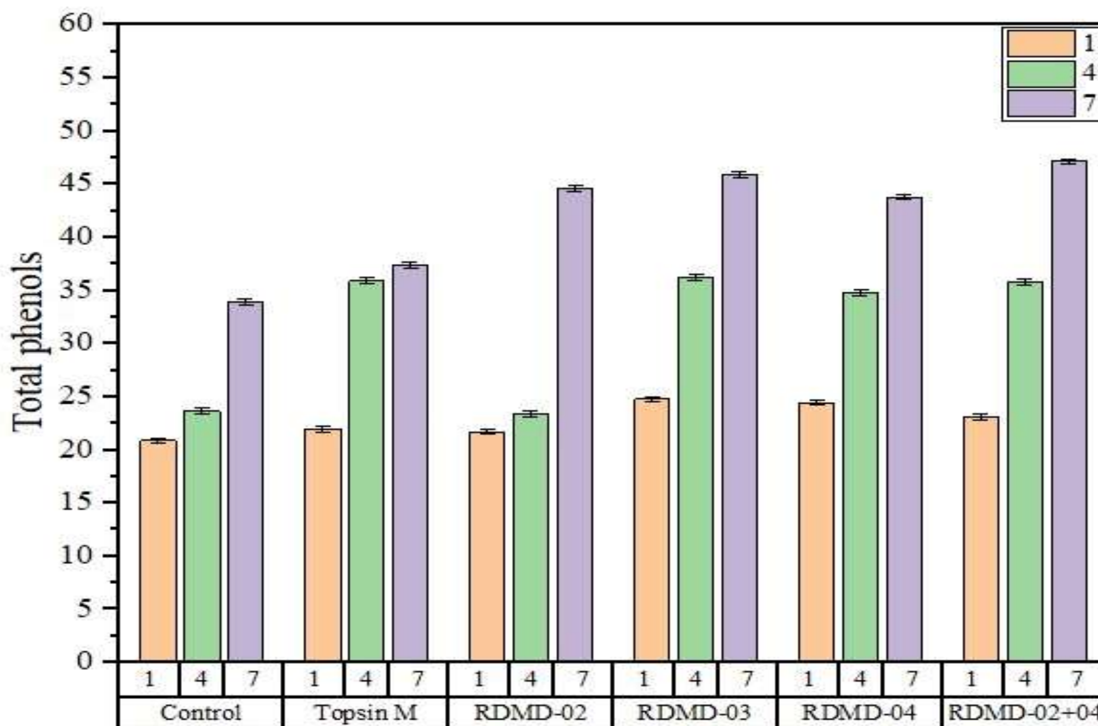

Fig. 8. Effect of fluorescent *Pseudomonas* species on the Total Phenols of apples stored 23 ± 4 ºC.
The $LSD_{(0.05)}$ for treatments is 10.68 and time is 7.55. Difference higher than LSD values among means in the graph is significant at $p<0.05$

DISCUSSION

The post-harvest diseases become the key curtail which cause paucity in export and domestic marketing of apple fruit consequently ameliorating its attribute of quality and shelf life along with enormous economic diminution (Harvey 1978; Ilyas *et al.*, 2007). Apple is found susceptible to postharvest fruit decay and 50 % of losses occur due to post-harvest decay (Ahmad *et al.,* 2021). The techniques like hot water dips, emulsion coatings, vapor heat treatments and fungicidal treatments are explained by many pre and post-harvest disease controlling studies (Diaz Sobac *et al.,* 2000; Sopee and Sangchote, 2005). In this study, the effect of endophytic fluorescent *Pseudomonas* on shelf life, and quality of fruit was observed. Initially, the *Pseudomonas* species were isolated, differentiated, tested biochemically, and to determine their effectiveness in laboratory conditions the dual culture plate method was used. The strains which showed effective results in the dual culture plate method were applied on the surface of apple fruit and various physio-chemical parameters were measured thrice (1st day, 4th day, and 7th day). This research found that due to of slow and stable metabolic processes the weight of apple fruit was reduced and more weight loss was seen in control apple fruits. The apple fruits diameter was also changed and the firmness gradually decreased over the passing days due to the enzymatic breakdown of pectin. The sustenance of fruit's firmness is accountably depend upon the structural carbohydrates like starch and pectin substances (Manganaris *et al.*, 2008). In this study an increasing pattern in pH of apple fruits has seen. Due to increase in the content of vitamin C, this pH accretion could be done. Indistinguishable findings are described by Machado *et al.*. (2007) during the study of jabuticaba fruits storage sealed with the packaging of plastics kept at low temperature. The correlation of increase in vitamin C and rise in pH was done by them. In the apple fruit, there is a constant fall in citric acid of the fruit was observed. The greater significance in the degree of reduction in total titratable acidity in control (untreated fruit) in comparison of treated fruits was observed. The reason of this could be of enormous oxygen recuperation through the process of oxidation which results in its quick loss. During the period of storage decay tends to be enhanced. Ketsa *et al.* (2000) also reported a rapid increase in decay at different storage temperatures. Although, an eminent result was shown by the application of *Pseudomonas* treatment in comparison of both control and other treatments.

REFERENCES

Ahmad, K., M. Afridi, A. N. Khan and A. Sarward (2021). Quality Deterioration of Postharvest Fruits and Vegetables in Developing Country Pakistan. *Asian J. of Agri and Food Sci*. 9 (2): 83-26.

AOAC, A. (1994). Official methods of analysis, 16th Ed. Association of official analytical chemists. Washington DC, USA, Sci. Educ.

Booth, C. (1971). *The Genus Fusarium*, CAB, Commonwealth Mycological Institute, Kew, Surrey, England. pp. 237.

Diaz-Sobac, R., L. Prez-Flores and J. Vernon-Carter (2000). Emulsion coatings control fruit fly and anthracnose in mango (*Mangiferaindica cv. Manila*). *J. Hort. Sci. Biotechnol.,* 75*:* 126-128.

Drapeau, R., J. A. Fortin and C. Gagnon (1973). Antifungal activity of *Rhizobium*. *Canadian Journal of Botany*, 51(3): 681-682.

Droby, S. (2006). Biological control of postharvest diseases of fruits and vegetables: difficulties and challenges. *Phytopathol. Pol*., 39: 105-117.

Droby, S., M. Wisniewski, D. Macarisin and C. Wilson (2009). Twenty years of postharvest biocontrol research: is it time for a new paradigm?. *Postharvest biology and technology*, 52(2): 137-145.

Garrity, G. M. (2005). *Bergey's Manual® of Systematic Bacteriology: Volume 2, The Proteobacteria (Part C)*. D. J. Brenner, N. R. Krieg, and J. T. Staley (Eds.). Springer US.

Gould, W.D., C. Hagedorn, T. R. Bardinelli and R. M. Zablotswicz (1985). New selective media for enumeration and recovery of fluorescent *Pseudomonas* from various habitats. *Appl. Environ. Microbiol.*. 49: 28- 32.

Harvey, J.M. (1978). Reduction of losses in fresh fruits and vegetables. *Ann, Rev. Phytopathol*., 16: 321-341.

Ilyas, M. B., M. U. Ghazanfar, M. A. Khan, C. A. Khan and M. A. R. Bhatti (2007). Post harvest losses in apple and banana during transport and storage. *Pakistan Journal of Agricultural Sciences*, 44(3): 534-539.

Ketsa, S., S. Chidtragool and S. Lurie (2000). Pre-storage heat treatment and poststorage quality of mango fruit. *HortScience*, 35(2): 247-249.

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

Krieg, N. R. and J. G. Holt (1984). *Bergey's manual of systematic bacteriology*, vol 1. Williams and Wilkins. *Baltimore London*.

Lee, D. H., D. H. Kim, Y. A. Jeon, J. Y. Uhm and S. B. Hong (2007). Molecular and cultural characterization of *Colletotrichum*spp. causing bitter rot of apples in Korea. *The Plant Pathology Journal*, 23(2): 37-44.

Machado, N. P., E. F. Coutinho and E. R. Caetano (2007). Embalagensplásticas e refrigeraçãonaconservaçãopós-colheita de jabuticaba. *RevistaBrasileira de Fruticultura*, 29: 166-168.

Manganaris, G. A., A. R. Vicente, C. H. Crisosto and J. M. Labavitch (2008). Cell wall modifications in chilling-injured plum fruit (*Prunussalicina*). *Postharvest Biology and Technology*, 48(1): 77-83.

Medina-Córdova, N., S. Rosales-Mendoza, L. G. Hernández-Montiel and C. Angulo (2018). The potential use of *Debaryomyceshansenii* for the biological control of pathogenic fungi in food. *Biological Control*, 121: 216-222.

Muqeet, S., A. Habib, A., Mansha and Z, M., Amina (2020). Response of Various Apple Cultivars against Post Harvest Rotting. *Nature Scienc,*. 18(8): 14-18.

Nash, S. M. and W. C. Snyder (1962). Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium*in field soils. *Phytopathology*, 52(6): 567-572.

Nelson, P. E., T. A. Toussoun and W. F. O. Marasas (1983). *Fusarium species: an illustrated manual for identification.* Pennsylvania state university press.

Palmieri, D., G. Ianiri, T. Conte, R. Castoria, G. Lima and F. De Curtis (2022). Influence of Biocontrol and Integrated Strategies and Treatment Timing on Plum Brown Rot Incidence and Fungicide Residues in Fruits. *Agriculture*, 12(10): 1656.

Rehan, N., H. Farhat, H. A. Shafique, M. Aijaz and S. Shaheen (2024). Role of microbial inoculants for improving productivity and systemic resistance in Abelmoschus esculentus. *Physiological and Molecular Plant Pathology*, 102211.

Shah, H., N. Rubina, S. A. Ali, K. A., Hasan, A. Jehan, S. Viqar and S. Ehteshamul-Haque (2017). Protective role of epiphytic fluorescent *Pseudomonas* on natural postharvest decay of tomato at room temperature. *Journal of Applied Botany and Food Quality*, 90: 288-297.

Sheikh, A. H. and A. Ghaffar (1975). Population study of the sclerotia of *Macrophomina phaseolina* in cotton fields. *Pakistan Journal of Botany*, 7 (1): 13-17.

Shafique, H. A., V. Sultana, J. Ara, S. Ehteshamul-Haque and M. Athar (2015). Role of antagonistic microorganisms and organic amendment in stimulating the defense system of okra against root rotting fungi. *Polish Journal of Microbiology*, 64(2): 157-162.

Singleton, V. L. and J. A. Rossi (1965). Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. *American journal of Enology and Viticulture*, 16(3): 144-158.

Sopee, J. and S. Sangchote (2004). Effect of heat treatment on the fungus *Colletotrichumgloeosporioides* and anthracnose of mango fruit. *V International Postharvest Symposium,* 682: 2049-2056.

W. H. Organization (2003). *Fruit and Vegetable Promotion Initiative: A Meeting Report*, 25-27/08/03. Geneva: World Health Organization.

Wasim, E., S. Shaheen, H. A. Shafique and Y. M. Adnan (2022). Antagonistic potential of *Pseudomonas aeruginosa* against postharvest decay of apple (*Malus pumila* L). *Int. J. Biol. Res*, 10 (1): 33-40, 2022.

Wilhelm, S. (1955). Longevity of the *Verticillium* wilt fungus in the laboratory and field. *Phytopatholog*y, 45: 180-181.

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