Int. J. Biol. Res., 10 (1): 33-40, 2022.

ANTAGONISTIC POTENTIAL OF *PSEUDOMONAS AERUGINOSA* AGAINST POST-HARVEST DECAY OF APPLE (*MALUS PUMILLA* MILL.) FRUIT

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ABSTRACT

Post-harvest losses of fruits are the core cause of agricultural losses due to phytopathogenic attacks. These phytopathogens were traditionally used to control by the application of synthetic fungicides but it has environment perilous impacts. Several flexible alternatives are made to control post-harvest losses biologically as a microbial antagonist against phytopathogens like fungi and bacteria. For economic loss prevention due to post-harvest decay, a biological control method is the most perpetual solution and beneficial to the environment and humans, as well. In this study, *Pseudomonas aeruginosa* has shown effective results as a biological control agent against post-harvest losses to overcome yield loss and long-term storage of fresh fruits in current times. 5 strains namely, GDMD-01. GDMD-02, GDMD-08, GDMD-09, and RDMD-01 were isolated from apples. In fruit analysis, bacterial isolates (GDMD-01, GDMD-02, GDMD-09, and a combination of GDMD-08 and RDMD-01 of apples were applied on apple fruits and they worked excellently against post-harvest decay of apples. The isolates retained the total soluble solids (TSS), weight, titratable acidity, length, pH, diameter, and decay and rotting severity in the storage time period of apple fruits. Whereas, the integrated application of *Pseudomonas aeruginosa* isolates (GDMD-08 and RDMD-01) worked potentially in overcoming apple fruit post-harvest decay.

KEY-WORDS: Post-harvest decay, Endophytic bacteria, Bio-control, Agriculture losses, Phytopathogens, Root rotting fungi

INTRODUCTION

Apple (*Malus pumilla Mill.*) belongs to family Rosaceae which is widely cultivated. 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 during transportation. (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). Post-harvest losses of fruits are caused by pathogen assailment due to the mechanical injuries during transportation and of novice handling etc. The post-harvest losses of 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. It has been reported that bacteria such as *Pseudomonas cepacia,* Van Hall, *Pseudomonas syringae, Bacillus subtilis;* also yeast like *Candida sake, Rhodotorula glutinis, Debaryomyces hansenii* 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 digitatum, Penicillium italicum, Alternaria, Aspergillus, Botrytis, Fusarium, Geotrichum, Gloeosporium, Monilinia, Penicillium, Mucor, Colletotrichum* and *Rhizopus* (Medina-Cordova *et al.,* 2018). As, organic fruits have pre-eminent requisition due to their essential nutrients and antioxidant properties to human's health. Biological control is a promising alternative to

many opulent and parlous methods to post-harvest decay of fruits while maintaining economy of country. This research was design to observe the effect of *Pseudomonas aeruginosa* as bio-control on post-harvest decay of golden delicious apple variety due to phytopathogens.

MATERIALS AND METHODS

Sample collection

The fresh and healthy apples (Golden delicious) were collected from fruit market of North Nazimabad Karachi, Pakistan. Isolation of *Pseudomonas* was made from the same samples in the laboratory within 24 h.

Isolation of Endophytic *Pseudomonas* spp

For the isolation of the endophytic *Pseudomonas*, method reported by Shah *et al*. (2017) was followed. Briefly, 2 g of sterilized sample was ground using autoclaved pestle and mortar in 20 mL of 0.05 M phosphate buffer having pH of 6.5. The 0.5 mL of extract was poured into plates having S1 medium and kept at room temperature for 2 days. The bacteria that showed fluorescence under the UV light were purified on King's B medium. For the identification of *Pseudomonas* spp. the Manual of Bergey's was used (Garrity *et al.*, 2005).

IN VIVO

Application of Endophytic *Pseudomonas* species on apples

Three isolates of golden delicious variety of apples (GDMD-01, GDMD-02, and GDMD-09) and one combination of two isolates (GDMD-08 and RDMD-O1) were selected, and broths were prepared in King's B medium and incubated for 3 days.

For the application of endophytic *Pseudomonas* the fresh, disease-free, mature, and uniform size apples were collected, and before the treatment application they were surface sterilized. These fruits were dipped in the aqueous suspension of endophytic *Pseudomonas* isolates (GDMD-01, GDMD-02, and GDMD-09) and two isolates combination (GDMD-08 and RDMD-O1) and were air dried and placed in the baskets. Each treatment was replicated 4 times (three fruits per basket). The fruits only treated with water acted as a negative control, while 0.01% of Topsin-M solution has taken as a positive control. Room temperature estimation was 23 ± 4°C with 25-70% relative humidity range. The physiological parameters were observed after 2-days intervals i.e., 1st day, 4th day, and 7th day of treatment application.

Physiochemical testing of Apple

Weight loss

By following standard procedure described by AOAC (1995) the weight loss of apple fruit was calculated as:

% Weight loss = W1-W2/W1×100

Where:

W1= Initial apple weight

W2= Final apple weight on subsequent days of study

Total soluble solids (TSS)

The total soluble solids tomato fruit content (AOAC, 1995) was measured by Hand refractrometer instrument (Atago Co., Tokyo, Japan)

pH

Apple fruit pH was determined by the described standard method (AOAC, 1995).

Titratable acidity (TA)

Titration was performed by titrating 2.5 mL of apple juice against 0.1 N sodium hydroxide with the use of phenolphthalein as an indicator. The data expression was % citric acid as per described by standard method (AOAC, 1995).

% 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.

Decay/rotting percentage

Stored apple fruits decay percent determination was done by visual observations by following the calculation formula:

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

ANALYSIS OF DATA

One-way and two-way analysis of variance (ANOVA) with Steffens post hoc test was used for the conduction of statistical comparisons among all treatments. All tests assumptions were statistically verified.

RESULTS

Isolation of Endophytic *Pseudomonas* spp.

Four isolates of endophytic *Pseudomonas* spp. were isolated from golden delicious apples named GDMD-01, GDMD-02, GDMD-08, and GDMD-09 and identified using Bergey's manual, the isolates are shown in Table 1.

Table 1. Table showing source of Golden Delicious *Malus pumilla* (GDMD) strains, locality and species of *Pseudomonas.*

Serial No.	Culture	Fruit sources	Species	Locality
1	GDMD-01	Golden Delicious	*Pseudomonas aeruginosa*	North Nazimabad fruit market
2	GDMD-02	Golden Delicious	*Pseudomonas aeruginosa*	North Nazimabad fruit market
3	GDMD-08	Golden Delicious	*Pseudomonas aeruginosa*	North Nazimabad fruit market
4	GDMD-09	Golden Delicious	*Pseudomonas aeruginosa*	North Nazimabad fruit market
5	RDMD-O1	Red Delicious	*Pseudomonas aeruginosa*	North Nazimabad fruit market

Effect of fluorescent endophytic *Pseudomonas* on physiochemical properties of apples (golden delicious)
Weight loss

The weight loss of apple fruit increased with the passage of storage time, but less decline in weight has seen in the fruits treated with *Pseudomonas* treatments that were GDMD-09 and GDMD-08+RDMD-01 as in comparison of (control) treatment and Topsin-M as seen in Table 2.

Diameter

Pseudomonas treatments (GDMD-09 and GDMD-02) when applied to apple fruits stored at room temperature have shown best result in maintaining the diameter with the comparison of treatment (control) as shown in Table 3.

Length

In this study of one week experiment in which apples were stored at room temperature have retained length efficiently when treated with *Pseudomonas* treatments that were (GDMD-08+RDMD-01, GDMD-09 and GDMD-02) in comparison of (control) treatment as shown in Table 4.

pH

pH is one of the most significant quality parameter of apple fruits. In this study, apples were stored for 7 days at room temperature after being treated with *Pseudomonas* isolates. (GDMD-01, GDMD-02 and GDMD-08+RDMD-01) which showed significant increase in pH in comparison of control treatment and Topsin-M shown in Table 5. It has been stated that the rise in the value of pH abrogates the fruits senescence (Anthon *et al*., 2011).

Titratable acidity (TA)

The titratable acidity is an another imperative parameter to be observed for apple fruits. In this study the application of treatments (GDMD-01, GDMD-02 and combination of GDMD-08 + RDMD-01) ameliorate the level of titratable acidity in comparison of treatment (control) as shown in Table 6.

Table 2. Effect of fluorescent *Pseudomonas* species on % weight loss of apples stored at 23 ± 4 ºC.

Treatments	Weight loss (%)		
	Day-1	Day-4	Day-7
Control	0.00 ± 0.00	12.43 ± 0.37	17.99 ± 0.37
Topsin-M	0.00 ± 0.00	12.76 ± 0.37	15.84 ± 0.37
GDMD-01	0.00 ± 0.00	9.86 ± 0.37	10.77 ± 0.37
GDMD-02	0.00 ± 0.00	10.60 ± 0.37	11.49 ± 0.37
GDMD-09	0.00 ± 0.00	11.16 ± 0.37	11.32 ± 0.37
GDMD-08 +RDMD-01	0.00 ± 0.00	10.41 ± 0.37	10.83 ± 0.37
$LSD_{0.05}$	Treatments[1] = 6.81	Time[2] = 4.83	

[1]Difference higher than LSD values among means in the column is significant at $p<0.05$
[2] Difference greater than LSD values among means in a row are significant at $p<0.05$

Table 3. Effect of fluorescent *Pseudomonas* species on the Diameter (mm) of apples stored at 23± ºC.

Treatments	Diameter (mm)		
	Day-1	Day-4	Day-7
Control	6.93 ± 0.19	7.13 ± 0.19	7.13 ± 0.19
Topsin-M	6.93 ± 0.19	6.76 ± 0.19	6.43 ± 0.19
GDMD-01	6.80 ± 0.19	6.70 ± 0.19	5.86 ± 0.19
GDMD-02	6.46 ± 0.19	6.30 ± 0.19	5.70 ± 0.19
GDMD-09	6.26 ± 0.19	6.70 ± 0.19	5.80 ± 0.19
GDMD-08 +RDMD-01	6.16 ± 0.19	6.53 ± 0.19	5.56 ± 0.19
$LSD_{0.05}$	Treatments[1] = 0.40	Time[2] = 0.28	

[1]Difference higher than LSD values among means in the column is significant at $p<0.05$
[2] Difference greater than LSD values among means in a row are significant at $p<0.05$

Table 4. Effect of *Pseudomonas* species on the Length (mm) of apples stored at 23 ± 4 ºC.

Treatments	Length (mm)		
	Day-1	Day-4	Day-7
Control	6.10 ± 0.17	6.36 ± 0.17	6.13 ± 0.17
Topsin-M	6.56 ± 0.17	6.16 ± 0.17	6.20 ± 0.17
GDMD-01	6.46 ± 0.17	6.20 ± 0.17	5.86 ± 0.17
GDMD-02	5.73 ± 0.17	6.20 ± 0.17	5.70 ± 0.17
GDMD-09	5.66 ± 0.17	5.63 ± 0.17	5.80 ± 0.17
GDMD-08+RDMD-01	6.33 ± 0.17	5.90 ± 0.17	5.56 ± 0.17
$LSD_{0.05}$	Treatments[1] = 0.36	Time[2] = 0.25	

[1] Difference higher than LSD values among means in the column is significant at $p<0.05$
[2] Difference greater than LSD values among means in a row are significant at $p<0.05$

Table 5. Effect of fluorescent *Pseudomonas* species on the pH of apples stored at 23 ± 4 ºC.

Treatments	**pH**		
	Day-1	**Day-2**	**Day-3**
Control	4.33 ± 0.25	5.33 ± 0.25	5.0 ± 0.25
Topsin-M	4.33 ± 0.25	4.66 ± 0.25	5.0 ± 0.25
GDMD-01	4.66 ± 0.26	4.66 ± 0.25	4.73 ± 0.25
GDMD-02	4.01 ± 0.25	4.66 ± 0.25	4.80 ± 0.25
GDMD-09	4.33 ± 0.25	4.33 ± 0.25	4.66 ± 0.25
GDMD-08 +RDMD-01	4.66 ± 0.25	4.66 ± 0.25	4.70 ± 0.25
$LSD_{0.05}$	Treatments[1] = 0.51	Time[2] = 0.37	

[1]Difference higher than LSD values among means in the column is significant at $p<0.05$
[2] Difference greater than LSD values among means in a row are significant at $p<0.05$

Table 6. Effect of fluorescent *Pseudomonas* species on the Titratable acidity (% citric acid) of apples stored at 23 ± 4 ºC.

Treatments	Titratable acidity		
	Day-1	Day-4	Day-7
Control	0.20 ± 0.02	0.24 ± 0.02	0.27 ± 0.02
Topsin-M	0.17 ± 0.02	0.22 ± 0.02	0.26 ± 0.02
GDMD-01	0.16 ± 0.02	0.17 ± 0.02	0.30 ± 0.02
GDMD-02	0.16 ± 0.02	0.19 ± 0.02	0.25 ± 0.02
GDMD-09	0.17 ± 0.02	0.20 ± 0.02	0.21 ± 0.02
GDMD-08 +RDMD-01	0.16 ± 0.02	0.17 ± 0.02	0.20 ± 0.02
$LSD_{0.05}$	Treatments[1] = 0.05	Time[2] = 0.04	

[1] Difference higher than LSD values among means in the column is significant at $p<0.05$
[2] Difference greater than LSD values among means in a row are significant at $p<0.05$

Table 7. Effect of fluorescent *Pseudomonas* species on the Total soluble solids (TSS) of apples stored at 23 ± 4 ºC.

Treatments	Total soluble solids (% Brix)		
	Day-1	Day-4	Day-7
Control	6.03 ± 0.45	6.20 ± 0.45	6.50 ± 0.45
Topsin-M	7.03 ± 0.45	7.03 ± 0.45	7.16 ± 0.45
GDMD-01	6.03 ± 0.45	6.30 ± 0.45	7.73 ± 0.45
GDMD-02	5.60 ± 0.45	6.86 ± 0.45	8.10 ± 0.45
GDMD-09	5.0 ± 0.45	7.03 ± 0.45	7.20 ± 0.45
GDMD-08 +RDMD-01	6.04 ± 0.45	6.23 ± 0.45	7.76 ± 0.45
$LSD_{0.05}$	Treatments[1] = 0.92	Time[2] = 0.32	

[1]Difference higher than LSD values among means in the column is significant at $p<0.05$
[2] LSD values Difference greater than among means in a row are significant at $p<0.05$

Total soluble solids (TSS)

The total soluble solids of apple fruits are preeminently composed of sugar while containing pectin, organic acids (citric and malic acids), ascorbic acids, amino acids etc. as well, but the sugar content is known to be as quality parameter for composition and texture evaluation of fruits and vegetables (Kamiloglu, 2011). In this study total

soluble solids (TSS) were maintained by *Pseudomonas* isolates (GDMD-01, GDMD-02, GDMD-09 and GDMD-08+RDMD-01) comparatively of (control) treatment. While the treatments GDMD-02, GDMD-08+RDMD-01 and GDMD-01 increases the level of TSS in comparison of control and Topsin-M throughout the week as shown in Table 7.

DISCUSSION

The application of synthetic fungicides to control fungal phytopathogens has hazardous impacts on crops, environment, and humans (Nunes, 2012). Due to this, an alternative to control post-harvest losses caused by fungal phytopathogens was prioritized. In current times, the strategy of using chemical pesticides in controlling fungal pathogens causing post-harvest fruit losses has been replaced to overcome post-harvest decay biologically in which most commonly microbial antagonists are involved like bacteria. (Cuthbert *et al*., 2018). The diverse antagonistic mechanisms have been shown by bacteria against fungal phytopathogens, most specifically hydrolytic enzymes, resistant induction, and competition of nutrients, biofilms, and volatile compounds. (Dukare *et al*., 2018). The use of bacteria as a bio-control agent is considered to be very significant in management of organic production and cultivations, where their importance increases as post-harvest fungal diseases control (Beneduzi *et al*., 2012). Plant protection against fungal diseases has been procured by bio-control method and recently it is the most suitable and promising alternative in overcoming post-harvest fruit decay by protecting fruits against phytopathogenic attack (Ghazanfar *et al*., 2016). A variety of mechanisms are involved in the protection of plants from pathogenic attack by Bacterial bio-control agents (BCA). The direct interaction of BCA with pathogens is by antimicrobial compound secretion, by interfering with the virulence of pathogens and competing for nutrients and space. Various BCA are involved in the release and synthesis of metabolites like bacteriocins, antibiotics, cell wall degrading enzymes, biosurfactants, lipopeptides and microbial volatile compounds which cause a reduction in metabolism and growth of phytopathogens due to of their antimicrobial activity (Meena and Kanwar. 2015). Quorum sensing (QS) system of the pathogens may also get interfered with BCA and they also cause enzyme decline or inhibition of the synthesis of signal molecules which are used for the initiation of the infections. For example, QS inhibitors are produced such as pectinases, lactonases, and chitinases which cause the degradation of QS signal molecules impairing the infection of pathogen and cause a reduction in plant disease symptoms (Kalia *et al*., 2019). The eminently imperative antibiotics were produced by bacteria are iutrin, Bacillus subtilus produced an important antifungal peptide, *Pseudomonas* cepacia produced pyrolotinin and Myrothecium roridum produced trichothecene. (Yu and Lee. 2015).

In this study of apple fruit analysis, the weight loss of fruits has been observed where the fruits treated with GDMD-09 and GDMD-08+RDMD-01 has shown lesser weight loss in comparison to (control) treatment and Topsin-M whereas, fruits treated with GDMD-01 exhibited greater weight loss in comparison to (control) treatment and Topsin-M and the maximum weight reduction was observed in fruits treated with treatment GDMD-02 in comparison of treatment (control) and Topsin-M. The structure of the fruit skin and the nature of waxes present on the surface of the fruit skin are the two factors on which fruit weight loss depends (Babos *et al*., 1984; Veravrbeke *et al*., 2003). The loss of moisture is the reason behind the loss of turgor pressure and causes the decline of visual quality and this is followed by softening (Vander-Berg, 1981). The total soluble solids (TSS) are majorly comprised of sugars. Although amino acids, ascorbic acids, organic acids, pectin, etc., are also present, for quality parameters, sugar content is used for fruit and vegetable composition and texture evaluation (Kamiloglu, 2011). In this study, *Pseudomonas* treatments GDMD-02, GDMD-08+RDMD-01, and GDMD-01 were applied to apple fruits increasing the level of TSS in comparison to control and Topsin-M. Borji and Jafarpour (2012) demonstrated total soluble solids value of tomatoes raises from 5.1% at the mature green stage to 6.2% at the complete ripened stage. The sweetness level is determined by total soluble solids which are a part of a large portion of total solids (Magwaza and Opara, 2015). The treatment GDMD-02 among of all treatments of *Pseudomonas* has significantly increased the level of TSS in apple fruit in comparison to treatment (control) and Topsin-M. There are significant differences seen in the TSS present among different cultivars of apple fruit (Ali *et al*., 2004). The increase in the duration of fruit storage is directly proportional to the increase in the TSS ratio in all cultivars. The TSS in all cultivars increased with an increase in duration in the storage of fruits. The breakdown of starch which results in sugars is due to an increase in TSS (Beaudry *et al*., 1989) and its consumption during respiration causes a reduction in organic acids (Mahajan, 1994; Rivera *et al*., 2005; Ghafir, 2009). The treatments GDMD-01, GDMD-02, and GDMD-08+RDMD-01 of *Pseudomonas* applied to stored apples have shown a significant increase in pH in comparison to (control) treatment and Topsin-M. The progress of fruit toward the ripening stage depends upon the rise in the pH level of the fruit (Anthon *et al*., 2011). The titratable acidity and pH are correlated and are the most significant quality parameters. pH is indicated by the acid content of the fruit (Anthon *et al*., 2011). The treatments GDMD-01, GDMD-02 and GDMD-08+RDMD-01 of *Pseudomonas* have shown significant results in comparison with

treatment (control) and Topsin-M in decreasing titratable acidity. Commonly, due to of citric acid decline the decrease in TA has been observed with maturity and over maturity (Anthon *et al*., 2011). This study has showed that the use of isolates from *Pseudomonas* spp. as a biological control agent in preventing apple fruits from post-harvest decay while promoting its shelf life.

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