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Effect of *Pseudomonas fluorescens* formulations on decay and quality of mango (*Mangifera indica*) fruits during storage

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ABSTRACT

Anthracnose and stem-end rot caused by *Colletotrichum gleosporioides* and *Botryodiplodia theobromae*, respectively are the most important postharvest diseases of mango (*Mangifera indica* L.) causing huge economic losses. In this study, biocontrol efficacy of *Pseudomonas fluorescens* was evaluated against anthracnose and stem-end rot of naturally infected mango fruits during their postharvest storage. Physiologically mature mango fruits were treated with *P. fluorescens* formulations (10^7 cfu/ml and 10^8 cfu/ml) by dipping them for 5 min. Following treatment, fruits were air-dried and stored at ambient condition ($30^o \pm 2^oC$) for 12 days. Among the treatments, *P. fluorescens* 10^8 cfu/ml showed significant reduction (5.92%) in decay loss of mango fruit compared to control (24.52%). Respiration and ethylene evolution rates were also brought down by *P. fluorescens* treatments. Loss of firmness, total phenolics content and titratable acidity were also found lower in *P. fluorescens* treated fruits. No significant differences in a* and b* values of fruit colour was recorded between control and treated fruits while, the L* value was lowest in control fruits. The fruits treated with *P. fluorescens* @ 10^8 cfu/ml maintained highest total carotenoids and total soluble solids content than other treatments. Therefore, *P. fluorescens* @ 10^8 cfu/ml treatment may be used as a potential biocontrol agent in reducing anthracnose and stem-end rot, and maintaining desirable fruit quality attributes of mango during postharvest storage.

Key words: Disease, Mango, Postharvest, *Pseudomonas fluorescens*

Mango (*Mangifera indica* L.) is one of the most important tropical fruits and it is deemed to be the choicest of all indigenous fruits among millions of people in the Orient (Barman *et al*. 2015). Anthracnose and stem-end rot caused by *Colletotrichum gloeosporioides* and *Botryodiplodia theobromae*, respectively are the most prevalent and serious postharvest diseases of mango fruits during storage, causing major economic losses (Govender *et al*. 2005). The pathogens invade the immature fruits in the orchard and remain as quiescent infections. Due to latent infection, these are not visible at the time of sorting, grading and packing. However, when the fruits soften during the ripening process, the natural defence mechanism break down and latent infections become visible.

Management of these diseases relies primarily on the pre-harvest spraying of fungicides such as carbendazim and postharvest dipping in hot water containing fungicides (Swart *et al*. 2002). These practices have shown their effectiveness in preventing pre- and postharvest pathogen infections. However, due to adverse effects on human health as well as environment and development of pathogen resistance to pesticides, there is an urgent need to reduce use of synthetic fungicides (Sharma *et al*. 2009). The reduced sensitivity to thiabendazole fungicide against crown rot and anthracnose fungi has already been reported (Marin *et al*. 1996). This situation has prompted an intensified research effort world-wide to develop an alternative and potentially safer approach to control anthracnose and stem-end rot disease in mango. Control of these pathogens using microbial antagonists has emerged out as one of the most promising alternatives to synthetic fungicides (El Ghaouth *et al*. 2002). In this study, biocontrol efficacy of *Pseudomonas fluorescens* against anthracnose and stem-end rot of naturally infected mango and their effect on physico-chemical fruit quality attributes was investigated.

MATERIALS AND METHODS

Pure culture of *Pseudomonas fluorescens* was obtained from Department of Plant Pathology, Indian Agricultural

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Research Institute, New Delhi. Then it was streaked with a sterile bacteriological loop in petri dish containing potato dextrose agar medium. Then the plates were incubated at 27ºC for 4 days. Following growth, the cells were harvested, suspended in distilled water and adjusted to an absorbance of 0.222 at 550 nm with a spectrophotometer to give a 10^8 cfu/ml concentration of *P. fluorescens*. Lower concentration of *P. fluorescens* (10^7 cfu/ml) was obtained by 10 fold dilution.

Mango fruits (cv. Chausa) were harvested at mature stage from the orchard of Indian Agricultural Research Institute, New Delhi, India. Immediately after harvesting, fruits were transported to the laboratory and uniform sized healthy fruits, free from visual blemishes, pests and diseases were selected for the experiment. Total 162 fruits were selected for the experiment and divided into 3 lots of 54 fruits each for the treatments (3 replications and 3 fruits per replication). The treatments were performed by dipping the fruits in *P. fluorescens* formulations (10^7 cfu/ml and 10^8 cfu/ml) for 5 minutes while in case of control, fruits were dipped in distilled water. After giving treatments, fruits were air-dried and stored at ambient condition (30 ± 2ºC). Fruits from each treatment were sampled at random at 4 days interval and analysed for different physico-chemical quality parameters.

Mango fruits showing symptoms of disease irrespective of severity were considered as decay loss. The percent disease incidence was determined by the following formula: (X/Y) × 100, where X is the number of fruits showing disease symptom and Y is the total number of fruit kept for observations at the beginning of storage.

Firmness of mango was determined using a probe (2 mm diameter) attached to a Texture Analyzer (TA+Di) through penetrations and the force required to penetrate 10 mm inside the fruit moving at a speed of 2 mm/s was measured. Four puncture force measurements were made on equatorial fruit zones at intervals of 90º angle and the results were expressed in Newton (N).

Peel colour of mango fruit was determined using the Hunter Colour Lab System. The colour values were expressed as L* (0: dark, 100: white), a* (negative value: green, positive value: red) and b* (negative value: blue, positive value: yellow). To measure peel colour, four readings were taken from different positions of each fruit. Then the values were calculated from the mean of four determinations for each fruit.

Respiration rate of fruit was determined by placing the fruit in 1 l capacity hermetically sealed container for 1 hr. The head-space gas was sucked and the respiration rate was determined by using auto gas analyzer and results were expressed as ml CO_2/kg/h. Ethylene evolution rate was quantified by using gas chromatograph equipped with a flame ionization detector and Porapack-N (80/100 mesh) stainless steel column. The temperatures of injector, column and detector were adjusted to 110ºC, 60ºC and 275ºC, respectively and flow rates of N_2, H_2 and air were maintained as 30, 30 and 300 ml/min, respectively. The rate of ethylene evolution was expressed as µl C_2H_4/kg/h.

Total carotenoids content in the fruit pulp was determined by extracting carotenoid pigments from the fruit pulp with a mixture of petroleum ether and acetone (3:1) and assayed colorimetrically by spectrophotometer at 452 nm (Roy 1973). The results were expressed as mg/100 g FW (fresh weight). Total phenolics content of the fruit extract (80% ethanol) were determined by the method of Singleton *et al*. (1999) and expressed as gallic acid equivalent (µg GA equiv./g FW).

Total soluble solids (TSS) content was determined using refractometer and results were expressed as Degree Brix (ºB). Titratable acidity was determined by titration method (AOAC 2000). For this, 2 g of mango pulp was crushed with distilled water and a few drop of phenolphthalein solution was added as indicator. Finally, the mixture was titrated with 0.1 N NaOH up to pH 8.1 and results were expressed as percentage (%) of citric acid.

The present experiment was carried out in completely randomized design with three treatments and three replications. The data recorded from different treatments with respect to various parameters were subjected to analysis of variance (ANOVA) with treatment and storage time as sources of variation. Values of different parameters were expressed as the mean ± standard error. Mean comparison among treatments were performed using HSD Tukey's test. A difference was considered statistically significant ($P < 0.05$). The analyses were carried out with SPSS software package version 16.0 for windows.

RESULTS AND DISCUSSION

Effect on decay loss

In the present study, treatment with *P. fluorescens* was highly effective in minimizing decay loss of mango fruit during their postharvest storage. No incidence of diseases was observed in any of the treatments up to 3 days of storage (Fig 1). Fruits treated with *P. fluorescens* @ 10^8 cfu/ml were completely free from diseases to 6 days of storage and it was also negligible (0.35%) in *P. fluorescens* @ 10^7 cfu/ml treated fruits while control fruits showed 5.05% decay loss. After 12 days of storage, maximum decay loss of 24.52% was recorded in control fruits while it was minimum (5.92%) in fruits treated with *P. fluorescens* @ 10^8 cfu/ml. In the present study, postharvest treatment with *Pseudomonas fluorescens* was found highly effective in suppressing disease incidence in stored mango fruits. The highest degree of control was achieved by *P. fluorescens* (10^8 cfu/ml) treatment. It has been reported that *P. fluorescens* produced antimicrobial compounds like 2,4-diacetylphloroglucinol (DAPG), phenazines and hydrogen cyanide (Haas and Défago 2005). So, these antimicrobial compounds might have inhibited the growth of *Colletotrichum gloeosporioides* and *Botryodiplodia theobromae*, the causal organisms of anthracnose and stem-end rot, respectively. Besides that competitive exclusion of pathogens due to rapid colonization of the rhizosphere might also be an important factor in disease control (Ganeshan and Kumar 2005). Furthermore,

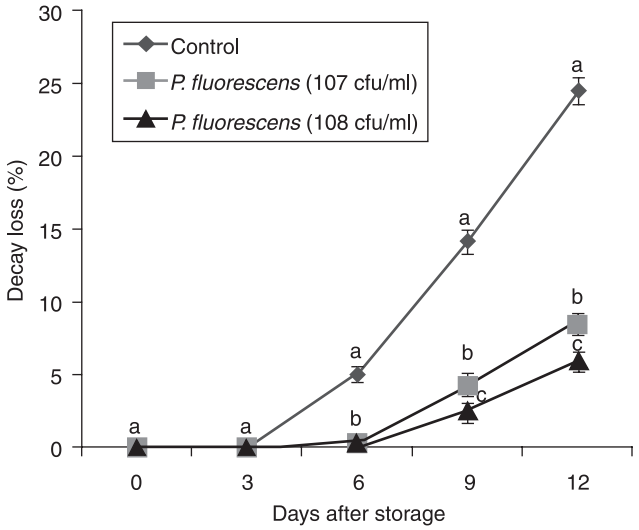

Fig 1 Effect of *Pseudomonas fluorescens* treatments on postharvest decay loss of mango.

the reduced disease incidence in *P. fluorescens* treated mango fruits compared to control was also due to the fact that these fruits maintained higher firmness owing to delay in ripening process.

Effect on fruit firmness

In the present study, irrespective of treatments fruit firmness decreased rapidly with the advancement of storage period. No significant difference in firmness was observed among the treatments up to 3 days of storage. After that, a marked decrease in fruit firmness was observed in control fruits. On the final day of storage, fruits treated with *P. fluorescens* (10^8 cfu/ml) retained highest firmness (9.23 N) with non-significantly followed by *P. fluorescens* (10^7 cfu/ml) treatment (8.09 N). However, it was lowest (5.52 N) in control mango fruits. The faster decrease in fruit firmness in control mango fruits was due to increased activity of cell wall degrading enzyme pectin methyl esterase (data not shown). Previous workers have also reported the association between fruit firmness and pectin methyl esterase activity (Barman and Asrey 2014).

Effect on peel colour

A marked increase in L*, a* and b* values with the onset of ripening was recorded under all the treated mango fruits during their storage (Table 1). Control fruits showed faster increase in L* value compared to other treatments while it was slowest in *P. fluorescens* (10^7 cfu/ml) treated fruits. After 9 days of storage, control fruits showed slight decline in L* value (59.835 ± 0.345) while in other treatments, continuous increasing trend up to end of the experiment was observed. Up to 6 days of storage, rapid increase in a* and b* values were recorded indicating change in green colour and development of yellow colour, respectively. However, after 12 days, no significant difference in a* and b* values were recorded between control and treated fruits.

Effect on respiration and ethylene evolution rates

In this study, respiration rate of mango fruit significantly influenced by the treatments. In control mango fruits, respiratory climacteric peak was observed at 6th day of storage however, fruits treated with *P. fluorescens* do not exhibited respiratory climacteric up to end of the experiment (Fig 2). After 12 day of storage, highest respiration rate of 241.18 ml CO_2/kg/h was recorded in control fruits while it was lowest (168.97 ml CO_2/kg/h) in *P. fluorescens* (10^8 cfu/ml) treated fruits exhibiting about 42% lower respiration rate than control. Likewise, treatment with *P. fluorescens* was found highly effective in suppressing the ethylene evolution throughput the storage period. On 6th day of storage, a typical climacteric peak of ethylene was observed in control and *P. fluorescens* (10^7 cfu/ml) treated fruits but

Table 1 Effect of *Pseudomonas fluorescens* treatments on peel colour (L*, a* and b* value) of mango

Treatment	Days after storage				
	L* value				
	0	3	6	9	12
Control	45.893±0.277Aa	47.981±0.389Ba	54.943±0.327Ca	60.394±0.376Da	59.835±0.345Da
P. fluorescens (10^7 cfu/ml)	45.718±0.237Aa	47.180±0.300Ba	50.809±0.642Cc	58.361±0.407Db	62.915±0.149Eb
P. fluorescens (10^8 cfu/ml)	45.868±0.248Aa	47.285±0.271Ba	51.182±0.467Cc	59.657±0.316Da	63.272±0.218Eb
			a value*		
Control	-11.315±0.235Aa	-7.558±0.322Bb	-4.059±0.439Cc	-1.455±0.415Dc	2.648±0.505Ed
P. fluorescens (10^7 cfu/ml)	-11.455±0.215Aa	-5.275±0.290Ba	-2.598±0.257Cb	1.272±0.213Da	3.221±0.263Ed
P. fluorescens (10^8 cfu/ml)	-10.745±0.165Aa	-4.480±0.440Ba	-1.658±0.312Ca	1.913±0.207Db	3.729±0.223Ed
			b value*		
Control	21.910±0.545Aa	25.647±0.301Ba	32.338±0.220Ce	33.679±0.253Da	34.097±0.322Da
P. fluorescens (10^7 cfu/ml)	23.123±0.292Aa	26.327±0.262Ba	32.790±0.174Ce	33.997±0.138Da	34.466±0.301Da
P. fluorescens (10^8 cfu/ml)	22.108±0.452Aa	26.956±0.310Ba	33.291±0.235Ca	34.348±0.282Da	35.013±0.248Da

According to HSD Tukey's test, treatment values with similar capital letters are not significantly different ($P < 0.05$) during storage. For each sampling date, values with similar small letters are not significantly different ($P < 0.05$) among treatments.

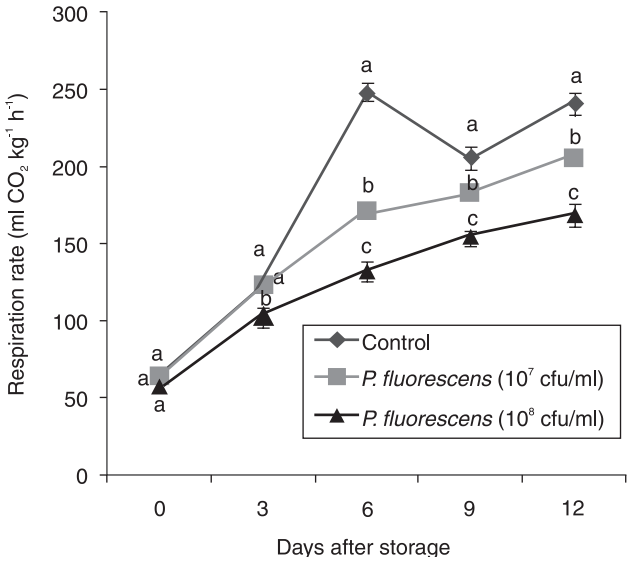

Fig 2 Effect of *Pseudomonas fluorescens* treatments on respiration rate of mango.

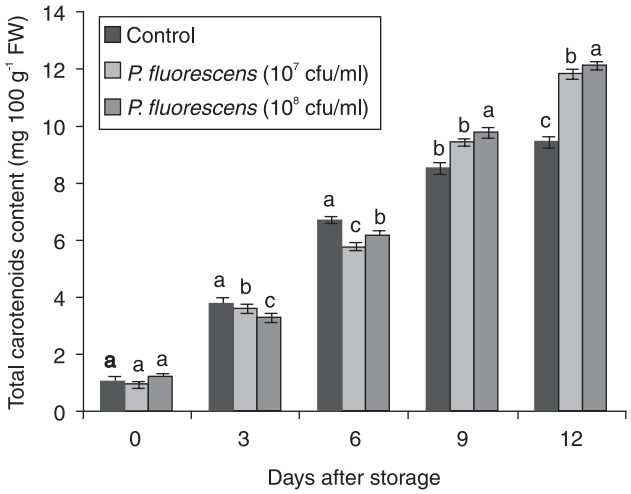

Fig 3 Effect of *Pseudomonas fluorescens* treatments on total carotenoids content of mango.

the magnitude of peak was much lower in biocontrol agent treated fruits than control. No such peak was observed in fruits treated with *P. fluorescens* (10^8 cfu/ml) up to end of the experiment. Maximum ethylene evolution after 12 days of storage was recorded in control fruits (2.15 µl C_2H_4/kg/h), while it was lowest (1.06 µl C_2H_4/kg/h) in *P. fluorescens* (10^8 cfu/ml) treated fruits. The control mango fruits exhibited significantly higher respiration and ethylene evolution rates compared to treated fruits. The suppressed respiration rate in *P. fluorescens* treated fruits might be attributed to lower disease incidence. Disease mediated increase of respiration rate have also been reported earlier (Zauberman and Barkai-Golan 1975). Likewise, higher ethylene evolution rate in control fruits might be attributed to faster ripening and senescence process, which might have triggered ethylene evolution, compared to other treatments. Moreover, development of diseases further aggravated higher ethylene evolution rate. Increase in ethylene evolution rate due to development of diseases has also been reported earlier by several workers.

Effect on total carotenoids and total phenolics content

The total carotenoids content in the fruit pulp increased gradually irrespective of treatments. Initially up to 6 days of storage, carotenoids content was recorded higher in control fruits (Fig 3). Later on fruits treated with *P. fluorescens* showed rapid increase in carotenoids compared to control. After 12 days of storage, maximum total carotenoids content (12.09 mg/100 g FW) was recorded in *P. fluorescens* @ 10^8 cfu/ml treated fruits with non-significantly followed by carotenoids (11.81 mg/100 g FW) in *P. fluorescens* @ 10^7 cfu/ml treated fruits while, it was lowest (9.41 mg/100 g FW) in control mango fruits. A rapid decrease in total phenolics content was noted in all the treated fruits. In general, phenolics content was significantly lower in control fruits than those which were treated with biocontrol agent. Among the treatments, *P. fluorescens* (10^8 cfu/ml) was proved most effective in retention of phenolic compounds (483.57 µg GA equiv./g FW) than other treatments. The control fruits showed maximum decrease in phenolics content reaching up to 314.36 µg GA equiv./g FW. The present study revealed that total carotenoids content increased progressively with the advancement of storage period. Initially higher carotenoids content in control mango fruits was attributed to faster ripening process, which led to higher synthesis of carotenoid pigments. Later on, control fruits showed higher incidence of anthracnose and stem-end rot that might have reduced the synthesis of carotenoid pigments. On the other hand, disease incidence was highly suppressed by *P. fluorescens* treatment, thus it maintained higher carotenoids content. The total phenolics content decreased with the increase in storage period. This decrease in phenolics content was due to the onset of ripening, which is a well known biochemical phenomenon (Zhu *et al*. 2009). However, fruits treated with *P. fluorescens* delayed the loss of phenolic compounds by delaying the ripening process. The delay in ripening of treated fruits might be due to reduced respiration and ethylene evolution rate. Further, lower disease incidence in *P. fluorescens* treated fruit also contributed to higher phenolics content.

Effect on total soluble solids and titratable acidity

In this study, TSS content showed no significant difference among the treatments up to 3 days of storage (Table 2). Thereafter, increase in TSS content in fruits was recorded up to 9 days of storage. Later on, control fruits showed a decrease in TSS content. At final day of storage, fruits received *P. fluorescens* (10^8 cfu/ml) treatment retained highest TSS (28.60 ± 0.30ºB), while no significant difference in TSS content was recorded between control (26.40 ± 0.70ºB) and *P. fluorescens* (10^7 cfu/ml) treated fruits (26.54 ± 0.26ºB). A continuous decrease in TA was recorded in fruits under all the treatments. No significant differences in TA were noted up to 6 days of storage among

Table 2 Effect of *Pseudomonas fluorescens* treatments on total soluble solids and titratable acidity of mango

Treatment	Days after storage				
	Total soluble solids (°B)				
	0	3	6	9	12
Control	12.05±0.75Aa	15.06±0.24Ba	22.60±0.30Ca	27.05±0.55Da	26.40±0.70Da
P. fluorescens (10^7 cfu/ml)	12.40±0.20Aa	15.57±0.33Ba	21.02±0.38Cb	26.17±0.43Da	26.54±0.26Da
P. fluorescens (10^8 cfu/ml)	11.35±0.55Aa	15.18±0.22Ba	21.56±0.34Cb	26.56±0.44Da	28.60±0.30Eb
	Titratable acidity (%)				
Control	0.56±0.016Aa	0.42±0.016Bb	0.29±0.016Cb	0.14±0.016Db	0.08±0.032Eb
P. fluorescens (10^7 cfu/ml)	0.56±0.016Aa	0.43±0.016Bb	0.29±0.032Cb	0.19±0.032Dc	0.12±0.016Ec
P. fluorescens (10^8 cfu/ml)	0.56±0.016Aa	0.43±0.016Bb	0.26±0.032Cb	0.15±0.016Db	0.08±0.016Eb

According to HSD Tukey's test, treatment values with similar capital letters are not significantly different ($P < 0.05$) during storage. For each samplingdate, values with similar small letters are not significantly different ($P < 0.05$) among treatments.

the treatments (Table 2). Thereafter, fruits treated with *P. fluorescens* (10^7 cfu/ml) retained highest TA (0.12 ± 0.016%) while it did not differ significantly among control (0.08 ± 0.032%) and *P. fluorescens* (10^8 cfu/ml) (0.08 ± 0.016%) treated fruits. The increase in TSS with subsequent storage period might be due to hydrolysis of starch into simple sugars, which in turn may lead to an increase in TSS. Among the treatments, control fruits exhibited initially a rapid increase in TSS up to 9 days of storage, later on it decreased. Initial increase in TSS might be attributed to faster ripening process but later it decreased due to higher respiration as well as higher incidence of anthracnose and stem-end rot diseases. Likewise, decline is titratable acidity with progressive increase in storage period was also due to natural ripening phenomenon.

This study suggests that postharvest decay loss of mango fruit can be minimized effectively by dipping the fruits in *Pseudomonas fluorescens* @ 10^8 cfu/ml formulation. This treatment was also found highly effective in delaying fruit ripening by suppressing respiration and ethylene evolution rates. Fruit quality attributes like total carotenoids, total phenolics and total soluble solids contents were also preserved by this treatment up to 12 days during storage at ambient condition.

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