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Effect of *Trichoderma* on postharvest quality of Harumanis mango

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Abstract

Trichoderma sp. is a biological control agent that has potential to control fruit diseases. This experiment evaluated the use of *Trichoderma* sp. as alternative to propicanacole, a synthetic fungicide used to control postharvest decay especially on mango. Harumanis mangoes were sprayed with 500 ppm propiconazole, at different concentrations of *Trichoderma* sp. and fruits without spraying as control. Fruits were stored at room temperature (25 °C) for assessment of quality and disease at day 0, 2, 4, 6 and 8. Spraying with different concentration of *Trichoderma* sp. did not affect mango skin and pulp colour, SSC, pH, TTA and vitamin C, however, there was significant effect on weight loss and disease severity. The skin of Harumanis was easily infected by fungus as early as 4 days of storage. *Trichoderma* sp. at 1 x 10^3, 1 x 10^6 and 1 x 10^8 conidia/ml concentration cannot provide the required level of protection against anthracnose of mango. The fungicidal application (propiconazole, 500 ppm) was effective in reducing anthracnose incidence in mango fruit stored at room temperature.

Keywords: biocontrol agents, propiconazole, control decay, alternative treatment, imperfect fungi

Introduction

Government has identified fruit production as one of the potential industries to be developed for this country. Under the National Key Economic Area (NKEA), government has targeted to produce 570,000 tonnes in 2020, with 94.7% dominated by tropical fruits out of which 84% is for export market. Through the effective implementation of Entry Point Project 7 (EPP7), government planned to increase the production of high quality fruits and vegetables and penetrate the premium market in the Europe and Middle East. The increase in fruit production must be supported with effective postharvest handling technology to ensure the quality and safety of fruit is maintained until they reach the consumer.

Mango (*Mangifera indica* L.) is one of the popular tropical fruits in the international market. Due to inadequate supply of mango, in 2007, Malaysia imported 25,700 tonnes of mangoes valued at USD5.2 million from Thailand (82%), India (6%), Philippines (4%) and Australia (3%) (APEDA 2007). Mango especially Harumanis is expanding very rapidly in recent years especially in the northern area of Malaysia such as Perlis and Kedah states.

However, the fruit has limited shelf-life (only 3 – 4 days at ambient) and created difficulties in marketing. Mango being a highly perishable fruit has a very short shelf-life at ambient temperature and reaches the respiration peak of ripening process on the 3rd or 4th day after harvesting (Narayana et al. 1996). The shelf-life of mango also

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varies among the varieties depending on the storage conditions. The shelf-life ranges from 4 – 8 days at room temperature and 2 – 3 weeks in cold storage at 13 ºC (Carrillo et al. 2000). This short shelf-life limits the long distance transportation of this fruit (Gomer-Lim 1997).

Anthracnose and stem-end rot diseases are considered major problems in storage and shelf-life of mango fruits. The diseases are controlled by fungicides such as benomyl, carbendazim or propiconazole. The use of synthetic chemicals to control postharvest diseases and deterioration has their limit due to carcinogenicity, teratogenicity, environmental pollution, effects on food and other side-effects on humans (Unnikrishnan and Nath 2002; Mari et al. 2003).

Biological control is a possible alternative to fungicides in a postharvest environment, whereby temperature and relative humidity are controlled (Sports and Sanderson 1994). The soil-borne fungus, *Trichoderma harzianum* has previously been reported to be an effective biocontrol agent (Benhamou and Chet 1996). Research has been conducted on the control of postharvest pathogens on a number of crops using antagonistic microorganisms such as *Trichoderma harzianum*. The use of *Trichoderma* sp. on crops include grapes (Dubois 1984), apples and strawberries (Tronsmo and Dennis 1977). The objective of this study was to evaluate the effectiveness of *Trichoderma* sp. on postharvest quality of Harumanis mango during storage at ambient temperature.

Materials and methods

Harumanis mango fruits used in this study were obtained from a commercial plot in Paya Kelubi, Perlis. The fruits were harvested at commercial maturity (12 weeks after bloom) and transported to a laboratory at Bukit Tangga, Kedah. Only fruits that were free from defects and of uniform maturity were chosen. The fruits were then washed using clean water and air-dried.

The various concentrations of *Trichoderma* sp. (1 x 10^3, 1 x 10^6 and 1 x 10^8 conidia/ml) and fungicide propiconazole (500 ppm) were applied to the fruits. Fruits without spraying were as control. The fruits were then air dried, packed in corrugated boxes and stored at 25 °C at 85 – 90% RH for 8 days. The experiment was carried out at 2 days intervals in triplicates, with each replicate consisting of three fruits.

Three fruit samples were weighed at the start and end of the experiment. The difference between initial and final fruit weight would be the total weight loss during storage intervals. The calculation was made in percentage on fresh weight basis. Fruit weight was taken daily using digital balance. The surface colour of flesh was measured with a colorimeter (Konica Minolta Chromameter Model CR-300) in the CIE L*, a*, b* and hue mode (Carreno et al. 1995).

The disease incidence was observed during storage period. The intensity of disease was assessed using a special score chart based on the area of infection (Bali Reddy et al. 2008).

0 = No infection
1 = <1% fruit surface infection
2 = 1 – 5% fruit surface infection
3 = 6 – 25% fruit surface infection
4 = 26 – 50% fruit surface infection
5 = >50% fruit surface infection

Total soluble solids (TSS) were determined directly from each sample by using hand refractometer (Atago PEL-1, Japan) and expressed as ºBrix. The acidity was determined as citric acid (g/100 g) by titrating 5 g of fresh pulp sample against 0.1 N NaOH solution by following the AOAC method (Anon. 1990). pH was determined by taking 5 g of homogenised pulp sample according to Anon. (1990) method using a digital pH meter. Ascorbic acid content was measured by titrating with 2,6-dichlorophenolindophenol (Ranganna 1977).

Statistical analysis

Experiment was designed as Completely Randomised Design (CRD) with three replications. For data analyses, the Statistical Analysis System programme was used (SAS Inst. 1985), while Duncan Multiple Range Test (DMRT) test was used to compare the differences between treatments at 95% confidence level of each variable.

Results and discussion

Physical characteristics

Percentage weight loss of Harumanis mango during storage was significantly increased when treated with 1 x 10^3 conidia/ml of *Trichoderma* sp. (*Table 1*). The fruits produced the highest weight loss (8.88%) compared to other treatments. The percentage weight loss increased throughout storage time and the maximum was recorded after 8 days of storage. Result also showed that the percentage weight loss was more than 12% after 8 days at ambient.

The highest weight loss may be coincided with the highest incidence of anthracnose on fruits treated with *Trichoderma* sp. at 1 x 10^3 conidia/ml concentration. Water was also lost via surface cracks that developed during growth or as the results of mechanical handling of the fruits. The skin surface of mango is waxy, which limits water loss and fungi attack through the outer skin.

Significant interactions observed between storage period and treatments (*Table 1*) might also be due to moisture loss during long-term storage caused by transpiration of the fruit, which is a physiological process that continues during storage. Transpiration involves (i) the movement of water both as a liquid and vapour from the intercellular space to the cuticle, (ii) solubilisation and diffusion of water molecules in and across the cuticle membrane and (iii) desorption of water at the outer surface (Veraverbeke et al. 2003).

Skin colour

There was no significant effect on luminosity (L*), a*, b* and hue angle of Harumanis mango skin treated with different treatments *(Table 1)* indicating that different treatments did not affect skin colour of mango. During storage period, L* and hue angle value of Harumanis mango skin maintained while the a* value reduced to –17.31 and b* value (27.67 – 34.49) increased where it showed green colour but slowly turned to light yellow from day 0 to day 8. The skin colour of Harumanis mango remained green even after the fruits reached the ripening stage.

Flesh colour

The luminosity (L*), a*, b* and hue angle of mango flesh has no significant effect with the different treatments (*Table 1*.) The L* value, hue angle and a* value decreased during the storage period, while the b* value increased. Low h° values in fruits coincide with low chlorophyll content and indicating that the colour changed from green to yellow orange. Reduction in hue shows an increase in the yellowing of the flesh.

Anthracnose incidence

Lowest anthracnose incidence score (0%) was recorded in Harumanis mango treated with propiconazole (500 ppm) followed by fruit treated with *Trichoderma* sp. as compared to control (*Figure 1*). Maximum anthracnose incidence score (2.0) was recorded in fruit treated with *Trichoderma* sp. (1 x 10^3 conidia/ml) during the 8 days storage at ambient. The results indicated that *Trichoderma* sp. treatment cannot provide the required level of protection against anthracnose incidence.

Trichoderma sp. isolates on soil borne plant pathogenic fungi, have been shown to have the ability to inhibit soil borne pathogens by parasitising other fungi, and compete aggressively for nutrients and antibiotics (Klein and Everleigh 1998). Many commercial products of *Trichoderma* sp. are available for the management of

Table 1. Effect of and interaction between different treatments and storage periods of Harumanis mango

	Weight loss	Skin colour				Flesh colour			
	(%)	L*	a*	b*	Hue angle	L*	a*	b*	Hue angle
Different treatments (DF)									
Control	7.17b	60.06a	–18.83a	31.63a	121.19a	79.36a	–8.49a	56.52a	98.85a
Propiconazole (500 ppm)	6.92b	58.46a	–18.65a	29.54a	122.53a	79.69a	–8.58a	54.83a	99.22a
Trichoderma (1 x 10^3)	8.88a	60.27a	–18.32a	31.73a	120.37a	78.90a	–6.72a	55.66a	109.24a
Trichoderma (1 x 10^6)	7.34ab	60.37a	–18.29a	31.40a	120.73a	79.59a	–8.14a	54.45a	98.79a
Trichoderma (1 x 10^8)	7.13b	60.37a	–18.63a	31.94a	119.14a	79.81a	–8.70a	54.59a	99.52a
Storage duration (SD)									
0 day	0e	57.63b	–19.12b	27.67b	124.98a	84.27a	–12.98c	48.10d	105.32a
2 days	3.92d	61.03a	–19.09b	29.59b	123.33ab	80.22b	–9.66b	52.14c	100.67a
4 days	9.11c	59.79ab	–18.14ab	34.06a	117.92cd	76.78c	–6.07a	58.82ab	107.97a
6 days	11.08b	59.44ab	–18.07ab	30.42b	120.86bc	78.99b	–6.77a	57.20b	96.81a
8 days	13.32a	61.63a	–17.31a	34.49a	116.87d	77.09c	–5.15a	60.79a	94.85a
Interaction									
DF x SD	*	ns	ns	ns	ns	ns	ns	ns	ns

Means with the same letters are not significantly different at $p < 0.05$

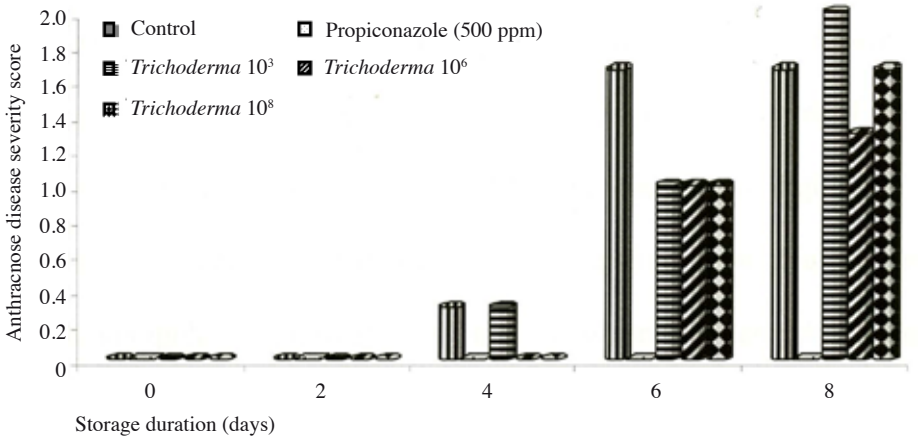

Figure 1. Effect of Trichoderma sp. treatment on anthracnose disease severity of Harumanis mango during storage at ambient

soilborne plant diseases. *Trichoderma* sp. was not effective in controlling antrachnose incidence on mango due to the culture not being viable and active in the liquid formulation.

Kolombet et al. (2008) showed that for *Trichoderma* sp. to remain active in liquid media, it needs the addition of starch as a food base, reduction of metabolic activity by lowering the pH of the biomass paste and the addition of small amounts of copper. Oxygen availability is important in maintaining biomass activity and competitiveness. Hence, there is a need to study on the extent of the shelf-life of *Trichoderma* sp. in liquid formulation.

The fungicidal application (propiconazole, 500 ppm) was effective in reducing anthracnose incidence in mango fruit during storage. Anthracnose is an important postharvest disease that cause losses in the production of tropical and subtropical fruits. There are many reports about using fungicides for its control i.e. carbendizim, mancozeb and propiconazole (Mortuza et al. 2003) and benomyl and bavistin (Wasker and Masalkar 1997).

Biochemical changes

There were no significant effect on total TSS content, pH, total titratable acidity (TTA) and ascorbic acid content of Harumanis mango treated with different treatments (*Table 2*). The TSS content was increased from 7.27 to 16.85 °Brix after 8 days of storage. The increase in TSS might be due to the alteration in cell wall structure and breakdown of complex carbohydrates into simple sugars during storage and ripening. This increase and decrease in TSS are directly correlated with hydrolytic changes in starch and conversion of starch to sugar being an important index of ripening process in mango and other climacteric fruits and further hydrolysis decreased the TSS during storage (Kays 1991).

The pH increased significantly during storage period in all treatments while the TTA decreased. Similar results reported by El-Ghaouth et al. (1991) showed that the decrease in acidity during storage demonstrated fruit senescence. It was determined as a small change in pH represents a large change in hydrogen ion concentration (Ball 1997). Results also showed that the decrease of TTA during storage might be due to the degradation of citric acid which could be attributed to increased activity of citric acid glyoxylase during ripening (Doreyappa-Gowda and Huddar 2001).

The ascorbic acid content increased significantly during storage duration of Harumanis. Ascorbic acid gradually

Table 2. Effect of and interaction between different treatments and storage periods on biochemical contents of Harumanis mango

	TSS (°Brix)	pH	TTA (%)	Ascorbic acid (mg/100 g)
Different treatments (DF)				
Control	13.81a	3.92a	0.73a	24.24b
Propiconazole (500 ppm)	13.67a	3.92a	0.76a	23.05b
Trichoderma (1 x 10^3)	13.49a	3.97a	0.72a	23.45b
Trichoderma (1 x 10^6)	13.73a	3.98a	0.70a	25.15b
Trichoderma (1 x 10^8)	13.47a	3.93a	0.70a	40.44a
Storage duration (SD)				
0 day	7.27c	3.42d	1.15a	17.26d
2 days	10.60b	3.48d	1.02b	51.03a
4 days	16.68a	3.90c	0.67c	14.98d
6 days	16.74a	4.18b	0.53d	24.18c
8 days	16.85a	4.63a	0.24e	28.85b
Interaction				
DF x SD	*	*	*	*

Means with the same letter are not significantly different at $p < 0.05$

increased during growth and development of mango fruit and reached maximum value at ripeness (*Table 2*). The increasing trend of ascorbic acid during growth and development of mango fruits is an exception to what is generally demonstrated in many fruits.

Conclusion

The skin of mango was easily infected by fungi after 4 – 6 days of storage at ambient. *Trichoderma* sp. at 1 x 10^3, 1 x 10^6 and 1 x 10^8 conidia/ml cannot provide the required level of protection against disease incidence of mango. The fungicidal application (propiconazole, 500 ppm) was effective in reducing anthracnose incidence in mango fruit during storage. There is an urgent need to find the alternative control methods that do not rely on fungicide chemicals for postharvest diseases since pathogens build up resistance to these chemicals. Further research is required before the biocontrol application reported here can be used commercially.

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Abstrak

Trichoderma sp. ialah agen kawalan biologi yang berpotensi mengawal penyakit pada buah. Kajian telah dijalankan bagi menilai keberkesanan penggunaan *Trichoderma* sp. sebagai alternatif kepada propicanacole, racun kulat sintetik yang digunakan untuk mengawal kerosakan lepas tuai pada buah mangga. Mangga Harumanis disembur dengan 500 ppm propiconazole pada kepekatan yang berbeza dan buah tanpa semburan bertindak sebagai kawalan. Buah kemudiannya disimpan pada suhu bilik iaitu 25 °C bagi penilaian kualiti dan penyakit pada hari 0, 2, 4, 6 dan 8. Semburan dengan kepekatan *Trichoderma* sp. yang berbeza tidak mempengaruhi kulit mangga dan isi warna, SSC, pH, TTA dan vitamin C tetapi telah menunjukkan perbezaan signifikan pada kehilangan berat dan tahap serangan penyakit. Kulit Harumanis mudah dijangkiti oleh kulat seawal 4 hari penyimpanan. *Trichoderma* sp. pada kepekatan 1 x 10^3, 1 x 10^6 dan 1 x 10^8 conidia/ml tidak dapat memberikan tahap perlindungan terhadap kejadian penyakit antraknos dan reput pangkal mangga. Penggunaan racun kulat (propiconazole, 500 ppm) berkesan mengurangkan kejadian penyakit antraknos pada buah mangga semasa penyimpanan.