Secondary Metabolites Application of Two *Pseudomonas fluorescens* isolates and Two *Trichoderma Harzianum* Isolates in Combination Against Postharvest Anthracnose in Papaya

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Mizar Muarifah[1], Loekas Soesanto[1]*, Murti Wisnu Ragil Sastyawan[2], Endang Mugiastuti[1], Noor Farid[1]

[1]*Faculty of Agriculture, Jenderal Soedirman University, Jl. Dr. Suparno, Purwokertyo 53123, Indonesia*
[2]*Faculty of Industrial Technology, Diponegoro University, Jl. Prof. Soedarto, Semarang 50275, Indonesia*

**Corresponding author, email:* lukassusanto26@gmail.com

ABSTRACT

The occurrence of papaya anthracnose is a significant post-harvest ailment, necessitating the effective disease management. The aim was to determine the ability of secondary metabolites combination of *Pseudomonas fluorescens* and *Trichoderma harzianum* isolates against the disease. A completely randomized design was used for *in vitro* experiments and a randomized block design for *in vivo* experiments. The treatments consisted of *P. fluorescens* P60 and *T. harzianum* T10, *P. fluorescens* P60 and *T. harzianum* T213, *P. fluorescens* P32 and *T. harzianum* T10, *P. fluorescens* P32 and *T. harzianum* T213 secondary metabolites and fungicides (a.i. maneb). The observed variables included the pathosystem component and papaya character and organoleptic test. The *in vitro* test results showed that *P. fluorescens* P60 and *T. harzianum* T10 and *P. fluorescens* P60 and *T. harzianum* T213 secondary metabolites inhibited the pathogen growth by 48.1075 and 43.4625%, respectively. The secondary metabolites of *P. fluorescens* P60 and *T. harzianum* T10 *in vivo* test results could delay the germination time by 12.63% and reduce the invasion area by 44.29%. All secondary metabolites had no effect on sugar content, hardness and sensory test. The combined secondary metabolites of *P. fluorescens* and *T. harzianum* are safe and does not affect papaya fruit quality.

Keywords: Combined application; Fruit quality; Organic control; Postharvest disease

ABSTRAK

Antraknosa pepaya adalah penyakit pascapanen utama dan pengendalian penyakit secara aman diperlukan. Tujuan penelitian adalah mengetahui kemampuan kombinasi metabolit sekunder dua isolat Pseudomonas fluorescens dan dua isolat Trichoderma harzianum dalam mengendalikan penyakit antraknosa pada buah pepaya pascapanen. Rancangan acak lengkap digunakan untuk pengujian in vitro dan rancangan acak kelompok untuk pengujian in vivo. Perlakuan yang diberikan adalah kontrol, metabolit sekunder P. fluorescens P60 dan T. harzianum T10, P. fluorescens P60 dan T. harzianum T213, P. fluorescens P32 dan T. harzianum T10, P. fluorescens P32 dan T. harzianum T213 dan fungisida (a.i. maneb). Variabel yang diamati adalah tingkat hambat, waktu inkubasi, luas serangan, kekerasan, kadar gula dan uji sensori. Hasil uji in vitro menunjukkan bahwa P. fluorescens P60 dan T. harzianum T10 dan P. fluorescens P60 dan T. harzianum T213 menghambat pertumbuhan patogen masing-masing sebesar 48,1075 dan 43,4625%. Hasil uji in vivo menunjukkan bahwa metabolit sekunder P. fluorescens P60 dan T. harzianum T10 mampu menunda waktu perkecambahan sebesar 12,63% dan mengurangi luas invasi sebesar 44,29%. Semua metabolit sekunder tidak berpengaruh terhadap kadar gula, kekerasan dan uji sensorik. Aplikasi kombinasi metabolit sekunder P. fluorescens dan T. harzianum untuk mengobati penyakit antraknosa pada buah pepaya aman dan tidak memengaruhi kualitas buah papaya.

Kata kunci: *Aplikasi gabungan; Kualitas buah; Pengendalian secara organik; Penyakit pascapanen*

INTRODUCTION

Papaya is a type of fruit widely developed in Indonesia. Papaya functions as a fresh fruit a processed vegetable, is good for the eyes, helps with weight loss, is a bile laxative, makes urination easier, and promotes breast milk production. Papaya fruit is a vitamin A and C source, containing amines, riboflavin, calcium, iron, potassium, magnesium, and sodium (Aravind et al., 2013; Santana et al., 2019). Papaya production in 2015 was 851,527 tons and tended to increase 2019 as much as 986,991

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tons, or there was an 11.20% growth from production in 2018 (Ministry of Agriculture RI, 2022).

Papaya fruit anthracnose is a major post-harvest disease that can reduce fruit quality and yield (Sarkar, 2016; Kadam et al., 2019). According to Alberida et al. (2014), anthracnose causes rotten fruit during storage and transportation, thus affecting the papaya fruit trade. Losses caused by anthracnose in papaya papaya fruit cause pre- and postharvest losses of up to 40-100% (Jayathunge et al., 2011). Post-harvest disease control is generally performed with synthetic pesticides (Thambugala et al., 2020). However, synthetic fungicides in postharvest fruit products are highly discouraged. This is because postharvest fruit products are directly consumed, and growing concern about health hazards and environmental contamination due to the use of chemicals calls for the development of alternative strategies to combat postharvest fruit diseases (Talibi et al., 2014).

Biological control is an alternative control method to reduce the use of synthetic pesticides (Thambugala et al., 2020). *Pseudomonas fluorescens* is a biological control bacteria widely used to control plant diseases (Soesanto et al., 2019), including postharvest diseases (Talibi et al., 2014; Sudha et al., 2021). In addition, the antagonist fungus *Trichoderma harzianum* has also been widely used (Soesanto et al., 2020; Zin & Badaluddin, 2020). The combined use of *P. fluorescens* and *T. harzianum* was able to prevent *Xanthomonas oryzae* pv. *oryzae* 4.74% (Damanik et al., 2013) and inhibited the growth of the fungus *Colletotrichum* in cocoa (Aini et al., 2013).

Antagonistic bacteria *P. fluorescens* can produce secondary metabolites that play a role in killing directly or only inhibiting pathogens (Soesanto et al., 2019). *T. harzianum* is capable of producing secondary metabolites that can inhibit the growth of other fungi or even kill them (Braun et al., 2018; Soesanto et al., 2020). The aim of this study was to investigate the ability of *P. fluorescens* P60 and P32 secondary metabolites together with *T. harzianum* T10 and T213 to prevent anthracnose disease and their effect on postharvest papaya fruit quality.

MATERIALS AND METHOD

The study was conducted at the Plant Protection Laboratory of the Faculty of Agriculture, Jenderal Soedirman University of Purwokerto for four months.

Preparation and Propagation *T. harzianum*

T. harzianum T10 (Soesanto et al., 2005) and T213 (Santoso et al., 2007) were plated on Petri dishes containing potato dextrose agar (PDA) and then incubated for 5 days. Propagation of *T. harzianum* isolates was performed in potato dextrose broth (PDB) followed by shaking (Daiki Orbital) at 150 rpm for 7 days at room temperature.

Preparation of *T. harzianum* Secondary Metabolites

The mycelia or fungal conidia and the supernatant from the culture of *T. harzianum* in PDB were separated by centrifugtion at 5,000 rpm for 10 minutes at 4^{o}C, then filtered through Whatman No. filter paper 1 (Soesanto et al., 2010; Wang et al., 2015).

Propagation of *P. fluorescens* isolates

Propagation of *P. fluorescens* was carried out with golden snail broth and placed in sterile jerry cans (Soesanto et al., 2019). Antagonist suspension of *P. fluorescens* was shaken (Daiki Orbital Shaker) for 3 days at 150 rpm at room temperature (Soesanto et al., 2010).

Separation of secondary metabolites of *P. fluorescens*

The supernatant was produced by centrifuging

P. fluorescens suspension (Sigma) at 3000 rpm for 6 minutes. The supernatant formed was taken, separated from the pellet, and ready for use (Han et al., 2012).

Anthracnose Pathogen Isolation

The anthracnose pathogen C. *gloeosporioides* was isolated from anthracnose-infected papaya fruits in the field and grown in a Petri dish on PDA medium supplemented with 50 ug/L streptomycin (Zhang et al., 2017), and incubated at 26-28°C for 7 days (Syabana et al., 2015). Identification of C. *gloeosporioides* was carried out based on several literatures (Peres et al., 2008; Rangkuti et al., 2017).

Propagation of *C. gloeosporioides*

Propagation of C. *gloeosporioides* was carried out in a PDA. Inoculation was carried out using C. *gloeosporioides* taken with a 3 mm cork drill, transferred into the PDA, incubated for 7 days, and ready for use.

Papaya Fruit Preparation and Treatment

The papaya fruit used was cv. Calina from community papaya plantations in Sumbang Village, Sumbang District, Banyumas Regency. The papaya fruits used were healthy, homogeneous in size and shape, and physiologically ripe (Gayosso et al., 2010). The fruits were cleaned and washed once, disinfected with 70% alcohol, and then air-dried (Ragavi et al., 2019). Papaya fruits were pricked with a sterile preparation needle with a puncture depth of about 0.2–0.3 cm and an area of 1 cm^2 (Proto et al., 2022). C. *gloeosporioides* was attached to the puncture, which was taken using a cork drill (0.6 cm in diameter), then sprayed with the solution according to the treatment, and covered with damp cotton. Papaya was placed in a sterile plastic box and stored at room temperature (Mukhtar et al., 2019).

Experimental Design

This study was conducted in two phases: *in vitro* and *in vivo*. *In vitro* testing was performed in a completely randomized design with five treatments, and *in vivo* testing was performed in randomized blocks of five treatments. The treatments were the same, including the control, *P. fluorescens* P60 and *T. harzianum* T10, a combination of *P. fluorescens* P60 and *T. harzianum* T213, *P. fluorescens* P32 and *T. harzianum* T10, *P. fluorescens* P32 and *T. harzianum* T213 and fungicide (a.i. maneb) with five copies.

Observed Variables

The variables observed were incubation period, attack area, sugar content, fruit hardness, and organoleptic. The incubation period was calculated in days after pathogen inoculation (dai). The attack area was measured in mm^2 with millimeter paper every day. Sugar content was measured in units of ^{0}Brix with a refractometer at the beginning and end of the observation. The hardness level was calculated in units of lbs with a penetrometer at the beginning and end of the observation. The organoleptic test was performed on the last day by 5 panelists, and the variables included color, texture, aroma, taste, and preferences.

Data Analysis

Data on incubation period, attack area, sugar content, and fruit hardness level were analyzed using analysis of variance (F test) at the 5% level. If the analysis showed a significant effect between treatments, then the data were subjected to an LSD test at 5%. Color, texture, aroma, taste, and preference data were analyzed descriptively.

RESULTS AND DISCUSSION

In vitro effects of combined secondary metabolites of *P. fluorescens* and *T. harzianum*

Based on Table 1, the combined treatments of *P. fluorescens* secondary metabolites significantly

Table 1. Effect of *P. fluorescens* and *T. harzianum* secondary metabolites on the inhibition of *C. gloeosporioides in vitro*

Treatment	Inhibition growth (%)
control	0 d
P. fluorescens P60 and *T. harzianum* T10	48.11 a
P. fluorescens P60 and *T. harzianum* T213	43.46 ab
P. fluorescens P32 and *T. harzianum* T10	37.98 b
P. fluorescens P32 and *T. harzianum* T213	38.45 b
Fungicide (a.i. maneb)	9.99 c

Remark: Means followed by different letters within the same variable are significantly different at the 5% error level in the LSD test.

affected inhibition diameter. The treatment of secondary metabolites of *P. fluorescens* and *T. harzianum* could prevent the growth of *C. gloeosporioides* with different values of 37.98-48.12%. The secondary metabolite of *P. fluorescens* P60 in combination with *T. harzianum* T10 showed the highest inhibition.

The highest inhibition is presumably because the compounds in the combined secondary metabolites can interact with each other in inhibiting the growth of pathogens. According to Soesanto et al. (2010), secondary metabolites are toxic and can inhibit the growth of pathogens, affecting plant resistance. Wallace et al. (2018) also stated that *P. fluorescens* isolates had the same type of interaction with pathogens in the form of competition for nutrients, producing antibiotics, siderophores, and cyanide acid. Meanwhile, the mechanism of inhibition of *T. harzianum* is competition for space and nutrients, as well as microparasites (Tyśkiewicz et al., 2022). The results of the *in vitro* test showed the existence of a clear zone, the zone of inhibition. The formation of an inhibition zone indicates the working of the antibiosis mechanism (Herliyana et al., 2013). The clear zone means secondary metabolites contain antibiotics (Khokhar et al., 2012). The application of secondary metabolites of *P. fluorescens* can be combined with the secondary metabolites of *T. harzianum* (Rajeswari, 2019). It was further said that the combination gave better results than when applied alone. In addition, the application of fungicides gave much smaller inhibition results than the application of antagonistic microbial secondary metabolites. This shows that synthetic chemical fungicides cannot overcome postharvest diseases of papaya fruit (Tonutti et al., 2016). Applying synthetic chemical functions in postharvest products has a negative impact (Feliziani & Romanazzi, 2013; Rani et al., 2017).

In vivo effects of combined secondary metabolites of *P. fluorescens* and *T. harzianum*

Combined use of secondary metabolites between *P. fluorescens* and *T. harzianum* against postharvest anthracnose in papaya showed significant effects on the disease components (incubation period and area of attack) but no significant impact on the physical components (hardness and sugar content) of papaya fruits (Table 2).

Table 2. Effect of *P. fluorescens* and *T. harzianum* secondary metabolites on *C. gloeosporioides* growth *in vivo*

Treatment	Incubation period (dai)	Attack area (mm^2)
control	4.08 b	991.58 a
P. fluorescens P60 and *T. harzianum* T10	4.67 a	552.33 b
P. fluorescens P60 and *T. harzianum* T213	4.58 ab	606.08 ab
P. fluorescens P32 and *T. harzianum* T10	4.08 b	790.33 ab
P. fluorescens P32 and *T. harzianum* T213	4.08 b	840.00 ab
Fungicide (a.i. maneb)	4.67 a	655.58 ab

Remark: Means followed by different letters within the same variable are significantly different at the 5% error level in the LSD test; dai = days after inoculation.

Incubation period

Based on Table 2, the longest incubation time of the combined secondary metabolites of *P. fluorescens* P60 and *T. harzianum* T10 was the same as when using the fungicide with the active ingredient maneb, which could delay incubation 12.63% compared to control. It is suggested that secondary metabolites of *P. fluorescens* P60 may be related to *T. harzianum*, thus suppresses *C. gloeosporioides* and causes a slower onset of symptoms.

The fungal suppression is following the opinion of Santoso et al. (2007), who reported that the longest incubation period of *C. gloeosporioides* was in *P. fluorescens* P60. Inhibition by *P. fluorescens* is a result of various mechanisms, including antibiosis, namely the presence of the antibiotic 2,4-diacetylfluroglucinol (Phl) that it produces (Rieusset et al., 2020; Zboralskia & Filion, 2020). Meanwhile, the secondary metabolites of *T. harzianum* contain various bioactive compounds (Özkale, 2017). Differences in the incubation period in the treatment of biological agents can be caused by the antagonist's resistance factor in suppressing the pathogen's incubation period. The shorter the incubation period, the higher the suitability of the host-pathogen. According to Ali et al. (2017), the incubation period is influenced by several factors, including the host, environment, and pathogen. The secondary metabolites of *P. fluorescens* P60 gave better results when combined with the secondary metabolites of *T. harzianum* T10. The better result indicates the combination's suitability or synergism between the two types of secondary metabolites. This condition is supported by the opinion of Rajeswari (2019), suggesting that a specific combination of *T. viridae* and *P. fluorescens* may have greater effectiveness in inhibiting the pathogen in the biocontrol of Fusarium wilt compared to individual strain.

Attack area

Based on Table 2, the control area was more significant than the attack area in the treatments. In contrast, the smallest invasion area was found using secondary metabolites of *P. fluorescens* P60 and *T. harzianum* T10, which reduced the invasion area by 44.29% compared to the control. This result is consistent with data from longer incubations. It is believed that the combined secondary metabolites of *P. fluorescens* P60 and *T. harzianum* T10 can better prevent the attack of *C. gloeosporioides*.

This greater ability is because *T. harzianum* produces some bioactive compounds, such as enzymes (proteases, 1,3 β glucanase, cellulose, and chitinase), antibiotics, IAA hormones, and can absorb metals (Fe, Mn, and Zn). *T. harzianum* is also able to produce volatile and non-volatile compounds that can inhibit the growth of plant pathogens. These compounds include trichodemin, paracelicine, trichotoxin, gliotoxin, acetaldehyde, and viridian, which play a role in microparasitism (Tyśkiewicz et al., 2022). Meanwhile, *P. fluorescens* P60 has several mechanisms that suppress or inhibit the growth of the pathogen. One of them is the mechanism of producing antibiotics, including phenazine and 2,4 diacetyl phloroglucinol, which have broad antiviral, antibacterial, antifungal, and antihelmintho properties, which inhibit fungal growth. Pyrolnithrin (Prn) inhibits the growth of different fungi and bacteria but does not kill the target organisms (Rieusset et al., 2020). The combined secondary metabolites of *P. fluorescens* P60 and *T. harzianum* T10 are compatible and have synergistic activity against anthracnose pathogens. The synergistic effect is in accordance with Rajeswari's (2019) report that the specific combination of *T. viride* and *P. fluorescens* can have a more significant effect on inhibiting pathogens than individual strains. The combined of *P. fluorescens* P60 and *T. harzianum* T10 secondary metabolites were also not significantly different from the fungicide (a.i. maneb), suggesting that the combination could be an alternative for fungicide replacement.

Fruit sugar content

All combined *P. fluorescens* and *T. harzianum* secondary metabolites were not significantly different from the controls and the fungicides used in papaya fruit's sugar content and hardness (Tabel 3).

Applying combined secondary metabolites did not affect the increase in papaya fruit sugar levels.

It is suspected that fruit physiological factors influence fruit sugar levels and will increase with increasing fruit maturity so that the rise in sugar levels does not depend on treatment but on fruit ripening. This situation follows the opinion of Khadivi-Khub (2014), who states that physiological metabolism, including fruit dissolved sugar, dissolved solids, pectin, and enzymes in fruit, is closely related to fruit growth and development. The sweet taste of fruit is due to an increase in the content of simple sugars and a decrease in phenolic compounds. The higher the dissolved solids content, the sweeter the fruit (Datta & Bora, 2019).

Papaya is a climacteric fruit with a short life span (Fabi et al., 2014). The general trend in fruit during storage is an increase in sugar content, followed by a decrease (Tigist et al., 2013). Suketi et al. (2010) added that the total dissolved solids content of the fruit flesh increased with the expansion of the yellow color on the surface to a level of 80 %, after which it decreased with the expansion of the skin color due to the hydrolysis of sugars into organic acids used for the respiration process.

Fruit hardness

The reduction of the level of hardness in the combined *P. fluorescens* and *T. harzianum* secondary metabolites showed no different results compared to the control and fungicide (Table 3). Treatment of secondary metabolites has not been able to affect the decrease in hardness of papaya fruit. The decrease of papaya hardness is presumably due to pathogenic infections that attack each treatment, causing the fruit to become softer. The reduction in fruit hardness can also be caused by respiration and transpiration (Paul & Pandey, 2014). The process of respiration results in the breakdown of carbohydrates into simpler compounds. This process causes tissue rupture in the fruit; the fruit becomes soft and undergoes ripening, resulting in the degradation of semicellulose and pectin, thereby causing a change in hardness. The process of respiration in fruit that is attacked by pathogens is higher when compared to fruit that is not attacked by pathogens, so the fruit decomposes more quickly (Zhang et al., 2021).

Effects of Secondary Metabolites Application on Papaya Fruit Orgnoleptics

Color

A total of 100 % of the panelists rated the fruit flesh as orange based on their assessment of the color of the fruit flesh in control and the combined *P. fluorescens* P32 and *T. harzianum* T10 secondary metabolites. Meanwhile, based on the assessment of the color of the fruit flesh in the combined *P. fluorescens* P60 and *T. harzianum* T10, *P. fluorescens* P60 and *T. harzianum* T213, and *P. fluorescens* P32 and *T. harzianum* T213 secondary metabolites, a total of 40, 20, and 40 % of the panelists rated the fruit as orange to black, respectively. The secondary metabolites have not been able to affect the change in the color of papaya fruit. The unable effect is presumably because the discoloration is caused by the ripening of the papaya fruit and influenced by the physiological processes of the fruit in the climacteric fruit. According to Tripathi et al. (2016), climacteric fruit ripening is guided by a number of biochemical events, which contain changes in sugar, acidity, color, texture, and volatile aroma that are important for sensory qualities. Several senescence-associated physiological changes occur in the next maturation stage, leading to membrane weakening and cell death. The first sign of fruit ripening is the loss of green color (Moreno et al., 2020). The chlorophyll content of ripe fruit gradually decreases. This causes the fruit to change its color to brownish-yellow. According to Abacı & Asma (2013), fruit ripening includes a series of biochemical, physiological, and structural changes,

such as hydrolysis of starch, degradation of chlorophyll, production of carotenoids, anthocyanins, and phenols, accumulation of sugars and organic acids, modification of the structure and composition of cell wall polysaccharides, color changes, and changes in taste and texture.

Papaya fruit aroma

Based on the panelist's assessment of fruit aroma, the combined application of secondary metabolites showed varied results. Most of the panelists rated the fruit aroma as felt and very pronounced. Treatment with *P. fluorescens* and *T. harzianum* secondary metabolites failed to affect papaya fruit aroma. The unable effect is presumably because the ripening of the papaya fruit causes the change in aroma. The distinctive aroma around ripe fruit usually comes from aliphatic alcohol ester compounds and short-chain fatty acids. Several terpenoid compounds cause the odor emitted by oranges, bananas, manganese, and papaya (Thibaud et al., 2020). The degree of ripeness is the primary physiological factor that influences the production of essential substances, but the aroma composition is strongly influenced by environmental conditions during ripening (Perotti et al., 2014).

Papaya flavor

Based on the panelists' assessment of the fruit taste, all treatments showed that most of the panelists rated the taste of fruit as less sweet and sweet. Based on Table 3, the final sugar content ranged from 8-9 °Brix. The less papaya sweet is thought to cause the treated papaya to have a less sweet taste. According to Saryoko et al. (2004), papaya fruit with a sweet taste has a sugar content ranging from 11–13 °Brix. The secondary metabolites have not been able to affect changes in the taste of papaya fruit. The unable effect can also be seen in the results of the statistical analysis of sugar content (Table 2), showing that the results were not significantly different. Changes in taste are thought to be due to fruit ripening and pathogenic infections, causing the fruit to reduce its sugar content. Pathogenic infections in fruit cause an increase in fruit maturity, and fruit maturity plays a crucial role in controlling fruit quality so that fruit decomposes more quickly (Garcia-Benitez et al., 2017). An increase in the respiration rate causes a decrease in the amount of glucose in the tissues so that the sugar content of the fruit will decrease (Bravdo, 1968; Rakhmankulova, 2022). The decrease sugar content causes the fruit to taste less sweet. Fruit that is injured or damaged can spur increased respiration. Respiration uses substrates like sugar, starch, cellulose, pectin, fat, and protein as ingredients in biological oxidation. The high respiration rate is accompanied by a short shelf life, indicating a decline in food ingredients' quality and value (Rovira et al., 2019).

Table 3. Effect of *P. fluorescens* and *T. harzianum* secondary metabolites on sugar content and fruit firmness

Treatment	Increasing sugar content (%)	Decreasing fruit hardness (%)
control	42.50 a	72.56 a
P. fluorescens P60 and *T. harzianum* T10	42.73 a	69.47 a
P. fluorescens P60 and *T. harzianum* T213	41.95 a	72.03 a
P. fluorescens P32 and *T. harzianum* T10	43.42 a	67.39 a
P. fluorescens P32 and *T. harzianum* T213	43.69 a	65.22 a
Fungicide (a.i. maneb)	42.48 a	63.31 a

Remark: Means followed by different letters within the same variable are significantly different at the 5% error level in the LSD test.

Papaya's preference

Based on the panelists' assessment of their preference for fruit, most considered they liked the tested papaya. The use of *P. fluorescens* and *T.*

harzianum secondary metabolites has not been able to influence panelists' preference for papaya fruit. The unable effect is presumably because the panelist's preference level can be obtained from his preference for the tested papaya's color, texture, aroma, or taste. The treatment of secondary metabolites of *P. fluorescens* and *T. harzianum* could not influence the organoleptic tests of color, texture, aroma and taste. According to Karabulut et al. (2018), the higher the degree of ripeness of the fruit, the higher the water content, total dissolved solids, color value and preference for fruit aroma and texture. However, vitamin C content, total acidity and hardness decrease.

CONCLUSIONS

The secondary metabolites of *P. fluorescens* P60 and *T. harzianum* T10 or *P. fluorescens* P60 and *T. harzianum* T215 were able to inhibit C. *gloeosporioides in vitro* by 48.11 and 43.46%, respectively. The combined secondary metabolites application of *P. fluorescens* P60 and *T. harzianum* T10 was able to postpone the incubation period *in vivo* by 12.63%. It was able to suppress the area of attack of C. gloeosporioides *in vivo* by 44.29%. The combined secondary metabolites application of *P. fluorescens* and *T. harzianum* did not affect sugar content, fruit firmness, color, texture, aroma, taste, or papaya fruit preference. The combined use of *P. fluorescens* and *T. harzianum* secondary metabolites in the treatment of papaya fruit anthracnose is considered safe and does not affec papaya fruit quality.

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