

Alternative control of post-harvest diseases in Tainung 1 papaya¹

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ABSTRACT

Controlling post-harvest papaya diseases without using agrochemicals is a challenge for producers. This study aimed at evaluating the effect of clove essential oil, biological fungicide (Trichodermil®), resistance inducer (Cob Sistem®) and chemical fungicide (Imazacure®) on the *in vitro* control of phytopathogenic fungi isolates from papaya as well as on the post-harvest quality of Tainung 1 papaya. The *in vitro* experiment was conducted in a complete randomized design, with five fungal species x five treatments and five replications. The *in vivo* experiment was conducted in a complete randomized design, with five treatments x five storage times, five replications and three fruits per replication. The fruits were stored under refrigeration at 10 ± 2 °C and 90 ± 5 % of relative humidity and evaluated at 0, 7, 14, 21 and 28 days of storage, plus two shelf life days at 25 ± 2 °C, to simulate marketing conditions. The inhibition of mycelial growth was evaluated in the *in vitro* experiment, while the diseases occurrence and post-harvest quality of the fruits were evaluated in the *in vivo* experiment. The clove essential oil and Trichodermil® were as efficient as Imazacure® in inhibiting the mycelial growth of *Alternaria* sp., *Colletotrichum gloeosporioides* and *Rhizopus* sp. The treatments with clove essential oil, Trichodermil® and Imazacure® were similar in controlling the pathogens up to 21 days of storage. The treatments had no effect on the fruits soluble solid contents.

KEYWORDS: *Carica papaya* L.; *Syzygium aromaticum*; *Trichoderma harzianum*; copper phosphite.

INTRODUCTION

Brazil is the second world largest producer of papaya (*Carica papaya* L.) fruits, being the production of the country 1,424,650 tons, in 2016 (FAO 2016).

Anthraxnose, peduncle rot and alternaria fruit spot are among the main post-harvest diseases that cause losses in the marketing of this fruit (Rezende & Fancelli 1997, Suárez-Quiroz et al. 2013). The use of

RESUMO

Controle alternativo de doenças pós-colheita em mamão Tainung 1

O manejo de doenças pós-colheita de mamão sem o uso de agrotóxicos é um desafio para os produtores. Objetivou-se avaliar o efeito do óleo essencial de cravo-da-índia, do fungicida biológico Trichodermil®, do indutor de resistência Cob Sistem® e do fungicida químico Imazacure®, no controle *in vitro* de fungos fitopatogênicos isolados de mamão e na qualidade pós-colheita de mamão Tainung 1. O experimento *in vitro* foi conduzido em delineamento inteiramente casualizado, com 5 fungos x 5 tratamentos e cinco repetições. No experimento *in vivo*, utilizou-se delineamento inteiramente casualizado, com 5 tratamentos x 5 tempos de armazenamento, cinco repetições e três frutos por repetição. Os frutos foram armazenados sob refrigeração a 10 ± 2 °C e 90 ± 5 % de umidade relativa e avaliados nos tempos 0, 7, 14, 21 e 28 dias, mais dois dias de tempo de prateleira a 25 ± 2 °C, para simular condições de comercialização. Avaliou-se a inibição do crescimento micelial para o experimento *in vitro* e, para o *in vivo*, a ocorrência de doenças e a qualidade pós-colheita dos frutos. O óleo essencial de cravo-da-índia e Trichodermil® foram tão eficientes quanto o Imazacure® na inibição do crescimento micelial de *Alternaria* sp., *Colletotrichum gloeosporioides* e *Rhizopus* sp. Até 21 dias de armazenamento, os tratamentos com cravo-da-índia, Trichodermil® e Imazacure® foram iguais, quanto ao controle de patógenos. Os tratamentos não propiciaram diferença quanto aos sólidos solúveis dos frutos.

PALAVRAS-CHAVE: *Carica papaya* L.; *Syzygium aromaticum*; *Trichoderma harzianum*; fosfito de cobre.

chemical products is the most common method for controlling diseases in papaya. However, products registered for this crop are not very efficient, and consumers are increasingly worried about problems related to the continuous and intensive use of agrochemicals in fruit species (Dantas et al. 2004, Suárez-Quiroz et al. 2013).

Thus, alternatives that are efficient, viable and cause less environmental impacts and risks to human

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health are intensively studied. Some products based on agents used for biological control, plant essential oils and resistance inductors have been efficient for controlling diseases in various pathosystems, including fruit plants such as papaya. These products have no deleterious effects as chemical pesticides (Combrinck et al. 2011, Catão et al. 2013, Hamini-Kadar et al. 2014, Lubaina & Murugan 2015, Valenzuela et al. 2015).

This study aimed at evaluating the effect of clove [*Syzygium aromaticum* (L.) Merrill and Perry] essential oil, biological fungicide (Trichodermil®), resistance inducer (Cob Sistem®) and chemical fungicide (Imazacure®) on the *in vitro* control of phytopathogenic fungi isolates from papaya, as well as on the post-harvest quality of Tainung 1 papaya fruits.

MATERIAL AND METHODS

The *in vitro* experiment was conducted at the Universidade Federal Rural do Semiárido, in Mossoró, Rio Grande do Norte state, Brazil, from July to August 2015. The *in vivo* experiment was conducted in a packing house in the Flamengo farm, in Tibau, Rio Grande do Norte state, and Icapuí, Ceará state, both in Brazil, where the treatments were applied, followed by refrigerated storage at 10 ± 2 °C and relative humidity of 90 ± 5 %.

The pathogens used in the *in vitro* experiment were *Alternaria* sp., *Colletotrichum gloeosporioides*, *Fusarium* sp., *Lasiodiplodia theobromae* and *Rhizopus* sp., which were isolated from papaya fruits (Tainung 1 cultivar). The isolates were identified based on visual morphological characteristics in an optical microscope, using a dichotomic key (Barnett & Hunter 1998). The clove essential oil used was obtained by hydrodistillation. The products used to control the pathogens were a biological fungicide based on *Trichoderma harzianum* conidia (Trichodermil®, 48 g L⁻¹), a resistance inducer based on copper phosphite (2.0 % of P₂O₅ and 4.5 % of Cu) (Cob Sistem®) and a chemical fungicide based on imazalil (Imazacure®, 500 g L⁻¹). Among the tested products, only the Imazacure® fungicide is commonly used in treatments of fruits to prevent post-harvest diseases. The products were incorporated into a potato-dextrose-agar (PDA) culture medium, autoclaved at 120 °C for 30 min (Nascimento et al. 2016). The rates used per liter of culture medium

were 3 mL (clove essential oil), 6 mL (Trichodermil®), 2 mL (Cob Sistem®) and 2 mL (Imazacure®). These rates were determined based on previous experiments, except for Imazacure®, which was based on the manufacturer recommendation. Petri dishes with PDA culture medium without the products were used as a control.

After 7 days of growth, a 7-mm mycelial disk from the border of the fungal colonies from each fungal isolate was transferred to the center of the Petri dishes containing the PDA and the respective treatment.

These plates were kept in a BOD incubator at 26 ± 2 °C, for 5 or 7 days. Then, the mycelial growth of the fungi was measured with a ruler in diametrically opposite directions. The obtained values were used to calculate the percentage of inhibition of mycelial growth (IMG), using the formula $IMG = (\text{control growth} - \text{treatment growth} \times 100) / \text{control growth}$ (Bastos 1997).

The experiment was conducted in a complete randomized design, with 5 treatments (clove essential oil, Trichodermil®, Cob Sistem®, Imazacure® and a control), 5 fungal species (*Alternaria* sp., *C. gloeosporioides*, *Fusarium* sp., *L. theobromae* and *Rhizopus* sp.) and 5 replications. The means were compared by de Scott-Knott test at 5 %.

The fruits were harvested at the maturation stage (2-25 % of yellow peel surface surrounded by light green) and taken to the packing house, where they were selected, washed in water, disinfested with 1.5 % sodium hypochlorite and weighed using a digital scale (Welmy BCW6). The treatments were applied with manual sprayers to the point of dripping. The solutions applied were prepared using distilled water for the control and dilutions with distilled water with clove essential oil (3 mL L⁻¹), Trichodermil® (6 mL L⁻¹) and Cob Sistem® (2 mL L⁻¹). Imazacure® was applied according to the manufacturer recommendation for post-harvest treatments of fruits, which consists in the immersion of the fruits in a solution of distilled water and Imazacure® (2 mL L⁻¹). All fruits were left to dry at room temperature (25 ± 2 °C, for 30 min) and then immersed in a coating wax based on polyethylene and shellac (Citrosol®). Subsequently, the fruits were packed in plastic boxes and maintained in a cold room at 10 ± 2 °C and 90 % of relative humidity for 28 days. The evaluations were performed at 0, 7, 14, 21 and 28 days of storage, plus two shelf life days at a temperature of 25 ± 2 °C.

The experiment was conducted in a complete randomized design, with 5 treatments (clove essential oil, Trichodermil[®], Cob Sistem[®], Imazacure[®] and a control), 5 storage periods (0, 7, 14, 21 and 28 days) and 5 replications, with each replication consisting of 3 fruits.

The fruit external aspect was evaluated using a 1 to 5 visual scale, considering the presence of defects, namely, absence of injuries, spots, wilting and depressions (1); light (2), mild (3) and severe (4) presence of these problems; and extreme deterioration of the fruit (5) (Peixoto 2005). Fruits with grades higher than 3 were considered unfit for consumption. The fruit weight loss was evaluated by the difference between the initial and final weights and the severity of post-harvest diseases by using a 1 to 4 visual scale, considering the percentage of the fruit surface area that was affected: 0 % (1), less than 10 % (2), between 10 % and 20 % (3) and greater than 20 % (4) (Peixoto 2005). The fruit pulp titratable acidity was evaluated according to the methodology described by the Instituto Adolfo Lutz (IAL 1985). The pulp was homogenized to determine its soluble solid contents using a digital refractometer (Biobrix ATC 104-D), and pH using a digital pH meter (Quimis).

The data were subjected to variance analysis, using the statistical software Assistat 7.7, and the means were compared by the Scott-Knott test at 5 %. The regression analysis was performed for the storage period factor.

RESULTS AND DISCUSSION

The alternative products used to control the pathogens affected significantly the mycelial growth of the evaluated fungi. The clove essential oil and

Trichodermil[®] were as efficient as the Imazacure[®] chemical fungicide in inhibiting the mycelial growth of *Alternaria* sp., *Colletotrichum gloeosporioides* and *Rhizopus* sp. (Table 1).

The clove essential oil completely inhibited the *L. theobromae* and *Rhizopus* sp. growth (Table 1). Similarly, Combrinck et al. (2011) found a complete mycelial growth inhibition of *L. theobromae* isolates from avocado and mango, when using clove essential oil at rates of 2 mL L⁻¹ and 3 mL L⁻¹. The antifungal activity of the clove essential oil is attributed to the eugenol, which is one of its main compounds (Wang et al. 2010, Rana et al. 2011, Ascenção & Mouchrek Filho 2013). According to previous studies, the use of clove essential oil causes the disorganization of cell contents, hyphae fragmentation, decreased turgidity and destabilization and rupture of the cell membrane in fungi (Wang et al. 2010, Costa et al. 2011).

Trichodermil[®] inhibited the growth of *Alternaria* sp. in 89 %, as well as the growth of *C. gloeosporioides* and *Rhizopus* sp. in 100 % (Table 1). Lubaina & Murugan (2015) evaluated the *in vitro* mycelial growth of *Alternaria sesame* using *T. harzianum* and found an inhibition rate of 96.7 %.

Cob Sistem[®] inhibited in 40 %, 45 %, 54 % and 83 % the mycelial growth of *C. gloeosporioides*, *Alternaria* sp., *Fusarium* sp. and *L. theobromae*, respectively, and had no effect on *Rhizopus* sp. (Table 1). Phosphites are used as resistance inducers to activate the plant defense mechanisms, and have a varied direct effect on pathogens (depending on the phytopathogen) and phosphite rate and type (Catão et al. 2013, Ogoshi et al. 2013, Alexandre et al. 2014).

The interaction between the factors evaluated was significant for the fruit external aspect, weight loss, pH, titratable acidity and disease severity; and

Table 1. Mycelial growth inhibition of pathogens isolated from papaya fruits (Tainung 1 cultivar), using Imazacure[®], clove essential oil, Trichodermil[®] and Cob Sistem[®].

Pathogen	Treatment*							
	Imazacure [®]		Clove essential oil		Trichodermil [®]		Cob Sistem [®]	
	%**	asen√(x/100)	%	asen√(x/100)	%	asen√(x/100)	%	asen√(x/100)
<i>Alternaria</i> sp.	100	90 aA	73	68 bA	89	80 aA	45	42 bB
<i>Colletotrichum gloeosporioides</i>	100	90 aA	87	79 bA	100	90 aA	40	39 bB
<i>Fusarium</i> sp.	100	90 aA	80	72 bB	80	72 bB	54	50 bC
<i>Lasiodiplodia theobromae</i>	100	90 aA	100	90 aA	84	67 bB	83	66 aB
<i>Rhizopus</i> sp.	100	90 aA	100	90 aA	100	90 aA	0	0.0 cB
CV (%)	19.17							

*Averages followed by the same lowercase letters in the columns and capital letters in the rows do not differ statistically from one another by the Scott-Knott test at 1 %;

** percentage of mycelial growth inhibition.

the storage period factor had an independent effect on the total soluble solid contents.

The external aspect of the fruits decreased during the storage period in all the treatments (Figure 1).

This decrease is normal during the storage period, due to the fruit senescence process. However, the treatments tended to reduce it. This result was already found by other authors working with papaya, regardless of the treatments applied (Rocha et al. 2005, Fernandes et al. 2010). Fruits treated with Imazacure®, clove essential oil and Cob Sistem® were statistically different from the control. Imazacure® was the best treatment at 28 days of storage, differing significantly from the others. However, fruits treated with clove essential oil and Cob Sistem® had grades lower than 3, indicating that these fruits were within the acceptable standards for trading.

The post-harvest diseases increased with the storage time. This is normal, due to the fruits natural senescence and loss of resistance along the ripening period (Figure 2).

However, fruits treated with Trichodermil®, Cob Sistem®, clove essential oil and Imazacure® showed a lower disease severity than the control fruits (Figure 2).

Fruits from all treatments presented post-harvest diseases from 14 days of storage. The phytopathogens found in the injuries of the papaya fruits were *Alternaria* sp., *C. gloeosporioides*, *Fusarium* sp., *Geotrichum* sp. and *L. theobromae*. Regarding the pathogens control, the treated fruits differed significantly from the control fruits at 21 days of storage, and the fruits treated with clove essential

oil, Trichodermil® and Imazacure® were statistically equal (Figure 2). *T. harzianum* (Trichodermil®) has been reported as efficient in the control of diseases in different pathosystems. Its parasitism and production of secondary metabolites have been pointed out as the main mechanisms of action for this product (Lubaina & Murugan 2015).

The fruit weight loss increased with increasing storage time (Figure 3).

The fruits treated with Imazacure®, clove essential oil, Trichodermil® and Cob Sistem® had a similar weight loss at 14 days of storage, differing from the control (Figure 3).

Treated fruits and control fruits had a similar weight loss at 0, 7 and 21 days of storage (Figure 3). The weight loss of the fruits treated with Imazacure®, clove essential oil and Cob Sistem® were statistically similar at 28 days of storage.

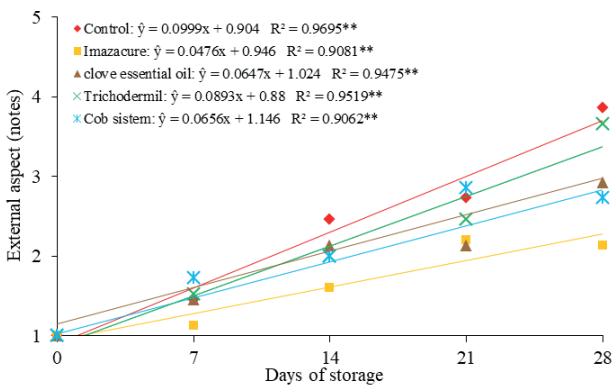


Figure 1. External aspect of the papaya fruits (Tainung 1 cultivar), using Imazacure®, clove essential oil, Trichodermil® and Cob Sistem®.

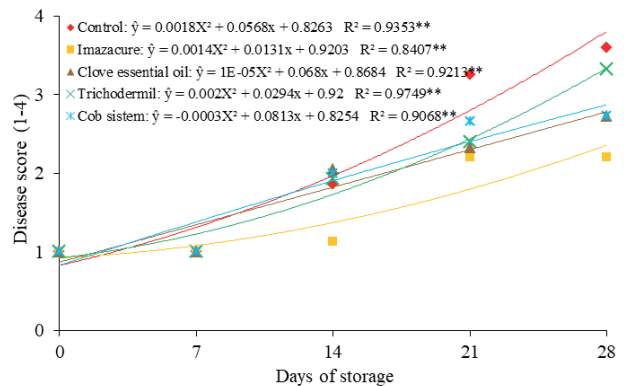


Figure 2. Severity of post-harvest diseases of the papaya fruits (Tainung 1 cultivar), using Imazacure®, clove essential oil, Trichodermil® and Cob Sistem®.

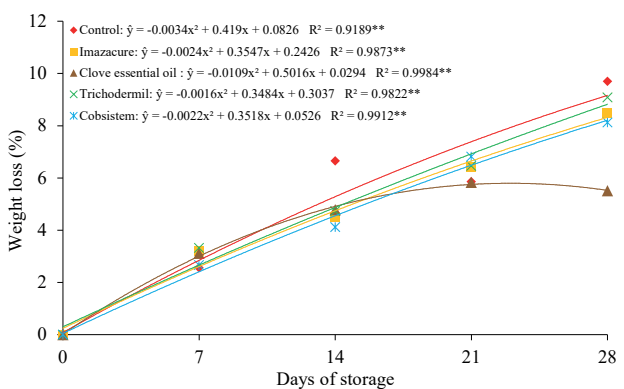


Figure 3. Weight loss of the papaya fruits (Tainung 1 cultivar), using Imazacure®, clove essential oil, Trichodermil® and Cob Sistem®.

The pH of the fruits varied during the storage times, regardless of the treatment applied (Figure 4).

These changes are common during ripening and are attributed mainly to the degradation and synthesis of organic acids (Almeida et al. 2006). Similarly, Pimentel et al. (2011) found variations for pH in papaya fruits (Hawaii cultivar) during 6 days of storage, when subjected to treatments with cassava and potato starch. The pH of the fruits treated with Imazacure® (5.55), clove essential oil (5.58), Trichodermil® (5.54) and Cob Sistem® (5.51) were statistically equal at 28 days, but differed from that of the control, which had a pH of 5.64. Fagundes & Yamanishi (2001) evaluated papaya fruits (Solo cultivar) and found a pH of 5.20 to 5.71, which are close to those found in the present study, denoting that the pH had no changes due to the applied treatments.

The titratable acidity varied during the storage periods (Figure 5).

According to Yamanishi et al. (2005), changes in this parameter are common due to maturation processes. Almeida et al. (2006) found a varied titratable acidity in papaya fruits of the Golden cultivar during the storage period.

The fruits treated with Imazacure®, clove essential oil, Trichodermil® and Cob Sistem® had a higher titratable acidity than the control fruits at 7 days of storage (Figure 5). The fruits treated with Cob Sistem® had a higher titratable acidity than those from the other treatments and the control fruits at 14 and 28 days of storage. The percentages of titratable acidity found in the present study were similar to those reported by Almeida et al. (2006), who found

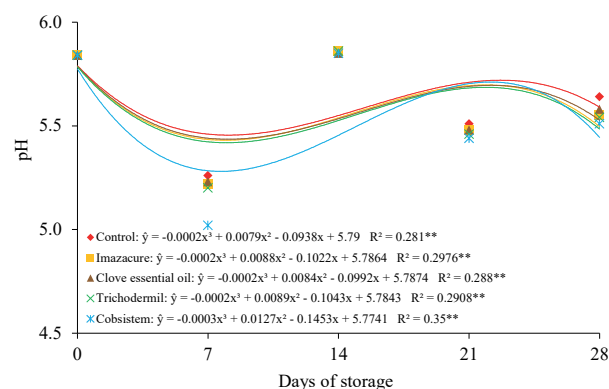


Figure 4. pH of the papaya fruits (Tainung 1 cultivar), using Imazacure®, clove essential oil, Trichodermil® and Cob Sistem®.

0.08-0.19 % of citric acid in papaya fruits of the Golden cultivar.

The fruits had similar total soluble solid contents, regardless of the treatments. They decreased from 0 to 7 days of storage, but had a low variation from 7 to 28 days (Figure 6).

The increase for sugar content in papaya fruits is only observed while the fruits are attached to the plant (Ruggiero et al. 2011). Total soluble solid contents vary little after harvesting, since these fruits have a low starch content to be hydrolyzed (Jacomino et al. 2007). Similarly, Souza et al. (2014) found little variation in total soluble solid contents in papaya fruits of the Tainung 1 cultivar stored under 25 °C and 80 % of relative humidity for 7 days; and Jacomino et al. (2007) found total soluble solid contents of 9.92-10.21 °Brix in papaya fruits of the Golden cultivar.

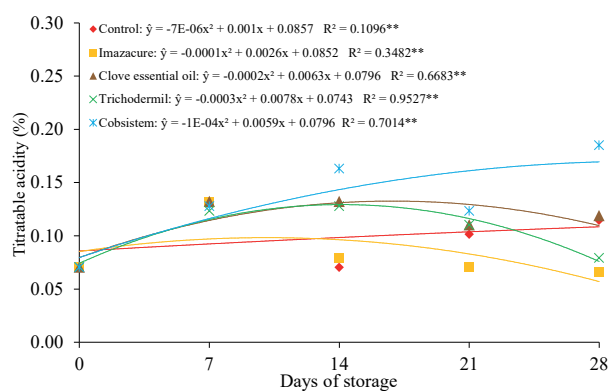


Figure 5. Titratable acidity of the papaya fruits (Tainung 1 cultivar), using Imazacure®, clove essential oil, Trichodermil® and Cob Sistem®.

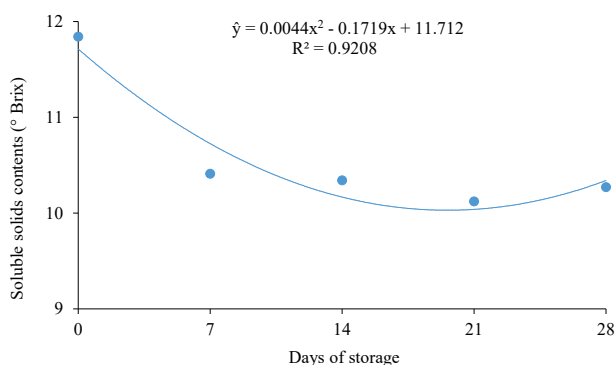


Figure 6. Total soluble solids contents of the papaya fruits (Tainung 1 cultivar), using Imazacure®, clove essential oil, Trichodermil® and Cob Sistem®.

CONCLUSIONS

1. The clove essential oil and the Trichodermil® biological fungicide were as efficient as the Imzacure® chemical fungicide in inhibiting the mycelial growth of *Alternaria* sp., *Colletotrichum gloeosporioides* and *Rhizopus* sp., *in vitro*;
2. The control of pathogens in the fruits treated with clove essential oil, Cob Sistem® and Imzacure® was similar up to 21 days of storage;
3. The alternative treatments do not alter the pH, titratable acidity and soluble solids contents of papaya fruits of the Tainung 1 cultivar.

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