

## POST-HARVEST STORAGE OF PAPAYA FRUITS COATED WITH EXTRACTS OF LEAVES AND FRUITS OF NEEM<sup>1</sup>

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**ABSTRACT** – The objective of the present work was to evaluate the use of extracts of leaves and fruits of neem (*Azadirachta indica* Juss) as coating for papaya Formosa fruits aiming to maintain their quality during post-harvest storage. A completely randomized experimental design in a 5x5 factorial arrangement was used, with 3 replications of 2 papaya fruits per plot. The treatments consisted of five coatings with extracts of neem leaves and fruits (leaf extracts at 5 and 10%, fruit extracts at 5 and 10% and a control treatment without coating) and five storage periods (0, 3, 6, 9 and 12 days) under mean temperature of 30.4 °C and relative humidity of 42%. The fruit weight loss, external appearance, pulp firmness, titratable acidity and soluble solids were evaluated, and the fungi species on the fruit surface of each storage were identified and quantified. The treatments with extract of neem leaves and fruits at 5% had papaya fruits with best external quality and provided best control of growth of phytopathogenic fungi. Regarding external appearance, papaya fruits coated with extracts of neem leaves and fruits at 5% had a shelf life of 12 days, while those coated with extracts at 10% had a shelf life of 9 days.

**Palavras-chave:** *Azadirachta indica* Juss. Coating. Quality. Fungi.

## ARMAZENAMENTO PÓS-COLHEITA DE FRUTOS DO MAMOEIRO RECOBERTOS COM EXTRATOS DAS FOLHAS E FRUTOS DO NIM

**RESUMO** – O presente trabalho teve como objetivo avaliar uso de extrato à base de folhas e de frutos do nim (*Azadirachta indica* Juss) como recobrimento na manutenção da qualidade pós-colheita do mamão Formosa. O delineamento utilizado foi o inteiramente casualizado em esquema fatorial 5 x 5, no qual o primeiro fator constou dos tipos de recobrimentos a base de folhas e frutos do nim (controle TC, extrato das folhas a 5% - T1, extrato das folhas a 10% - T2, extrato dos frutos a 5% - T3 e extrato dos frutos a 10% - T4) e o segundo fator dos tempos de armazenamento (0, 3, 6, 9 e 12 dias) a temperatura média de 30,4 °C e UR de 42%, com 3 repetições de 2 frutos por parcela. A cada tempo de armazenamento foram avaliadas perda de massa, aparência externa, firmeza da polpa, teor de acidez titulável, sólidos solúveis, percentual de fungos na superfície dos frutos e identificação de espécies de fungos. O extrato das folhas e dos frutos de nim a 5% apresentaram os melhores resultados por manter a qualidade externa dos frutos, além de controlar o crescimento dos fungos fitopatogênicos. Com base na aparência externa, os frutos de mamão tratados com extratos das folhas e dos frutos do nim a 5% tiveram vida útil de 12 dias. Os frutos recobertos com extratos das folhas e dos frutos a 10% apresentaram vida útil de 9 dias.

**Palavras-chave:** *Azadirachta indica* Juss. Revestimento. Qualidade. Fungos.

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<sup>1</sup>Received for publication in 08/30/2016; accepted in 05/24/2017.

Paper extracted from masters dissertation of the first author.

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## INTRODUCTION

Papaya is a climacteric fruit of high ethylene production and high to very high respiratory rate, whose characteristics promote organoleptic alterations during its maturation, and make this fruit very perishable (COSTA; BALBINO, 2002; PEREIRA et al., 2006; SOUZA et al., 2014).

One of the problems in the storage of papaya is the fruit rotting, which causes large post-harvest losses. According to Liberato and Tatagiba (2001), the fungi *Colletotrichum gloeosporioides* Penz. and *Phoma caricae papaya* are responsible for the major post-harvest papaya diseases, anthracnose and dry rot. The control of these diseases has been achieved through continuous chemical spraying programs, but the use of these products causes several undesirable consequences when used improperly. Thus, alternatives to control possible post-harvest diseases and meet the needs of producers and consumers have been studied, with emphasis on the use of extracts with antimicrobial activity, aiming to reduce the use of pesticides (BRITO et al., 2011; FERREIRA et al., 2014; NASCIMENTO; NERY; RODRIGUES, 2008).

The use of herbal plants is one of these alternatives, considering the great variability of existing species, low cost of these products, easy availability in rural properties and mainly, absence or low risks of contamination for the environment, animals and humans (AGNOLIN et al., 2010). Neem (*Azadirachta indica* Juss) is the most used plant of insecticidal action due to the contents of several substances with biological activity (azadirachtin, meliantriol, limonene, odoratone and other triterpenoids) in its leaves, bark, fruits and seeds; and its low cost, low polluting potential and low residual potential compared with synthetic products, thus presenting low risk of intoxication for mammals and birds (QUINTELA; PINHEIRO, 2004).

According to Wang et al. (2010), the growth of pathogens in plum and pear fruits can be reduced with the use of neem extracts. The fungicidal effect of neem was also observed in pineapple (RIBEIRO et al., 2011), tomato (LIMA et al., 2011; OLADIMEJI et al., 2013), apple (LEITE et al., 2009) and orange (AL-SAMARRAI; SINGH; SYARHABIL, 2013). However, studies on the use of extracts of leaves and fruits of neem in the control of post-harvest diseases in Papaya Formosa Tainung-01 are scarce. The use of alternative substances to control post-harvest papaya diseases may reduce the use of fungicides, providing greater protection to the consumer's health, helping to preserve the environment and maintaining fruit quality for a longer post-harvest period.

The objective of the present work was to evaluate the use of extracts of leaves and fruits of neem (*Azadirachta indica* Juss) as coating for papaya Formosa fruits aiming to maintain their quality

during post-harvest storage.

## MATERIAL AND METHODS

Fruits of the papaya Formosa Tainung-01 with first signs of skin yellowing (maturation stage 1) were manually collected from an orchard in Quixeré, State of Ceará (CE), Brazil. These fruits were packed in plastic containers and transported to the Pilot Fruit and Vegetable Processing Plant of the Federal Institute of Education, Science and Technology of Ceará, Campus Limoeiro do Norte, Brazil. They were then selected, sanitized with active chlorine solution (100 ppm) and divided into five groups: control (TC=uncoated), coating with extracts of neem leaves (T1=5%, T2=10%) and coating with extracts of neem fruits (T3=5%, T4=10%).

Neem leaves with petioles were collected, distributed in trays and dehydrated in a forced-air circulation oven at 65° C for 48 h. Subsequently, the dried leaves were ground to obtain the powder with a mill (Willye, Star FT 50).

Samples of 50 and 100 g of powdered leaves were diluted in 1 L of water to each concentration (5 and 10%) to prepare the extracts used in the treatments T1 and T2. The resulting solutions were filtered with a cotton cloth to obtain only the aqueous extract, to which 1.5 mL of adhesive spreader (Activate<sup>®</sup>) was added to improve its fixation on the papaya fruit surface.

Green and mature fruits of neem were harvested manually to elaborate the extracts used in the treatments T3 and T4. These fruits were sanitized with active chlorine solution (5 ppm) and ground in an industrial blender (FAK<sup>®</sup>) to obtain a dough, which was filtrated through a cotton cloth to obtain the aqueous extract. Aliquots of 50 and 100 mL of this extract were diluted in water with 1.5 mL of adhesive spreader.

The coating process consisted of immersing the fruits (for one minute) in the aqueous extract of leaves or neem fruits, according to the different concentrations. The control fruits (TC) were immersed for one minute in water containing only the adhesive spreader. Afterwards, all fruits were subjected to natural drying, stored in expanded polystyrene trays and stored at mean temperature of 30.4 °C and relative humidity of 42% for 12 days.

The fruit weight loss (%) of each storage period (0, 3, 6, 9 and 12 days) were evaluated in a semi-analytical balance, considering the difference between the initial weight of the fruit. Fruit pulp firmness (N) was determined using a manual penetrometer (McCormick model FT 327) with an 8 mm diameter ferrule. Fruit external appearance was visually evaluated, using a subjective scale from 1 to 5 (LIMA et al., 2004), considering the absence or presence of defects, wilt and fungal attack symptoms. Soluble solids content (°Brix) was

determined in the filtered juice using a digital refractometer. Titratable acidity (% of malic acid) was evaluated by titration of 10 mL of the sample with NaOH solution (0.1 N) (IAL, 2008). Incidence of fungi (%) were determined by visual analysis, performed at each storage period, using the fungi percentage classification adopted by Azevedo (1998), with some adaptations. The identification of fungal species was performed considering the morphological characteristics of their reproductive structures, evaluated under an electronic microscope.

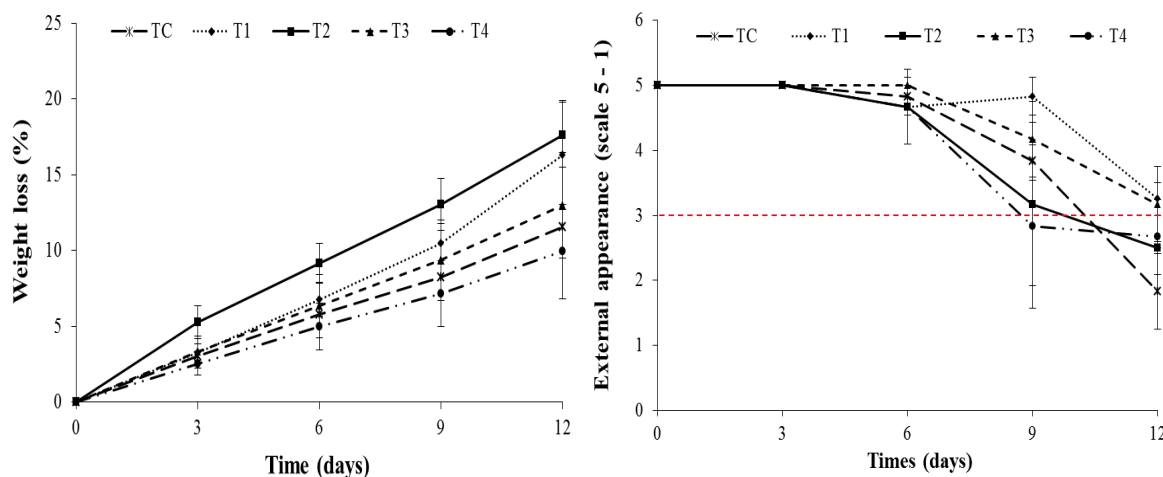
A completely randomized experimental design in a 5x5 factorial arrangement was used consisting of different types of coatings (TC = control, T1 = extract of the neem leaves at 5%, T2 = extract of the neem leaves at 10%, T3 = extract of neem fruits at 5% and T4 = extract of neem fruits at 10%) and storage period (0, 3, 6, 9 and 12 days), with 3 replicates of 2 fruits per plot, totaling 150 fruits. The results were evaluated by comparing the means of the treatments followed by the standard deviation (n = 3).

## RESULTS AND DISCUSSION

There was a gradual increase in the means of weight loss over the storage period, especially for TC fruits (Figure 1A). Fruits in T1 and T3 had the highest weight loss on the 12<sup>th</sup> day, with 16.32 and 17.64%, respectively. Fruits in T4 had a lower weight loss, reaching 9.94% at the end of the storage period. According to Chitarra and Chitarra (2005), 10% is the maximum acceptable weight loss for

fruits and vegetables to not compromise their appearance. The papaya fruits covered with extract of neem fruits at 10% had a weight loss of 9.94%, thus within this criterion. The positive effect of minimizing weight losses is the maintenance of a film on the fruit surface, which protects it against moisture loss. Neem extract also reduced the fresh weight loss in oranges after eight weeks of storage (AL-SAMARRAI; SINGH; SYARHABIL, 2013). On the other hand, Carnellosi et al. (2009) found increases in weight loss of papaya fruits when treated with essential oils of *Eucalyptus citriodora* L. and *Cymbopogon citratus* DC. *Staf* (lemon grass).

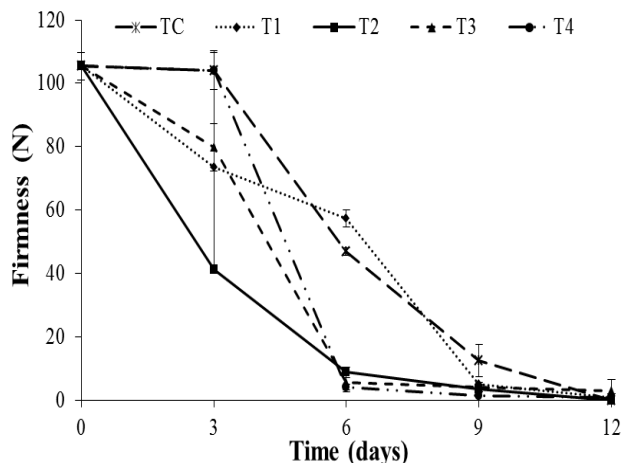
The external quality of the fruit decreased throughout the storage period, with mean grade results at the end of the experiment of 1.83 (TC), 3.25 (T1), 2.50 (T2), 3.17 (T3) and 2.67 (T4) (Figure 1B). The fruits covered with extracts of neem leaves and fruits at 5% (T1 and T3) presented the best external quality for the storage periods. According to Lima et al. (2012), the external appearance of Tommy-Atkins mangoes was compromised by direct application of fennel extract, with grades below the accepted limit after 12 days of storage in BOD chamber at 10±5 °C and relative humidity of 88±2%. The use of extracts on the fruit surface can promote a bright appearance, since some extracts contain essential oils; and form a protective barrier to the attack of microorganisms. On the other hand, the external fruit quality may reduce due to wilting caused by alterations in their physiological processes, leading to the wrinkle of the fruit due to weight and volume loss, depreciating its appearance and reducing its shelf life.



**Figure 1.** Fruit weight loss (A) and external appearance (B) of papaya Formosa Tainung-01 coated with extracts of neem leaves and fruits, stored at 30.4 °C and relative humidity of 42% for 12 days. TC = control, T1 = extract of the neem leaves at 5%, T2 = extract of the neem leaves at 10%, T3 = extract of neem fruits at 5% and T4 = extract of neem fruits at 10%.

The initial pulp firmness of the fruits was 105.37 N (Figure 2), which reduced especially from the 3<sup>rd</sup> day of storage. At this day, fruits in T2 (extract of neem leaves at 10%) had the lowest pulp firmness (41.7 N) compared with the control, which presented the highest pulp firmness. This result was probably because the extract affected the tissues of the fruits softening the fruit pulp. According to Lima et al. (2012), mangoes coated with fennel extracts (1.5%) also have lower firmness than the control;

and fruits coated with fennel (1.5%) plus cassava starch (3.0%) resulted in firmer fruits up to the 12<sup>th</sup> (mangoes harvested with good agricultural practices) and 6<sup>th</sup> day of storage (mangoes harvested without good agricultural practices). Martins et al. (2005) found similar results regarding the decrease of fruit pulp firmness during storage in papaya Golden applying extracts of fennel and hydrothermal treatments.



**Figure 2.** Fruit pulp firmness of papaya Formosa Tainung-01 coated with extracts of neem leaves and fruits, stored at 30.4 °C and relative humidity of 42% for 12 days. TC = control, T1 = extract of the neem leaves at 5%, T2 = extract of the neem leaves at 10%, T3 = extract of neem fruits at 5% and T4 = extract of neem fruits at 10%.

The titratable acidity of the fruits evaluated had a small oscillation (Figure 3A), with the initial malic acid of 0.10% increasing in all treatments, especially from the 3<sup>rd</sup> day of storage, with a subsequent decrease on the 9<sup>th</sup> day of storage, since papaya is a fruit that does not present uniformity during the maturation process. According to Costa and Balbino (2002) this increase in pulp titratable acidity is probably due to the formation of galacturonic acid, as a consequence of pectin hydrolysis by the enzymes pectinamethylsterase and polygalacturonase. The acidity increases with fruit ripening until 75% of yellow skin surface; Then, the acidity decreases, except inside the mesocarp, where titratable acidity increases until the fruit reaches full yellowing.

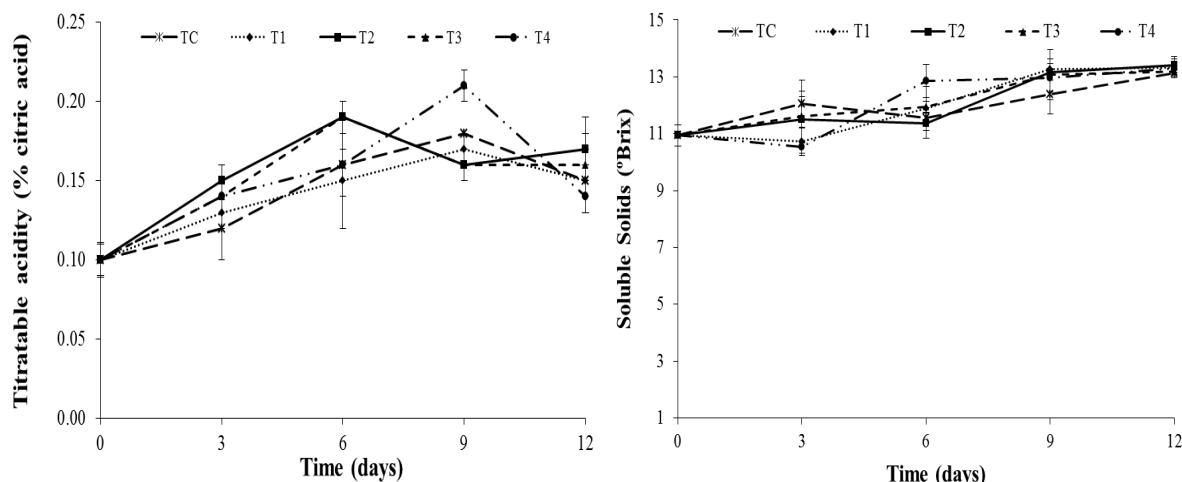
The fruits had a small increase in soluble solids contents throughout the storage period (Figure 3B). The soluble solids decrease in fruits in T1 and T4 by the 3<sup>rd</sup> day of storage, presenting contents of 10.72 and 10.53 °Brix, respectively.

Fruits in T2 had the highest soluble solids content at the end of the storage period, probably due to their faster ripening, and loss of moisture, which were also responsible for their lower pulp firmness (Figure 2). The soluble solids found in the present study are higher than those found by Souza et al. (2014) for Papaya Formosa Tainung-01 coated with

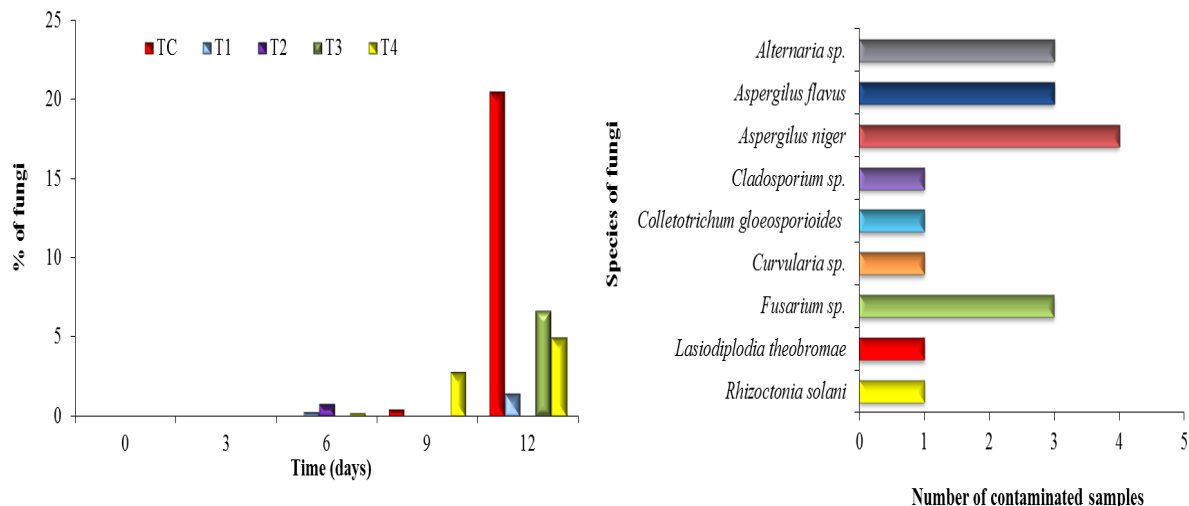
cassava starch. The highest concentration extract of neem leaves (10%) may have accelerated some metabolic reactions that occur during maturation, since this is an organic compound. The soluble solids contents found are close to those found in papaya Formosa coated with an edible cassava starch-based coating (PEREIRA et al., 2006).

Growth of fungi occurred from the 6<sup>th</sup> day of storage (Figure 4A). At this day, fruits in T2 had 0.83% of incidence, with later absence of fungi development. Fruits coated with the extracts presented reductions in the development of fungi compared with fruits in the control treatment, which presented 20.5% of incidence fungi at the end of the experiment. The fungi species with higher incidence in the evaluated samples were *Alternaria* sp., *Aspergillus flavus*, *Aspergillus niger* and *Fusarium* sp. (Figure 4B).

Lima et al. (2011) evaluated the efficiency of plant extracts in the control of nymphs of *Bemisia tabaci* biotype B and found that the use of neem oil was one of the most efficient. Oladimeji et al. (2013) evaluated the control of *Fusarium verticilloides* (Saccardo), with aqueous extracts of *Azadirachta indica* and *Vernonia amygdalina*, and found that both extracts can be used for preservation of tomato fruits, prolonging their shelf life.



**Figure 3.** Titratable acidity (A) and soluble solids (B) of papaya Formosa Tainung-01 coated with extracts of neem leaves and fruits, stored at 30.4 °C and relative humidity of 42% for 12 days. TC = control, T1 = extract of the neem leaves at 5%, T2 = extract of the neem leaves at 10%, T3 = extract of neem fruits at 5% and T4 = extract of neem fruits at 10%.



**Figure 4.** Percentage of fungi (A) and identification of fungus species (B) in papaya Formosa Tainung-01 coated with extracts of neem leaves and fruits, stored at 30.4 °C and relative humidity of 42% for 12 days. TC = control, T1 = extract of the neem leaves at 5%, T2 = extract of the neem leaves at 10%, T3 = extract of neem fruits at 5% and T4 = extract of neem fruits at 10%.

The growth of pathogens *Monilinia fructicola*, *Penicillium expansum*, *Trichothecium roseum* and *Alternaria alternata* in plum and pear fruits can also be reduced with the use of neem extracts (WANG et al., 2010). Tests involving extracts and residues of neem leaves showed inhibition of vegetative growth in many pathogens of the genus *Fusarium* (*F. oxysporum* f. sp. *ciceri* (Fock.), *F. oxysporum* f. sp. *Cepae* (Hanzawa.), *F. oxysporum* (Snyder & Hansen), *F. solani* Mart.) and from other fungi genera (*Aspergillus*, *Sclerotinia*, *Pyricularia*, *Rhizoctonia*, *Penicillium*). Some reports confirm the antifungal potential of this plant, denoting its efficiency in the control of diseases, especially those caused by fungi (MOSSINI; KEMMELMEIER, 2005). On the other hand, Venturoso et al. (2011) evaluated the antifungal activity of extracts on the development of

phytopathogens and found that the extract of neem favored the growth of *Fusarium solani*, confirming the results found in the present work.

### CONCLUSIONS

The use of extracts of neem leaves or fruits at 5% presented the best results on maintaining the external quality, and controlling the growth of phytopathogens in papaya fruits at post-harvest.

Based on the external appearance, papaya fruits treated with extracts of neem leaves or fruits at 5% had a shelf life of 12 days, while fruits treated with extracts of neem leaves and fruits at 10% had a shelf life of 9 days. The use of neem extracts reduced the development of fungi during the storage period compared with the control. The fungi species with

the highest incidence in the samples evaluated were *Alternaria* sp., *Aspergillus flavus*, *Aspergillus niger* and *Fusarium* sp.

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