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TOTAL PHENOLIC CONTENT IN ′SUPER HADEN′ MANGO FRUITS DAMAGED BY ANTHRACNOSE AND TREATED IN POSTHARVEST

Contenido de fenoles totales en frutos de mango ′Super Haden′ dañados por antracnosis y tratados en poscosecha

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ABSTRACT. Total phenolic content in the exocarp of fruits is related to their own defense mechanisms against fungal pathogen attack. *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. causes anthracnose in mango (*Mangifera indica* L.) thereby high postharvest losses. The aim of this study was to evaluate the total phenolic content in damaged and postharvest treated 'Super Haden' mango fruits, to make evident its relationship with the defense mechanisms against pathogen infection. Exocarp samples were taken from healthy and anthracnose-damaged (mild, moderate and severe levels) fruits as well as from those postharvest treated with hot water (53 °C for 5 min), polyethylene wax (10 % ST) plus imazalil (800 mg L^{-1}), polyethylene wax (10 % ST) plus imazalil (800 mg L^{-1}) plus two bags of Conserver 21 and a control without application. Samples were lyophilized and the total phenolic content was determined by the method suggested by Slinkard and Singleton (1977) with some modification. Anthracnose-affected fruits showed a lower total phenolic content: 59,25 at the mild degree, 58,63 at the moderate and 56,52 mg of galic acid g^{-1} fresh weight at the severe level, showing significant differences between damage levels and health fruits. Postharvest treatments increased total phenolic content. Hot water and the combination of polyethylene wax plus imazalil showed values of 37,58 and 37,11 mg of galic acid g^{-1} fresh weight respectively, compared with the control value of 33,94 mg galic acid g^{-1} fresh weight, showing a correspondence with a lower disease occurrence.

Key words: anthracnose, exocarp, phenol, mango

RESUMEN. El contenido de fenoles totales en el exocarpio de los frutos tiene relación con los mecanismos de defensa propios del fruto frente al ataque por patógenos fúngicos. *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. es causante de la antracnosis en el mango (*Mangifera indica* L.) y ocasiona elevadas pérdidas en poscosecha. El objetivo de este trabajo fue evaluar el contenido de fenoles totales en frutos de mango 'Super Haden' dañados y tratados en poscosecha y evidenciar su relación con los mecanismos de defensa frente a la infección por patógenos. Se tomaron muestras de exocarpios de frutos sanos y con niveles de daños ligeros, moderados y severos por la antracnosis, así como en frutos tratados en poscosecha con agua caliente (53 ºC por 5 min), cera polietilénica (10 % ST) más imazalil (800 mg L^{-1}), cera polietilénica (10 % ST) más imazalil (800 mg L^{-1}) más dos bolsas de Conserver 21 y un testigo sin aplicación. Las muestras se liofilizaron y se determinó el contenido de fenoles totales por el método sugerido por Slinkard y Singleton (1977) con modificaciones. Los frutos afectados por la antracnosis presentaron menores contenidos de fenoles totales en el grado ligero fue de 59,25, en el moderado de 58,63 y en el severo 56,52 mg de ácido gálico g^{-1} de masa fresca, mostrándose diferencias significativas entre los grados y frutos sanos. Los tratamientos poscosecha incrementaron el contenido de fenoles totales. El agua caliente y la combinación de cera polietilénica con imazalil mostró valores de 37,58 y 37,11 mg de ácido gálico g^{-1} respectivamente, en comparación con el testigo que fue 33,94 mg de ácido gálico g^{-1}, existiendo una correspondencia con la disminución de la incidencia de la enfermedad.

Palabras clave: antracnosis, exocarpio, fenol, mango

INTRODUCTION

Phenolic compounds are secondary metabolites characterized by being formed by phenol -aromatic rings units attached to one or more hydroxyl groups. These biomolecules are located in all parts of the plants and its concentration varies throughout the

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growing season; also involved in various functions, such as nutrient uptake, protein synthesis, enzyme activity, photosynthesis, formation of compounds and the defense against biotic and abiotic factors (1).

Defense mechanisms activated in plants include the rapid production of reactive oxygen species (ROS), alterations in the constitution of the cell wall, accumulation of secondary metabolites such as phytoalexins, activation and peptides synthesis and defense proteins, among others (2, 3).

Among the most important phenolic compounds, phenolic acids, flavonoids, tannins and other less common compounds such as stilbenes and lignans are distinguished (4, 5).

The evolution of phenolic compounds during the stage of post-harvest or ripening in fruits, has been a topic of interest to researchers because they can be involved in the defense mechanisms of the fruits to infection from certain fungi (6, 7).

In absence of damage or pathogen attack, defense mechanisms of the fruit can be induced by physical or chemical elicitors (8). Physical elicitors include high and low temperatures, the gamma and ultraviolet radiation (9, 10, 11). Among the chemical products are as chitosan, benzothiadiazole (BTH), harpin and 1-methylciclopropane (12, 13, 14, 15) and others such as oxalic acid and calcium chloride (16).

The *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc fungus is considered very polymorphous and polyphagous, by the way they cause infection in fruits, classified as a fungus of latent infection, it produces enzymes (polygalacturonase and pectatelyase) that degrade the cell wall and causes the disease known as anthracnose in Mango (*Mangifera indica* L.) (17, 18, 19).

The incidence and severity of damage anthracnose in mango fruits affect their commercial quality and cause high losses during storage and marketing (20). Postharvest control is effected by the (hydrothermal) and physicochemical methods or a combination of both (21).

In Cuba, the mango is a one of most produced fruit, representing more than 30 % of the total production of fruits[A] and there is great interest in increasing its export as fresh fruit, with the cultivar 'Super Haden' typical of the country and more distributed in all production regions, but losses during postharvest are high, because among other reasons, the incidence of anthracnose. However, there are few studies related to the latent infection process of the fungus and disease development during the ripening of fruits and their resistance induction mechanisms to the application of treatments for postharvest control anthracnose. The aim of this study was to determine total phenols in 'Super Haden' mango fruit with varying degrees of severity for anthracnose and after post-harvest treated.

MATERIALS AND METHODS

The fruits of 'Super Haden' mango (*Mangifera indica* L.) were collected with physiological maturity of a plantation located in the Scientific and Technological Base Unit Alquízar (UCTB, Alquízar), Artemisa province, located in 32' N 82 ° and 22 ° 47' O, 11 m above middle sea level, on a Ferrasol eutric soil with flat topography slope 0 (22).

Determination of total phenols in the exocarp of the damaged fruits by anthracnose

Symptoms of anthracnose were identified as Ploetz (23) and damage to the fruit exocarp were determined by arbitrary rating scale according Mulkay *et al*. (24). The results were expressed in the percentage of fruit damage (Table).

Total phenols in the exocarp for classified fruit, according to arbitrary scale, were determined by the colorimetric Folin Ciocalteu (25) with slight modifications. Three samples (one fruit per sample and classification) were collected and lyophilized for further analysis. The resulting extracts were analyzed by spectrophotometry. Absorbance was measured with a Shimadzu UV-180 at a wavelength of 765 nm and total phenolics were calculated based on the slope of the linear portion of the gallic acid curve pattern and expressed as mg of gallic acid g^{-1} of dry mass.

[A] MINAGRI. *Reporte Estadístico*. La Habana, Cuba, 2010.

Arbitrary rating scale of damage due to anthracnose in the mango 'Super Haden fruit exocarp

Classification	Description
Sane	Without damages in the exocarp
Mild	Less than 10% of the affected exocarp surface the and spots smaller than 1 cm in diameter
Moderate	Less than 10% of the affected exocarp surface and stains greater than 1 cm diameter
Severe	More than 10% of the affected exocarp surface and stains greater and smaller than 1 cm in diameter

Determination of total phenols in the exocarp of the fruit treated postharvest

The fruits were washed with Tropicleaner® detergent 0,01 %, and rinsed and subjected to the following treatments:

- Immersion in hot water 53 °C x 5 min (AC)
- Sprinkling with FECUNDAL mixture 50 % (active ingredient imazalil 50 % W/V) at the concentration of 800 mg L^{-1} plus polyethylenic wax (10 % total solids) (I+C).
- Sprinkling with FECUNDAL mixture 50 % (active ingredient imazalil 50 % W/V) at the concentration of 800 mg L^{-1} plus polyethylenic wax (10 % total solids) + two bags of Conserver 21 within the packing (I+C C21)
- Control without application (T)
- Subsequently, the fruits were dry, they were packed in corrugated cardboard boxes of 4 kg and stored for 15 days at 14±1 °C and 85-90 % RH

At the end of the cold storage were determined:

- The incidence of anthracnose by the percentage of affected fruits
- The effectiveness of treatments by Abbot's formula (26)
- Total phenols as described above, three samples of healthy exocarps fruits per treatment (one fruit per sample) were taken and lyophilized for further analysis.

Experimental design

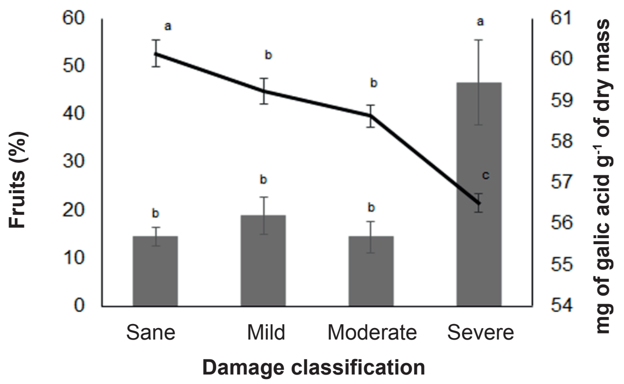
A completely randomized experimental design was used. For each assessment of damages by anthracnose and treatments three repetitions (30 fruits x repetition) were performed. The statistical processing of the results was performed using a simple classification ANOVA. Means were compared by Tukey test (p≤0,05).

RESULTS AND DISCUSSION

Determination of total phenols in the exocarp of fruits damaged by anthracnose

Mango fruits were classified as healthy, with mild, moderate and severe damage anthracnose. On exocarp round spots, sagging, dark brown to black, accompanied by a mass salmon, as a result of the acervulus fungus maturation (23) were developed. The percentage of fruits with severe damage was more significant than the mild, moderate and healthy damage. In analyzing the content of total phenols in fruits with severe damage, there were minor differences between the amounts with mild and moderate damage and these in turn with healthy (Figure 1).

Those related to the high percentage of mango fruits with severe damage by anthracnose results could be due to the presence of favorable conditions for the development of the disease, such as temperatures between 20 and 30 °C for 12 hours and relative humidity over 95 % conditions, plus the existence of abundant source of inoculum in the plantation, favoring latent infections naturally fungus, *C. gloeosporioides* (27, 28).



Error bars represent ± standard deviation

Different letters indicate significant differences by Tukey test (p≤0,05)

Figure 1. Percentage of 'Super Haden' mango fruits and amount of total phenols, as rated by anthracnose damage after 15 days at 14±1 °C and 85-90 % HR

The decrease of total phenols in the exocarp of mango fruit with severe damage could be due to the higher percentage of affected exocarp area as a result of the fungus latent infection, besides the presence of a greater number of spots of size greater and less than 1 cm in diameter, indicating the status of the disease development , very different from that observed in the fruits with mild, moderate and healthy damage, where the amounts of phenols were higher.

The mango fruits present high content of phenolic compounds in the exocarp. The higher levels of these compounds are detected in juvenile stages of fruit development, resulting in a significant decrease during ripening.

Phenolic compounds are only induced when stressors are present. The biosynthesis of phytoalexins usually occurs in localized areas around the entrance area of the organism, being absent or only present in small amounts in healthy tissues (29).

Some authors report that changes in the content of phenols may be associated with the pathogen infection process and the characteristics of the defense materials as phytoanticipine, recently reported (5).

When the cell tissue is healthy and intact, the enzyme polyphenol oxidase (PPO) and their substrates, the phenols are in separate compartments (chloroplasts and vacuoles, respectively). However, when the cell is disrupted as a result of the infectious process by anthracnose (23), enzymes and substrates are contacted and the oxidation reaction of phenolic compounds happens, catalyzed by the enzyme PPO, causing quinones formation, which are polymerized and form black, brown and brown, pigments akin with symptoms of anthracnose.

Determination of total phenols in the treated fruit exocarp in postharvest

The incidence of anthracnose in mango fruits, treated postharvest decreased significantly; witnesses in the disease affected 66,66 %, so the effectiveness of the treatments was higher than 46,87 %, with no statistical differences (Figure 2).

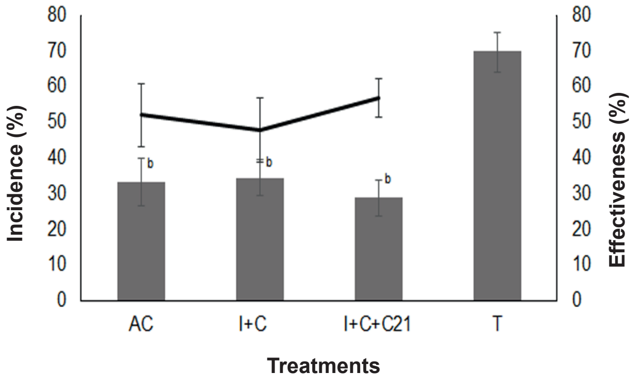
The effectiveness of hot water and imazalil fungicide for control of anthracnose in mango fruit, coincides with the point made by various authors regarding the effect of these on the quality and shelf life of different fruits[B] (30, 31, 32).

The three treatments caused a significant increase in phenol content, whereas in the control was 33,93 mg gallic acid g^{-1} of dry mass. The hydrothermal and the fungicide combination plus wax showed higher amounts of phenols, with statistical differences to the combination of the fungicide, wax and Conserver 21 (Figure 3).

The phenol increase in the exocarp of mango fruit with water at 53 °C x 5 min, are similar to those obtained by Talcott *et al*. (33). Different studies indicate the possible mechanisms of the effect of heat treatment on the induction of disease resistance and post-harvest fruit quality (34, 35, 36).

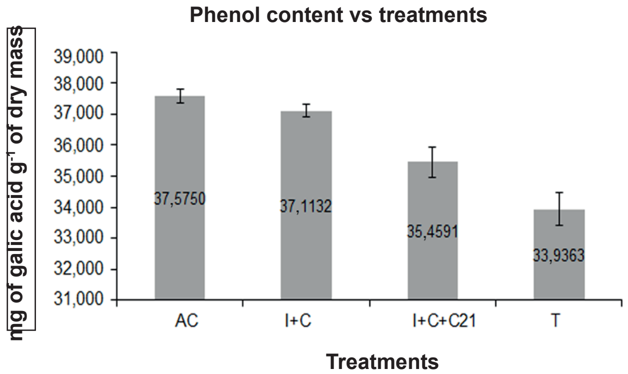
A primary mode of action of this treatment is the disinfection of fruit, being removed or destroyed spores or fungal mycelium (37).

In papaya fruits, immersed in water at 54 °C x 4 min, there was a reduction in the developmental stages of *C. gloeosporioides*, with a significant decrease in the incidence of anthracnose, fruit natural wax melted and cracks and stomata were covered and sealed by providing a mechanical barrier against pathogen attack; This treatment also induced expression of protein polygalacturonase (38), which can inhibit fungal endopolygalacturonase, considered as an important plant resistance factor to phytopathogenic fungi.



Error bars represent ± standard deviation
Different letters indicate significant differences by Tukey test (p≤0,05)

Figure 2. Incidence of anthracnose in 'Super Haden' mango fruit treated with hot water (AC) Imazalil+wax (I+C), Imazalil+wax+Conserver 21 (I+C+C21 and control fruits (T) and its effectiveness after 15 days at 14±1 °C and 85-90 % HR



Error bars represent ± standard deviation
Different letters indicate significant differences by Tukey test (p≤0,05)

Figure 3. Total Phenols in the exocarp of healthy 'Super Haden' mango fruit treated with hot water (AC) Imazalil+wax (I+C), Imazalil+wax+Conserver 21 (I+C+C21) after 15 days to 14±1 °C and 85-90 % HR

[B] Brecht, J.; Sargent, S.; Kader, A.; Mitcham, E.; Maul, F.; Brecht, P. y Menocal, O. *Manual práctico para el mejoramiento poscosecha del mango*. Inst. Copyright National Mango Board, Orlando, Florida, 2010, pp. 5-67.

Also, the application of hydrothermal treatment at 53 °C x 3 min in melon fruits (*Cucumis melo* L.) reduced rots caused by different fungi, there was increased activity of the hydroxylase cinnamata-4-amonoliasa phenylalanine enzymes, coumarata, CoA ligase, polifenoxidase and peroxidase, related to the defense mechanisms and increase the antifungal compounds and phenolic compound levels (39).

On the other hand, the Imazalil is a fungicide derived from imidazoles. It belongs to the group of inhibitors of the biosynthesis of ergosterol precursors (IBE), which inhibit enzyme-dependent of the cytochrome P-450, responsible for the demethylation of ergosterol. Acts directly on the pathogen, cell permeability affects the fungus and lipid biosynthesis. It causes antisporulant activity, reducing spore germination and inflammation distortion of germtubes and cytoplasm loss of cells germinated. It has demonstrated the sensitivity of various isolates of *Colletotrichum* spp. to this fungicide (40, 41).

The application of imazalil +wax and imazalil +wax + Conserver 21, (the latter is an absorber product of ethylene), could change the atmosphere surrounding the fruit, with the consequent reduction of respiration and ethylene production and the delay in ripening (42, 43, 44), which also results in a delay in the activity of different enzymes involved in biosynthetic pathways of phenolic compounds (45), such as polyphenol oxidase or peroxidase, primarily responsible degradation of these compounds (46).

The high content of total phenols in the exocarp of 'Super Haden' mango fruit treated postharvest, are in correspondence with the low incidence of anthracnose and effectiveness of these in its control. These results constitute a contribution to knowledge on the development and severity of anthracnose in 'Super Haden' mango fruits and effectiveness of post-harvest treatments to control, being important to determine other enzymes and related processes fruits and the disease manifestation.

CONCLUSIONS

- The amounts of total phenols were lower in the 'Super Haden' mango fruit severely damaged by anthracnose and higher in mild, moderate and healthy damage after 15 days of storage.
- The 'Super Haden' mango fruit, treated with hot water at 53 °C x 5 min, Imazalil 800 mg L^{-1} + Polyethylene wax (10 %) and Imazalil (50 %) 800 mg L^{-1}+ Polyethylene wax (10 %) + Conserver 21, induced higher amounts of total phenols in the exocarp after 15 days of storage

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