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Phytopathologia Mediterranea (2016) 55, 2, 276–284
DOI: 10.14601/Phytopathol_Mediterr-15214

SHORT NOTE

Cassava starch coatings for postharvest control of papaya anthracnose

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Summary. The effects of coating papaya fruit (*Carica papaya*) with cassava starch were studied to determine the best concentration and mode of action of this material in postharvest control of anthracnose. The concentrations of starch tested were 1%, 2%, 3% and 4%. These were prepared to give gel consistency. Surface sterilized papaya fruits were inoculated with conidia of *Colletotrichum gloeosporioides* and incubated for 48 h in a moisture chamber. The fruits were then treated with cassava gels and dried. During the following 14 d storage period, fruit maturation and anthracnose on the fruits were assessed, and electron microscopy was used to examine fruit epicarps. All cassava starch coating concentrations reduced fruit maturation and anthracnose, with the 2%, 3% and 4% coatings giving 100% disease control. The 2% starch coating is likely to be optimum, considering the lower cost efficiency of disease control. The mechanism of disease control provided by the coating is likely to be related to delay of ripening and the formation of a protective layer over the fruit.

Key words: *Colletotrichum gloeosporioides*, cassava biopolymer, alternative disease control.

Introduction

Anthracnose is a major limiting factor to papaya (*Carica papaya*) production worldwide (Rampersad, 2011). Control of this disease has typically relied on synthetic fungicides. Due to 'side-effects' of fungicides on humans and the environment, alternatives alone or in combinations are required for integrated disease management strategies. Wax combined with fungicides, heat treatments and fruit irradiation are currently used for anthracnose control. Antagonistic microorganisms and natural compounds [e.g. chitosan or plant derivatives (extracts, essential oils, isothiocynates)], could improve the control of anthracnose in papaya (Bautista-Baños *et al.*, 2013).

Polysaccharide-based coatings (PBC) have been widely explored for protection of fresh fruits and vegetables against moisture loss and to reduce respiration rates propitiating brightness and attractive product appearance (Azeredo, 2003). According to Luvielmo (2012), the most commonly used polysaccharides for preparation of edible fruit protection are: cassava starch, sodium alginate, pectin, carrageenan, chitosan and cellulose derivatives (methylcellulose, carboxymethyl cellulose and hydroxypropyl methylcellulose).

Starch coatings is an alternative for post-harvest conservation of fresh marketed and consumed fruits. Some authors have reported the effectiveness of biodegradable coatings based on cassava starch for postharvest conservation of strawberry (Cereda *et al.*, 1992), guavas (Oliveira and Cereda, 1999), tomato (*Lycopersicon esculentum* Mill.) (Damasceno *et al.*, 2003), acerola (Maciel *et al.*, 2004), Japanese cucumber (*Cucumis sativus* L.) (Reis *et al.*, 2006) and, papaya (Pereira *et al.*, 2006). Pereira *et al.* (2006) demonstrated that PBC could increase the storage life of

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ISSN (print): 0031-9465
ISSN (online): 1593-2095
www.fupress.com/pm
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papaya. However, reports of studies conducted with PBC aimed at controlling postharvest disease in papaya fruit cannot be found.

The aim of the present study were to evaluate cassava starch fruit coatings for postharvest control of anthracnose in papaya.

Materials and methods

This study was carried out at the Laboratory for Electron Microscopy and Ultrastructural Analysis (LME), Plant Pathology Department (DFP), Federal University of Lavras (UFLA), Brazil.

Homogenization of cassava starch was performed at the Laboratory of Grains, Roots and Tubers, Department of Food Science (DCA) at UFLA.

Preparation of cassava starch

Two kg of white cassava starch with clear appearance, produced by farmers in Porto Seguro, BA, Brazil, was placed in a homogenizer stirrer (TE-200/10 Tecnal Brazil) at 30 rpm for 15 min. An assay was conducted using a set of five sieves, with respective mesh size categories of 40, 60, 80, 100 and 140 mesh (Brazilian Association of Technical Rules), equivalent to, respectively, 0.42, 0.25, 0.177, 0.149 and 0.105 mm diameter mesh apertures.

Sample of 100 g taken from the homogenizer were placed on the top of the sieve assembly, arranged one above the other in increasing order of mesh size, with a collection tray at the bottom. The assay was performed during 15 min at maximum speed in a vibrator (ELKA Brazil). Fractions of the samples deposited on each sieve over the tray were collected and weighed using an analytical balance. This process was repeated in three trials. The percentage of material in each range of particle size was obtained from average amounts deposited.

Preparation of papaya fruits for coating

Fruits from papaya cultivar 'Sunrise Solo', in stage 2 of maturation (Ministério da Integração Nacional, 2000) from Alvorada Farm, São Mateus, ES, Brazil, were purchased through a fruit and vegetable dealer in Lavras, MG, Brazil. Fruits without disease symptoms and mechanical injuries were selected, washed in water with detergent, surface sterilized with sodium hypochlorite (2% NaOCl solution) for 3 min, rinsed with distilled water and then dried on paper towels. Average fruit weight was 623.5 g and average length (apex to base) was 14.5 cm.

Inoculum preparation and fruit inoculation

Colletotrichum gloeosporioides isolate CML 2339, supplied by Lavras Mycological Collection, Department of Plant Pathology, UFLA, was used as inoculum. The fungus was grown for eight days in potato dextrose agar plate, and 25 mL of sterile distilled water and glass beads were added to each plate. The plate was then shaken until the colony was disrupted, and the resulting inoculum suspension was filtered through gauze to obtain conidia and hyphal fragments. The inoculum suspension was assessed using a Neubauer chamber, and the concentration was adjusted to 2.0×10^5 conidia mL^{-1}. Three drops of Tween 20 were added as a spreader-sticker.

The papaya fruits at ripe stage 2 (up to 25% of fruit surface yellow) were each inoculated by making five holes (4 mm deep) together (Gomes *et al.*, 2012) with a histological needle (1 mm diam.) in three distinct regions of the fruit surface. A micropipette was used to dispense 15 µL of the inoculum on each of the three regions. Inoculated fruits were then placed in a moist chamber for 48 h.

Starch-based gel preparation and papaya fruit coating

Suspensions of cassava starch containing 1%, 2%, 3% and 4% (weight volume^{-1}) were heated at 80°C in a microwave oven, and shaken every 10 s to obtain coating-forming gels without granules. The total gelatinization time in the oven ranged from 1 to 2 min for 300 ml of the starch slurries.

The fruit coating was carried out by adding the gel at 25°C in a bath for 1 min, removing excess starch solution and then drying for 3 min, this process was repeated three times for each of the following treatments:

a) fruits coated with cassava starch gel at 1.0% concentration;
b) fruits coated with cassava starch gel at 2.0% concentration;
c) fruits coated with cassava starch gel at 3.0% concentration;
d) fruits coated with cassava starch gel at 4.0% concentration;

e) fruits not coated with cassava starch gel (experimental control).

After coating, the fruits were stored on a laboratory bench with environment ambient temperature and humidity to simulate natural shelf conditions of the Brazilian markets. An assessment of the coating uniformity on coated fruits was carried out, using 2% iodine tincture to detect the starch presence on the fruit surfaces.

Experimental design and statistical analyses

The experiment was of completely randomized design, where treatments and fruits were numbered and randomly distributed. There were five treatments (the different starch coating concentrations above), with three replicates each containing a total of 15 papaya fruits.

At the fourth day after inoculation, and for a further 10 d, the numbers of anthracnose lesions on the fruits were counted, to evaluate disease incidence.

SISVAR software (Ferreira, 2011) was used for statistical analyses of data collected, which were subjected to analysis of variance and means were compared using the Tukey test ($P<0.05$).

Analysis of cassava starch coatings with scanning electron microscopy

Fourteen days after inoculation, pieces of fruit epicarp measuring 2 × 2 cm were taken each with a flamed scalpel for analysis with a stereo electron microscope (SEM). These pieces were immersed for 1 week in fixing solution (2.5% glutaraldehyde, 2.5% paraformaldehyde in cacodylate buffer 0.05 M, pH 7.2 and 1% of $CaCl_2$ 0.1 M) in microcentrifuge tubes. The specimens were then each washed three times with cacodylate buffer for 10 min, and were then post-fixed in 1% osmium tetroxide in water for 1 h at room temperature in a fume hood. The samples were then washed three times in distilled water and then dehydrated in acetone gradient series of 25, 50, 75, 90 (one wash each) and 100% (three washes). The specimens were then placed in micropore capsules in a Petri dish containing acetone 100% and critical point dried in liquid CO_2, using Balzers Device CPD 030. The tissue pieces were then mounted on aluminum stubs using double-sided tape for sample adhesion. The epicarp surfaces of each specimen were placed uppermost for surface observation. The stubs were then gold-coated in an evaporator (Balzers SCD 050), and then observed in SEM (LEO EVO 40). Captured SEM images were edited using the software Corel Draw 12.

Results and discussion

Particle size samples of cassava starch

Samples of cassava starch used for gel preparation and subsequent coating formulations contained 48.3% of granules of 0.42 mm diam. retained on sieve 40; 11% of 0.25 mm diam. retained on sieve 60; 12% of 0.177 mm diam. retained on sieve 80, 12.3 % of 0.149 mm diam. retained on the sieve 100, 11% of 0.105 mm retained on sieve 140 and 5.4% smaller than 0.105 mm in diameter. Within this range of particle sizes, the time for water absorption and to form the gels was between 1 and 2 min in 300 mL preparations at 80°C.

Lesion incidence on papaya fruits

The first lesion was observed 4 d after inoculation for fruit without starch coatings (control treatment), and the lesion featured 0.21 cm in diameter. The lesion was round and depressed. At 6 d after inoculation, the first anthracnose symptoms were observed on starch coated fruits treated with the 1% starch coating. At that stage the non-coated control treatment had a mean of 14.8% incidence, whereas the fruits coated with 2%, 3% and 4% starch had no lesions (Table 1).

Disease incidence in the control treatment continued to increase until 12 d after inoculation, averaging 22.2% of anthracnose, whereas fruits coated with 1% starch stabilized at 9 d after inoculation at incidence of 7.4% (Table 1). Although the 1% treatment did not fully suppress anthracnose, it gave better control of the disease than for the uncoated fruits. No anthracnose developed on the fruits treated with the 2%, 3% or 4% starch coatings, and these treatments gave better control of the disease than the 1% coating and the nil coated control.

Interference of fruit ripening processes by the starch coatings could explain the efficient control of anthracnose in fruits coated with starch at 2%, 3% and 4% (Figure 1). Under these conditions, the fruits could remain resistant for longer periods because of phenolic compounds decrease during ripening (Chitarra and Chitarra, 2005), and these compounds are

Table 1. Mean proportions of incidence of anthracnose lesions on papaya fruits inoculated with *Colletotrichum gloeosporioides* (Control) or coated with different cassava starch concentration gel treatments, during evaluations performed over 11 d post-inoculation.

Treatment	Incidence (%)/day											
	4	5	6	7	8	9	10	11	12	13	14	Total
Control	3.7	-	11.1	3.7	-	-	-	-	3.7	-	-	22.2 a[a]
Coating 1%	-	-	3.7	-	-	3.7	-	-	-	-	-	7.4 b
Coating 2%	-	-	-	-	-	-	-	-	-	-	-	-
Coating 3%	-	-	-	-	-	-	-	-	-	-	-	-
Coating 4%	-	-	-	-	-	-	-	-	-	-	-	-

[a] Letters indicate difference ($P<0.05$) between control fruits (uncoated) and those coated with 1% cassava starch.

generally associated to resistance to plant pathogens (Rocha *et al*., 2011). After pathogen inoculation, *C. gloeosporiodes* probably remained quiescent without activation of pathogenicity factors, and did not compromise visual health of the fruit. According to Prusky (1996), for the decomposition processes in fruits and vegetables caused by pathogenic fungi, pathogenicity factors will be enabled to damage host tissues to release the necessary nutrients to maintain pathogen development. In parallel a pathogen must overcome host defenses to successfully invade host tissues.

Considering ripening stage observed after 48 h in humid chamber, all inoculated fruits were to ripening stage 3. At this point the fruits were coated and had their postharvest storage prolonged probably due to delays in ripening.

At the end of the experiment, the fruits coated with 3% and 4% starch progressed to ripening stage 4 [papaya fruits with 51–75% yellow surface (Brasil, 2000)], and they remained at this stage until the end (day 14). Those treated with 2% starch reached stage 5 (76 to 100% of yellow surface) whereas fruits coated with 1% starch showed some signs of senescence. Uncoated control fruits were completely ripe with tissue death, and were inappropriate for marketing (Figure 1).

Treatments with starch coating at 1% mature increased due to weak gel adhesion forming a non-uniform coating over the fruit. The texture consistency increased proportionally with the greater concentration of starch, but all coated fruits had reduced ripening compared with uncoated fruits. This was probably because the gel coat application partially fill the stomata on the fruit surfaces, reducing moisture loss (transpiration) and gas exchange (respiration) (Assis *et al*., 2009). However, at 12 d after coating, the fruits treated with with 3% or 4% starch coatings were less ripe (Figure 1) than those treated with with 1% or 2% coatings, which showed a degree of shrinkage (Figure 2), especially at the fruit apices and bases. These results indicate that a higher concentration of starch in the coating leads to more efficient ripening control, although the 3% and 4% coatings both gave similar results.

These results corroborate with those of Castricini *et al*. (2010) using the basic formulas of cassava starch at 1%, 3% or 5% concentrations for in "Golden" papaya fruits. They concluded that concentrations of 3% and 5% could provide better results regarding rates of fruit ripening. They also showed that these concentrations of cassava starch could reduce fruit weightloss.

At the end of the experiment (12 days after the coating), fruits were cut horizontally linearly and the pulp hardness of the coated were assessed as compared to the uncoated control fruits (Figure 3). These observations indicated that coating with cassava starch at more than 2% concentration promoted tolerance of the fruits to pathogen infection, as indicated by lack of injuries.

Pereira *et al*. (2006) evaluated the ripening of papaya fruits coated with edible cassava starch at room temperature, and showed that coatings at 1% and 3%

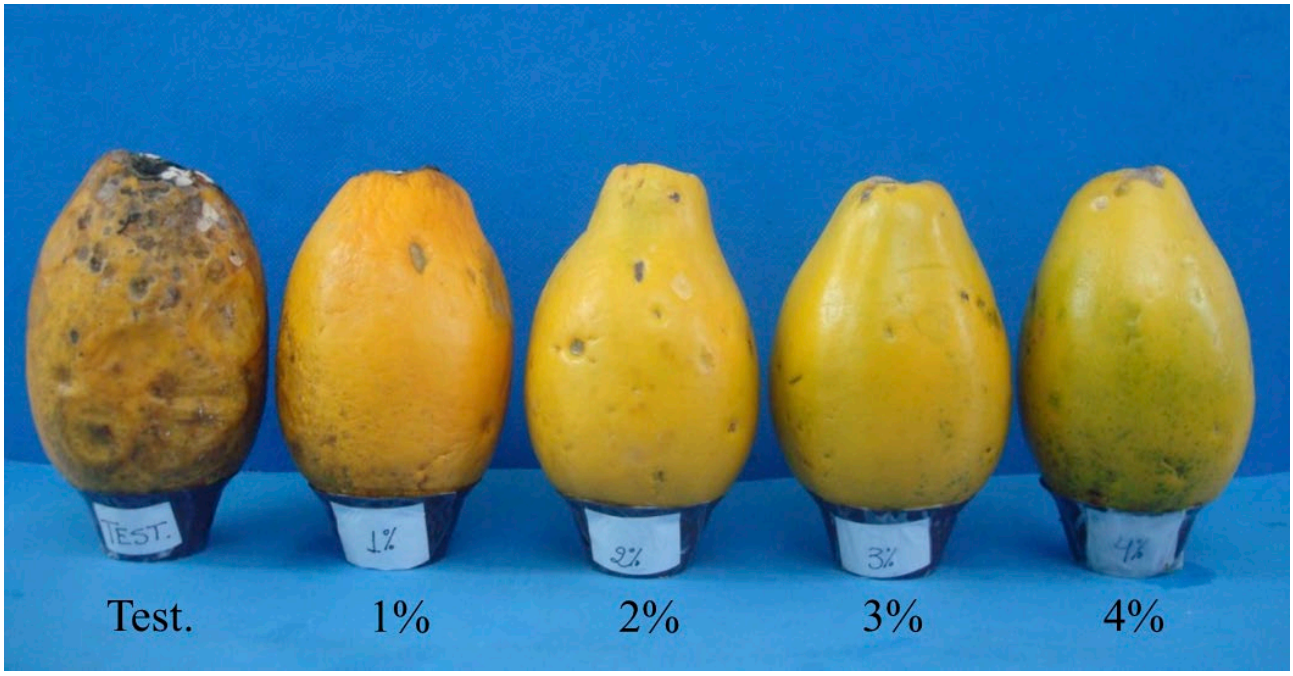

Figure 1. Papaya fruits inoculated with *Colletotrichum gloeosporioides* and coated with cassava starch. Control (Test.) and cassava starch coating of 1%, 2%, 3% and 4% at 14 d after inoculation.

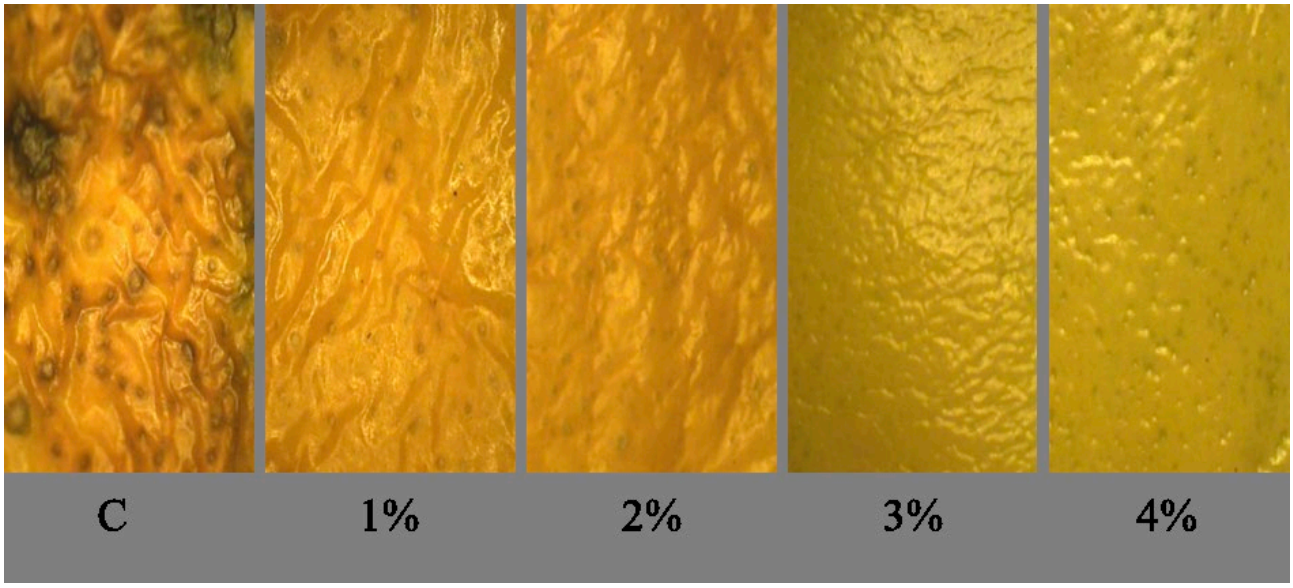

Figure 2. Electron micrographs showing details of papaya fruit epicarp texture, either uncoated or after treatment with cassava starch gel at different concentrations. C = Control (uncoated) and cassava starch coatings at 1%, 2%, 3% and 4% at 14 d after inoculation with *Colletotrichum gloeosporioides*.

starch concentrations could extend postharvest storage periods to four days without affecting the fruit quality. The coating treatments delayed fruit ripening, and changes in peel colour, titratable acidity, soluble solids and flesh hardness were significantly less than for untreated fruits.

During the coating formation a gradual darkening according to cassava starch concentration could occur (Figure 4). This intense darkness suggested by the literature was reached in fruit coated with gel 3% of cassava starch. According to Rocha *et al.* (2001), the test for iodine aqueous solution of potassium iodide detects the presence of starch products through the development of a dark coloration.

This is because in neutral aqueous solutions, normal spiral structure of starch products has the ability to react with iodine, producing helical inclusion complex with amylose molecules about six per circle, wherein the iodine is located in the central cavity of helix (Denardin and Silva, 2009).

For the iodine test, it was also observed a discontinuity color in fruits coated with 1% was bathed with the solution, showing poor adhesion of this coating. This irregularity of 1% coating, with undyed areas with iodine tincture (Figure 4), could prove areas non-covered by the coatings, might explain the incidence of disease in fruit coated with this concentration.

The coatings with 2%, 3% and 4% showed excellent adhesion, therefore when handling these fruits, their removal was not visually noticeable. Through the 2% iodine test, the coating fruit with 2% even entirely coated, showed no uniformity in the color, i.e. some areas were darker than others (Figure 4).

SEM observations

Surface stomata on samples of papaya fruit tissues were more obvious for untreated control fruit than in those treated with cassava starch coatings (Figure 5). This was probably due to lack of coating. Corroborating this observation, Assis (2009) reported partial filling of stomata from applied fruit coatings. The barrier coating of stomata is likely to restrict CO_2 and O_2 exchange that could delay ripening and prevent the development of *C. gloeosporioides*.

Large areas devoid of starch coating were observed on fruits treated with cassava starch at 1%. This would allow metabolic processes to occur at intermediate levels as compared to the other treatments. This poor adhesion of the coating could lead the incidence of anthracnose found at 6 d after inoculation with *C. gloesporiodes*, averaging 3.7% incidence of anthracnose (Table 1).

Detachment of the coating or the barrier opening was noted in the 2% starch coating treatments (Figure 5C), which could interfere with the fruit ripening processes, advancing to the subsequent stages compared with those for fruits coated with 3% and 4% coatings. However, the 2% coating were sufficient to control fruit ripening and anthracnose. The 3% coating treatment gave the most complete protective layer on fruit without detachment or cracking of the starch gel coating. (Figure 5D), with some streaks due to moisture loss. The coating with 4% starch, the most concentrated tested, while losing moisture, became rigid and developed cracks (Figure 5E).

The use of coatings based on cassava starch at concentrations above 2% has potential to control of diseases and delay ripening.

In conclusion cassava starch coatings may provide effective control of anthracnose on papaya fruits. The most appropriate concentration of cassava starch for fruit coating was shown to be 2%, considering the low cost and efficiency of disease control. The probable mechanism of disease control provided by the coating is related to delay of fruit ripening and the formation of a protective layer over the fruit.

Acknowledgements

We thank CAPES for providing a Master's scholarship of the first author and FAPEMIG for support of the Electron Microscopy and Ultrastructural Analysis Laboratory of the UFLA (CAG Project - APQ - 02616-12).

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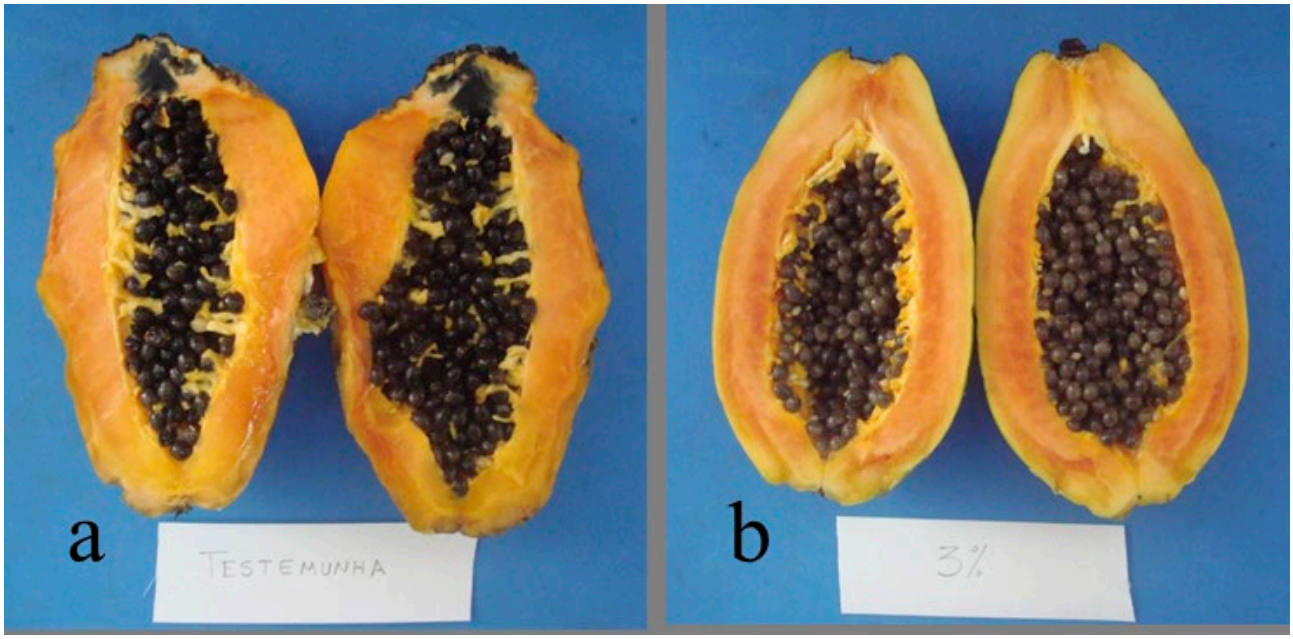

Figure 3. Sections of longitudinally cut of papaya fruits 12 d after coating: a) control fruit (only inoculated with *Colletotrichum gloeosporiopides*) and b) fruit coated with 3% starch-based gel.

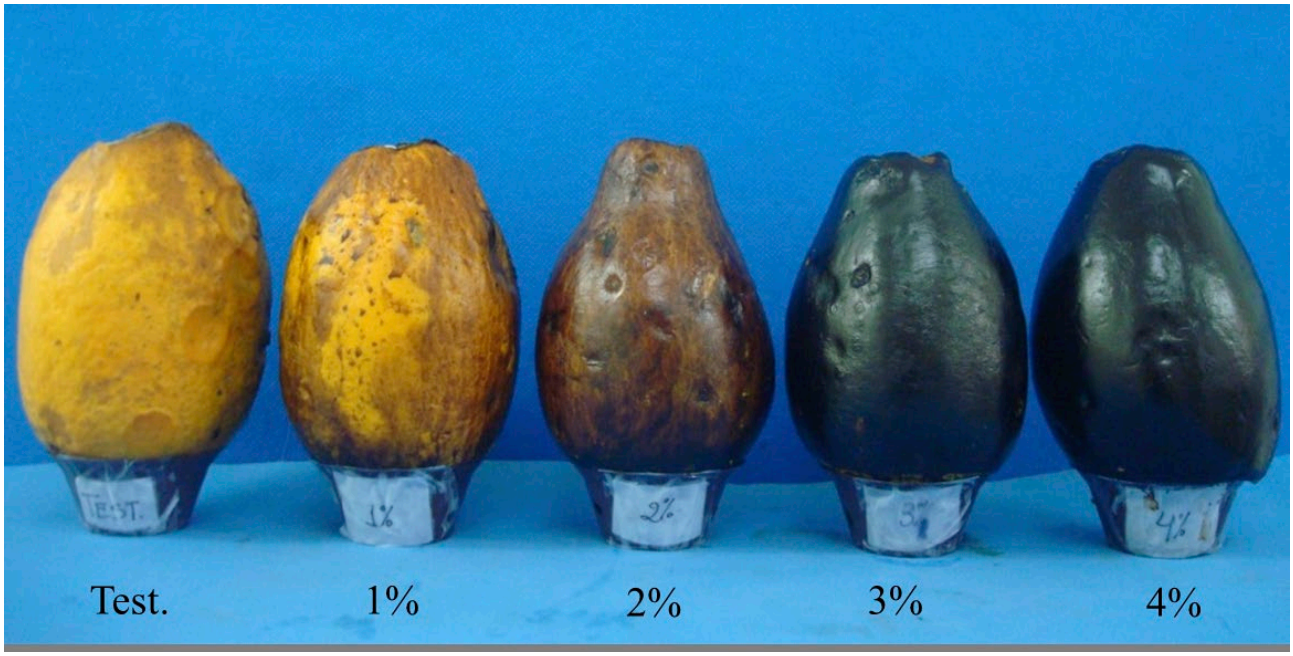

Figure 4. Papaya fruits treated with 2% iodine solution for evaluation of uniformity of distribution of cassava starch coatings.

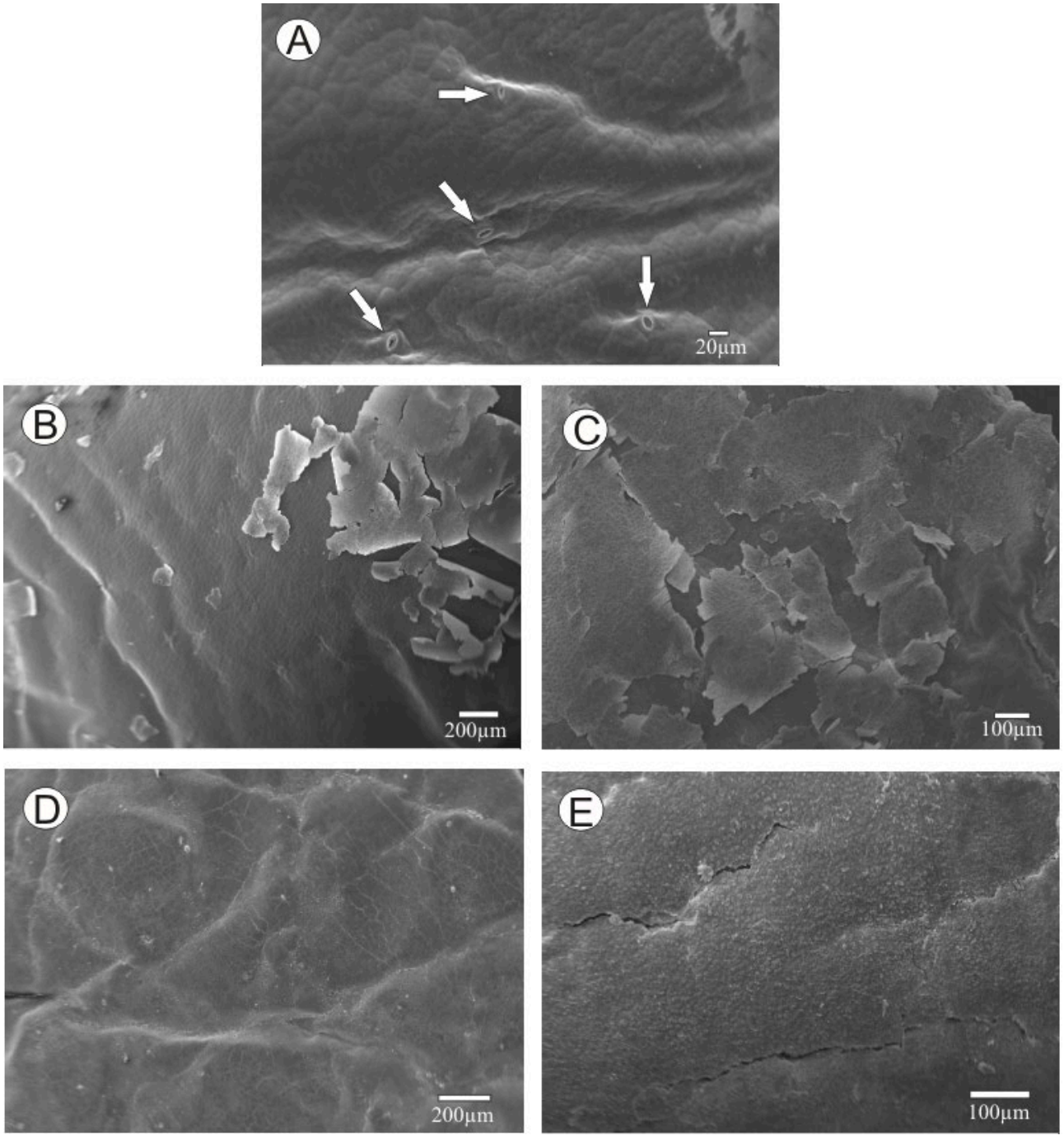

Figure 5. Scanning electron micrographs of papaya epicarps: A) non-coated fruit surface with visible stomata (arrows); B) fruit surface coated with cassava starch gel at 1% concentration; C) coated with 2% concentration; D) coated with 3% concentration, and E) coated with 4% starch gel concentration.

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Accepted for publication: January 5, 2016
Published online: July 29, 2016