ISSN impreso: 0258-5936
ISSN digital: 1819-4087

Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas
http://ediciones.inca.edu.cu

EVALUATION OF BIODEGRADABLE FILMS FOR POSTHARVEST CONTROL OF FUNGI IN PAPAYA

Evaluación de películas biodegradables en el control de hongos postcosecha de la papaya

Mónica Hernández López[1], Jessica Guillén Sánchez[2], Silvia Bautista Baños[1] and Dagoberto Guillén Sánchez[2]✉

ABSTRACT. There are several postharvest fungi that cause diseases and that greatly reduce the storage life of the papaya fruit. The objective of the study was to evaluate the antifungal effect of films formulated with chitosan, and other natural products. Chitosan films combined with beeswax, oleic acid and essential oils of thyme, cinnamon and clove were first evaluated as inhibitors of the *in vitro* mycelial growth of Rhizopus stolonifer, Colletotrichum gloeosporioides, Alternaria alternata, Fusarium oxysporum and Penicillium digitatum. The formulations that responded best *in vitro* were evaluated by immersion of the papaya fruits, determined their effect on the control of anthracnose (*C. gloeosporioides*) compared with Sportak (prochloraz) at 3 mL L^{-1} as commercial control. Films based on chitosan, beeswax/oleic acid and essential oils of cinnamon and clove 1 % (Treatments 9, 3, 21 and 25), completely inhibited the mycelial growth of the five fungi. The treatments applied by immersion of papaya fruits did not present a significant effect on the incidence and severity of the anthracnose, only the treatment with Sportak and Chitosan had effect after 17 days of storage at 14 ± 2 ºC. Regarding quality, the biodegradable films did not affect the content of total soluble solids in the fruits and weight loss.

Key words: antracnosis, *Carica papaya*, beeswax, chitosan

RESUMEN. Existen varios hongos postcosecha que causan enfermedades y que reducen en gran medida la vida de almacenamiento del fruto de papaya. El estudio tuvo como objetivo evaluar el efecto antimicótico de películas formuladas con quitosano y otros productos naturales. Las películas de quitosano combinadas con cera de abeja, ácido oleico y aceites esenciales de tomillo, canela y clavo, fueron evaluadas primeramente como inhibidores del crecimiento micelial *in vitro* de *Rhizopus stolonifer*, *Colletotrichum gloeosporioides, Alternaria alternata*, *Fusarium oxysporum* y *Penicillium digitatum*. Los formulados que mejor respondieron *in vitro* fueron evaluados por inmersión de los frutos de papaya, determinado su efecto en el control de antracnosis (*C. gloeosporioides*), comparado con Sportak (procloraz) a 3 mL L^{-1} como control comercial. Las películas a base de quitosano, cera de abeja/ácido oleico y aceites esenciales de canela y clavo al 1 % (Tratamientos 9, 3, 21 y 25), inhibieron completamente el crecimiento micelial de los cinco hongos. Los tratamientos aplicados por inmersión de frutos de papaya, no presentaron un efecto significativo sobre la incidencia y severidad de la antracnosis, solo el tratamiento con Sportak y Quitosano tuvieron efecto a los 17 días de almacenamiento a 14±2 ºC. En cuanto a calidad, las películas biodegradables no afectaron el contenido de sólidos solubles totales en los frutos y pérdida de peso.

Palabras clave: Antracnosis, *Carica papaya*, cera de abeja, quitosano

INTRODUCTION

The fruits of papaya (*Carica papaya* L.) are characterized by their great demand in the different international markets (1). Mexico ranked as the world's largest papaya exporter in 2014 with 131,391 tons. In the United States of six papayas bought, approximately eight come from Mexico; however, as a tropical product

[1]Departamento de Interacciones Planta-Insecto. Instituto Politécnico Nacional, Centro de Desarrollo de Productos Bióticos, Carr. Yautepec-Jojutla Km. 6, Col. San Isidro, CEPROBI 8, Morelos 62731, México.
[2]Escuela de Estudios Superiores de Xalostoc, Universidad Autónoma del Estado de Morelos, Nicolas Bravo s/n, Parque Industrial Cuautla, Ayala, Morelos, México.
✉ dagoguillen@yahoo.com

it has serious limitations in storage because it is a highly climacteric fruit, susceptible to mechanical damage and host of several microorganisms during storage, including fungi of the genus *Rhizopus*, *Fusarium*, *Penicillium* and others (2). The most common disease of papaya is anthracnose caused by the fungus *Colletotrichum gloeosporioides*, which causes losses ranging from 40 to 100 %, depending on the production area (3).

The fruit is infected in the pre-harvest and then damage occurs during storage (2,4,5). Some antifungal control alternatives, such as the ozone use (6), carrageenan and glycerol (7) and biodegradable coating films (8) have had good results to protect fruits and extend shelf life by inhibiting microorganisms (9). Chitosan, a natural compound obtained from chitin found in the outer layer of crustaceans such as crabs, shrimps, has been used for its antifungal effects against bacteria and fungi, and in turn has also been shown to be effective in controlling fungi in the cultivation of papaya both *in vitro* and *in situ* tests (2). Also this polymer, used as a coating added with antimicrobial agents (essential oils of clove, cinnamon, thyme, lemon, mint, rosemary, cedar, etc.), can provide the natural product with greater antimicrobial activity (10). In previous studies, where formulations based on chitosan at 10 mg mL^{-1} were used, mixed with essential oils of clove, cinnamon and thyme at 300 µg mL^{-1}, the decay caused by *R. stolonifer* was reduced by 50 % in tomato (10).

The objective of the trial was to evaluate the antimicrobial effect of formulations based on chitosan and other combined products such as beeswax, oleic acid and essential oils of thyme (*Thymus vulgaris* L.), cinnamon (*Cinnamomum verum* J.Presl) and cloves (*Syzygium aromaticum* (L.) Merr. & LMPerry): as well as its effect on the post-harvest quality of the papaya.

MATERIALS AND METHODS

Cultivation of fungi

For the experiments *in vitro*, the strains *Rhizopus stolonifer*, *Colletotrichum gloeosporioides*, *Alternaria alternata*, *Fusarium oxysporum* and *Penicillium digitatum* were used, which were obtained from the laboratory of the Post-harvest Technology Laboratory of Agricultural Products, of the Center for the Development of Biotic Products-IPN. From the fungal strains, spore suspensions were prepared at a concentration of 1x105 spores mL^{-1}. The inoculation was performed on papaya fruits to which four 0,5 mm wounds were made with a sterilized dissection needle, in each wound 50 µL of the spore suspension were placed. The inoculated fruits were placed in humid chambers, which consisted of unicel dishes on which wet absorbent paper was placed. Afterwards, the fruits were placed inside transparent polyethylene bags, sealed with a rubber band, to promote the development of the mycelium during 48 hours at 28 ± 2°C. After this time a portion of mycelium was taken from the fungus and placed in Petri dishes with Potato-Dextrose-Agar culture medium (PDA, Bioxon®) that were incubated for four days for *R. stolonifer*, 10 days for *C. gloeosporioides* and *P. digitatum* and 12 days for *F. oxysporum* and *A. alternata*, at a temperature of 28 ± 2°C and photoperiod 12:12 h (light: dark). These activated isolates were conserved by replacing them in PDA. For *in situ* studies, only the strain of *C. gloeosporioides* was selected.

Preparation of the chitosan solution

A solution of chitosan (Sigma Aldrich®), of 1 % (w/v) average molecular weight, with 1 % (v/v) acetic acid and distilled water was prepared. The solution was heated to 70 °C and stirred constantly for 24 h. The final pH was adjusted to 5,6 with 0,1 N NaOH.

Formulations of films

Different combinations of products such as films were used in their effect on the growth of the five fungi. 12,5 mL of 1 % (w/v) chitosan was placed in an Erlenmeyer flask on a grate with stirring at a temperature of 70°C for 1 minute. Once the solution was homogenized, 0,1 % beeswax and 1 % oleic acid were added, both in 0,3 % v/v glycerol. Subsequently the essential oil of thyme, cinnamon or clove (Essencefleur de México® Oils and Essences) was incorporated, at the concentrations of 0,1 % (12,5 µl), 0,25 % (31,3 µl), 0, 5 % (62,5 µl) and 1 % v/v (125 µl), for a total of 24 combinations.

In vitro evaluations

An inoculum portion of each strain (5 mm diameter) was placed in the center of Petri dishes with PDA culture medium, then coated with 1,0 mL of each formulation, according to the different treatments and incubated as required, described earlier. In the control, no formula was applied.

The radial growth of each fungus was measured with a ruler until the fungus reached its maximum growth in the plate.

In situ evaluations

For the *in situ* assay, the formulation that best inhibited the development of *C. gloeosporioides in vitro* was used. The fruits of papaya cv. Maradol red, were obtained from the Central Supply Cuautla Morelos, Mexico, with a uniform size and a maturity index 2, corresponding to the change of color of the fruit (1/2 of maturity) to the appearance of yellow tones (3/4 maturity), for all treatments. A 0,5 mm wound was applied to each fruit with a sterile dissection needle, and 50 µl of a spore suspension of *C. gloeosporioides* was inoculated at a concentration of 105 spores mL^{-1}. After 24 h the fruits were submerged in each of the two formulations and dried at room temperature (28±2°C). The control treatments consisted of immersing the fruits in running water and another one in the synthetic fungicide Sportak (prochloraz) at a concentration of 3 mL L^{-1}. Fruits were stored in a storage chamber at 14 ± 2°C and others at room temperature (28 ± 2 °C). For each of the treatments three repetitions with 10 fruits were used. The percentage of incidence and severity was evaluated at 13 and 17 days, then in fruits stored at 14 ± 2 °C and at 2 days in fruits stored at room temperature.

The percentage of incidence was determined by dividing the number of diseased fruits among the fruits evaluated and multiplied by 100. The severity was evaluated with a scale of 5 classes: 1: 0 %, 2: 1-25 %, 3: 26-50 %, 4: 51-75 %, and 5: 76-100 % of the surface of the fruit with symptoms by *C. gloeosporioides*.

At the end of the storage period (17 days), firmness was determined with a penetrometer on both lateral sides of each fruit and expressed in Newtons; the total soluble solids (TSS %) were determined from two samples of fruit pulp (10 g), which were placed in a gauze and squeezed, then a drop of each sample was placed in a refractometer and it was taken the reading. The weight loss (%) was calculated with the formula: Weight loss = (initial weight-final weight/initial weight) X 100; and the color was evaluated with a Baking meter colorimeter (Konica Minolta, model BC-10), measurements were made on both sides of the fruit, considering the chromatic coordinates L* (luminosity), a* (shades of green to red) and b* (shades of yellow to blue).

Statistical analysis

For *in vitro* and *in situ* studies a completely randomized design was used. The data were analyzed by means of variance analysis and Tukey test (α=0,05), with the Sigma Stat 3.5 program. Previously the premise of normality and homogeneity of the samples was calculated. For the *in vitro* test, six Petri dishes were used for each formulation. In the *in situ* trial the treatments had three repetitions with 15 fruits per repetition.

RESULTS

The *in vitro* effect of the biodegradable films on the five microorganisms evaluated is shown in Table 1. The formulations of chitosan with beeswax and oleic acid with the essential oils of cinnamon and clove at the concentration of 1 % completely inhibited the development of the mycelium of all the fungi evaluated, while the formulation based on chitosan with bee wax and with essential oil of thyme at 1,0 %, inhibited the development of *Fusarium oxysporum*, *Alternaria alternata* and *Penicillium digitatum*, the formulation with chitosan, acid oleic and thyme essential oil at 1,0 % inhibited the development of *R. stolonifer*, and *C. gloeosporioides*, *Alternaria alternata* and *Penicillium digitatum*. Likewise, the formulation that included oleic acid and 0,5 % clove essential oil inhibited the growth of 100 % *C. gloeosporioides*.

The formulations that achieved a medium inhibition (50 to 82 %) in *R. stolonifer* were those that were mixed with beeswax and 0,5 % clove essential oil, as well as those made with oleic acid and cinnamon essential oil and 0,5 % clove. Also in *Colletotrichum gloeosporioides* were the formulations with beeswax, thyme essential oil at 0,5 and 1,0 %, cinnamon essential oil at 0,5 % and clove essential oil at 0,25 and 0,5 % and finally the formulation with oleic acid and 0,5 % cinnamon essential oil. In *Fusarium oxysporum* only the formulations with oleic acid and 0,5 % clove and cinnamon essential oil achieved medium inhibition. Finally for *Penicillium digitatum* were the formulations made with beeswax and oleic acid and essential oil of thyme, cinnamon and clove 0,1; 0,25 and 0,5 %.

Formulations with inhibitions less than 50 % for *Rhizopus stolonifer* were those with beeswax and thyme essential oil at 0,1; 0,25; 0,5 and 1,0 %, cinnamon essential oil and clove at 0,1; 0,25 and 0,5 %; also the formulations with oleic oil and thyme essential oil at 0,1; 0,25 and 0,5 %, essential oil of cinnamon and clove at 0,1 and 0,25 %.

In *Colletotrichum gloeosporioides* the formulations were registered with bee wax, thyme essential oil at 0,1; 0,25 and 0,5 %, cinnamon essential oil at 0,1 and 0,25 % and clove essential oil at 0,1 %; in the same way the formulations with oleic acid and thyme essential oil at 0,1; 0,25 and 0,5 %, essential oil of cinnamon and clove at 0,1 and 0,25 %.

In *Fusarium oxysporum* the formulations were found with bee wax and essential oil of thyme, cinnamon and clove at 0,1; 0,25 and 0,5 %, as well as the formulations with oleic acid, essential oil of thyme at 0,1 ; 0,25; 0,5 and 1,0 %, cinnamon essential oil and clove at 0,1 and 0,25 %. In the same sense, for *Alternaria alternata*, inhibitions less than 50 % were achieved with formulations based on chitosan, both with beeswax and oleic acid with the three essential oils at 0,1; 0,25 and 0,5 % respectively.

The fungi that showed the highest tolerance to the formulations of chitosan, beeswax and oleic acid and essential oils of thyme, cinnamon and cloves were *Alternaria alternata, Rhizopus stolonifer* and *Fusarium oxysporum* and the most susceptible were Penicillium digitatum and *Colletotrichum gloesporioides*.

In fruits stored at controlled temperature at 13 days after treatment, the lowest incidence (10,0 %) of anthracnose was achieved with the formulations of chitosan, oleic acid and beeswax and cinnamon essential oil and clove 1 % , although they did not differ from the treatments where fungicide Sportak (10,0 %) or control (16,7 %) was applied. The highest incidence (40,0 %) was registered in the treatment with the formulation of chitosan, beeswax and thyme essential oil, following the treatments with the formulation of chitosan, oleic acid and thyme essential oil and the treatment with chitosan only (Table 2).

At 17 days after treatment, the incidence of the disease in fruits stored at controlled temperature increased considerably. The lowest incidence (13,3 %) was observed in the treatment with fungicide and with the chitosan 1 % (32,4 %) and the highest incidence (81,5 %) was with the chitosan treatment 1 % +beeswax 0, 1 % + thyme essential oil 1 %, being statistically equal with the treatments based on chitosan, oleic acid and beeswax and the essential oils of clove, cinnamon and thyme and the control (Table 2).

Table 1. Effect of chitosan-beeswax-essential oils films on the *in vitro* development of isolated papaya fungi after an incubation period at 28 ± 2 °C

Treatments	Mycelial growth (cm)				
	R,s	*C,g*	*F, o*	*A, a*	*P,d*
1. PDA control	8,3 a	8,3 a	8,3 a	8,3a	4,6 a
2. Q 1 %+CA 0,1 %+AET 0,1 %	8,3 a	7,9 a	7,9 a	8,2a	3,8 a
3. Q 1 %+CA 0,1 %+AET 0,25 %	8,3 a	8,0 a	7,8 a	8,3a	3,6 a
4. Q 1 %+CA 0,1 %+AET 0,5 %	8,3 a	4,0 b	7,7 a	7,6 a	2,8 a
5. Q 1 %+CA 0,1 %+AET 1 %	8,2 a	1,5 c	0 c	0 c	0 b
6. Q 1 %+CA 0,1 %+AEC 0,1 %	8,3 a	5,9 b	6,4 a	7,7 a	4,0 a
7. Q 1 %+CA 0,1 %+AEC 0,25 %	8,1 a	5,1 b	6,0 a	7,5 a	2,6 a
8. Q 1 %+CA 0,1 %+AEC 0, 5 %	5,3 a	2,3 c	5,7 b	7,1 a	2,4 a
9. Q 1 %+CA 0,1 %+AEC 1 %	0 c	0 c	0 c	0 c	0 b
10. Q 1 %+CA 0,1 %+AECL 0,1 %	8,3 a	5,7 b	7,1 a	7,6 a	2,7 a
11. Q 1 %+CA 0,1 %+AECL 0,25 %	8,3 a	4,1 b	6,5 a	6,6 a	2,6 a
12. Q 1 %+CA 0,1 %+AECL 0,5 %	3,4 b	1,9 c	4,8 b	5,8 b	2,1 a
13. Q 1 %+CA 0,1 %+AECL 1 %	0 c	0 c	0 c	0 c	0 b
14. Q 1 %+AO 0,1 %+AET 0,1 %	8,3 a	8,3 a	6,6 a	8,0 a	2,2 a
15. Q 1 %+AO 0,1 %+AET 0,25 %	8,3 a	6,7 a	7,2 a	7,8a	2,2 a
16. Q 1 %+AO 0,1 %+AET 0,5 %	8,3 a	7,8 a	6,2 a	7,4 a	2,1 a
17. Q 1 %+AO 0,1 %+AET 1 %	0 c	0 c	6,7 a	0 c	0 b
18. Q 1 %+AO 0,1 %+AEC 0,1 %	8,3 a	7,3 a	6,2 a	7,3 a	2,1 a
19. Q 1 %+AO 0,1 %+AEC 0,25 %	7,7 a	7,2 a	5,0 b	7,0 a	2,1 a
20. Q 1%+AO 0,1 %+AEC 0, 5 %	3,3 b	2,1 c	2,4 c	6,7 a	2,1 a
21. Q 1 %+AO 0,1 %+AEC 1 %	0 c	0 c	0 c	0 c	0 b
22. Q 1 %+AO 0,1 %+AECL 0,1 %	8,3 a	6,9 a	5,4 b	6,8 a	2,1 a
23. Q 1 %+AO 0,1 %+AECL 0,25 %	6,1a	4,8 b	4,7 b	6,6 a	2,2 a
24. Q 1 %+AO 0,1 %+AECL 0,5 %	3,1 b	0 c	3,0 c	6,7 a	2,4 a
25. Q 1 %+AO 0,1 %+AECL 1 %	0 c	0 c	0 c	0 c	0 b

Q: Chitosan 1%, CA: 0.1 % beeswax, AO: 1 % oleic acid, AET: thyme essential oil, AEC: cinnamon essential oil, AECL: clove essential oil. * Different letters in columns indicate significant differences (P <0.05). Tukey test (n = 6)

Table 2. Incidence (%) of anthracnose in papaya fruits artificially inoculated with *C. gloeosporioides* and treated with chitosan films, beeswax and oleic acid and essential oils, stored at controlled temperatures and environment

Treatments	Incidence (%)		
	day 2 TA	day 13 TC	day 17 TC
1. Control (Water)	91,1 a	16,7 c	59,9 a
2. Fungicide (Sportak)	70,0 b	10,0 c	13,3 b
3. Q 1%	61,1 b	26,7 b	32,4 b
4. Q1% + CA 0,1 % + AET 1%	100 a	40,0 a	81,5 a
5. Q1% + CA 0,1% + AEC 1%	100 a	13,3 c	73,3 a
6. Q 1% + CA 0,1% + AECL 1%	100 a	13,3 c	76,7 a
7. Q 1% + AO 1 % + AET 1%	67,8 b	26,7 b	61,5 a
8. Q1% + AO 1% + AEC 1%	88,6 a	10,0 c	60,8 a
9. Q 1% + AO 1% + AECL 1%	80,9 a	10,0 c	57,8 a

Q: chitosan; CA: beeswax; AO: oleic acid; AET: thyme essential oil; AEC: cinnamon essential oil; AECL: clove essential oil; TA: room temperature; TC: controlled temperature. * Equal letters in columns indicate statistical similarity by Tukey P≤ 0.05

The incidence of anthracnose only decreased slightly in the treatments with Sportak and Chitosan at 1 % at 17 days of storage with 13,3 and 32,4 % respectively, compared to the control that presented 59,9 %.

The anthracnose severity index, in fruits stored at room temperature, did not present significant differences between the treatments, ranged from 1,3 to 2,0, being the same as the control where an index of 2.0 was registered (Table 3) . In fruits stored at controlled temperature, at 13 days after treatment, the severity index was 1,1 to 1,5, but without statistical differences with the control, who registered a severity index of 1,3.

At 17 days after treatment, in fruit stored at controlled temperature, the lowest severity index was achieved in the Sportak (1,1) and 1% chitosan treatments (1,2), while the chitosan-based treatments , beeswax and essential oils of thyme and cinnamon, as well as the treatments based on chitosan, oleic acid and essential oils of thyme, cinnamon and clove presented an index that varied from 1,7 to 1,9, being statistically equal to control. The highest severity index (2.0) was reached in the treatment with chitosan, beeswax and clove essential oil.

The behavior of the total soluble solids was very similar, with values ranging from 8.9 ± 1.010 (T5) to 10,8 ± 0,898 (T6). The fruits with smaller firmness (6,9 ± 1,0) were obtained with the T6, very similar to the initial values (6,5 N) in comparison with the rest of the treatments. The lowest weight loss (4,6 ± 1,217) occurred with T8, similar to T2 (4,8 ± 1,476) and the highest weight loss (6,3 ± 0,719) corresponded to T4 (Table 4).

The determined values of brightness (L), chromaticity (C) and hue angle (hº) indicate that the fruits of the different treatments maintained similar brightness values and increased their chromaticity, decreasing their hue angle, in comparison with the initial fruits that they were not treated with a chromaticity of C = 34,8 and a hue angle hº = 87,5. The lower luminosity (49,3 ± 2,3) was observed with the T5 treatment and the greater luminosity (55,2 ± 3,5) was reached with the T7 treatment. The highest value of a * (18,9 ± 4,0) was observed with the T6 treatment and the lowest (7,6 ± 4,6) with the T2, in comparison with the other treatments and with the initial fruits (1, 53 ± 4.8). The initial fruits had values for b* of 34,8 ± 8,2, the highest value of b* (42,4 ± 4,8) was presented by T7 and the lowest value (37,0 ± 3,7) corresponding

Table 3. Severity index in papaya fruits artificially inoculated with *C. gloeosporioides* and treated with chitosan films, beeswax and oleic acid and essential oils, stored at controlled temperatures and environment

Treatments	Severity index		
	day 2 TA	day 13 TC	day 17 TC
1. Control (Water)	2,0 a	1,3 a	1,9 abc
2. Fungicide (Sportak)	1,9 a	1,1 a	1,1 c
3. Q 1 %	2,0 a	1,5 a	1,2 bc
4. Q 1 % + CA 0,1 % + AET 1 %	2,0 a	1,4 a	1,7 abc
5. Q 1 % + CA 0,1 % + AEC 1 %	2,1 a	1,1 a	1,8 abc
6. Q 1 % + CA 0,1 % + AECL 1 %	1,8 a	1,1 a	2,0 a
7. Q 1 % + AO 1 % + AET 1 %	1,3 a	1,5 a	1,9 abc
8. Q1 % + AO 1 % + AEC 1 %	2,0 a	1,2 a	1,8 abc
9. Q 1 % + AO 1 % + AECL 1 %	1,8 a	1,2 a	1,9 abc

Q: chitosan; CA: beeswax; AO: oleic acid; AET: thyme essential oil; AEC: cinnamon essential oil; AECL: clove essential oil; TA: room temperature; TC: controlled temperature. * Equal letters in columns indicate statistical similarity by Tukey P≤ 0.05

to T5. The yellow color was maintained in most of the treatments, including the initial fruits, with the exception of treatments T6, T8 and T9, which presented a yellow-orange color (Table 5).

DISCUSSION

In vitro studies in general showed that the best biodegradable films based on chitosan, beeswax or oleic acid, were those that were mixed with thyme oil, cinnamon and clove at the highest concentration (1,0 %), where Mycelial inhibition was 100 % in *R. stolonifer, C. gloeosporioides, F. oxy*sporum, *A. alternata* and *P. digitatum* compared to the control treatment, where the mycelial development was 8,3 cm. In *R. stolonifer* the films based on chitosan, beeswax and oleic acid with the essential oil of cinnamon and clove at the concentration 0,5 % also inhibited the mycelial development of this fungus, obtaining a mycelial growth of 3,1 to 5,3 cm.

In vitro studies with chitosan at 2,5 and 3,0 % reported a fungicidal effect on *R. stolonifer, C. gloeosporioides, F. oxysporum* and *P. digitatum*. Likewise, chitosan at 1,5 % inhibited mycelial growth in more than 50 % of *C. gloeosporioides, F. oxysporum, P. digitatum* and *R. stolonifer* isolated from papaya (4). In this study, *C. gloeosporioides* showed inhibition of mycelial growth when using biodegradable films based on chitosan, beeswax and / or oleic acid and essential oils of thyme, cinnamon and clove at the concentration of 0,5 and 1,0 %, its mycelial growth was 0; 1,5; 1,9; 2,1; 2,3 and 4,0 cm compared to the control (PDA) that obtained a mycelial growth of 8,3 cm, with the exception of the biodegradable film of chitosan, oleic acid and thyme oil at 0,5 % where the growth mycelial was the same as the control treatment. Chitosan at concentrations of 2,5 and 3,0 % achieved total mycelial inhibition on *C. gloeosporioides* isolated from papaya, during 7 days of incubation (2).

Table 4. Effect of the films of chitosan-beeswax and oleic acid -1 % essential oils in total soluble solids, firmness and weight loss in fruits artificially inoculated with *C. gloeosporioides* and stored for 17 days at controlled temperature (14 ± 2 °C)

Treatments	Total soluble solids	Firmness (N)	Weight loss (%)
1. Control (Water)	10,2 ± 1,100	9,1 ± 1,2	5,1 ± 1,008
2. Fungicide (Sportak)	9,6 ± 1,174	9,7 ± 0,3	4,8± 1,476
3. Q 1 %	10,1 ± 0,848	9,6 ± 0,6	5,3 ± 1,440
4. Q 1 % + CA 0,1 % + AET 1 %	9,6 ± 1,059	7,6 ± 2,3	6,3 ± 0,719
5. Q 1 % + CA 0,1 % + AEC 1 %	8,9 ± 1,010	7,4 ± 1,4	5,6 ± 2,396
6. Q 1 % + CA 0,1 % + AECL 1 %	10,8 ± 0,898	6,9 ± 1,0	5,1 ± 2,183
7. Q 1 % + AO 1 % + AET 1 %	9,5 ± 1,295	9,0 ± 1,5	5,3 ± 1,171
8. Q 1 % + AO 1 % + AEC 1 %	10,3 ± 1,370	9,5 ± 0,6	4,6 ± 1,217
9. Q 1 % + AO 1 % + AECL 1 %	10,5 ± 0,902	9,9 ± 0,2	5,9 ± 1,119

Q: chitosan; CA: beeswax; AO: oleic acid; AET: thyme essential oil; AEC: cinnamon essential oil; AECL: clove essential oil. Initial total soluble solids: 10.8 ± 0.9. Initial firming: 6.5N

Table 5. Values L*, a*, b* of the CIELAB scale for the determination Chromaticity and nuance angle of initial papaya fruits and the preventive effect of treatments inoculated with *C. gloeosporioides*, stored for 17 days at controlled temperature (14 ± 2 °C)

Treatments	Luminosity (L*)	a*	b*	Chromaticity (C*)	Nuance angle (h°)	Color of the graphic CIELAB
Initial fruits	52,8 ± 2,9	1,53 ± 4,8	34,8± 8,2	3 4,8	87,5	A
1. Control (Agua)	51,6 ± 3,3	11,3 ± 5,0	38,1± 8,0	39,7	73,5	A
2. Fungicide (Sportak)	50,8 ± 2,8	7,6 ± 4,6	40,7± 9,8	41,4	79,4	A
3. Q 1 %	49,9 ± 3,2	9,5 ± 4,3	37,3± 6,6	38,5	75,7	A
4. Q 1 % + CA 0,1 % + AET 1 %	51,9 ± 3,9	13,1 ± 5,3	42,0± 8,6	43,9	72,7	A
5. Q 1 % + CA 0,1 % + AEC 1 %	49,3 ± 2,3	13,3 ± 7,7	37,0± 3,7	39,2	70,2	A
6. Q 1 % + CA 0,1 % + AECL 1 %	49,6 ± 2,4	18,9 ± 4,0	38,3± 3,9	42,7	63,7	AN
7. Q 1 % + AO 1 % + AET 1 %	55,2 ± 3,5	12,5 ± 5,3	42,4± 4,8	44,2	73,7	A
8. Q 1 % + AO 1 % + AEC 1 %	54,0 ± 2,4	14,9 ± 7,2	42,1± 2,6	44,6	70,5	AN
9. Q 1 % + AO 1 % + AECL 1 %	50,6 ± 2,9	13,3 ± 6,4	40,2± 3,3	42,3	71,7	AN

L *, luminosity; a *, green / red; b *, yellow / blue; C *, [(a * 2 + b * 2) 1/2] chroma; h º0 (arctangent angle b * / a *), hue angle (0º: red-purple, 90º0 yellow, 180º: green, 270º: blue. A, yellow; AN, yellow orange. The analysis is a comparison of means Tukey P≤ 0.05

Films based on chitosan and beeswax with thyme, cinnamon and clove oils at 0,5 and 1,0 % were better at reducing the growth of *F. oxysporum*. The oils of cinnamon, clove and thyme at concentrations of 100-300 µg mL^{-1} presented the lowest values of mycelial growth (0-36 mm) in *Fusarium* sp. isolated from papaya fruit (11). The mycelial growth of *A. alternata* and *P. digitatum*, in interaction with biodegradable films based on chitosan, beeswax, oleic acid, and essential oils of thyme, cinnamon and clove presented a very similar behavior; the effect of the films based on chitosan, beeswax, oleic acid with 1 % oils showed a percentage of mycelial growth inhibition of 100 %.

The fungi that showed the highest mycelial inhibition *in vitro* were *P. digitatum* and *C. gloesosporioides* since the effect of inhibition of the covers was from the concentration of 0,5 and 1 % in all treatments performed. It is not known if this is due to the morphology of the fungus, its sensitivity or its behavior with chitosan, beeswax, oleic acid and essential oils.

The treatments that showed a better effect *in vitro* were the biodegradable film based on chitosan, beeswax, oleic acid, thyme essential oil, cinnamon and clove at the 1 % concentration.

The incidence of anthracnose in papaya fruits stored at room temperature, in most of the treatments was greater than 80 % and statistically equal to the control treatment, only the treatments with chitosan 1 % (61,1 %), chitosan + oleic acid + thyme essential oil (67,8 %) and Sportak (70,0 %) achieved minor incidents, but which are commercially considered very high incidences.

The incidence of anthracnose in papaya fruits stored at controlled temperature for 13 days varied from 10,0 to 13,3 % for treatments with chitosan, oleic acid or beeswax and clove and cinnamon essential oil, but were statistically equal to the control treatment (16,7 %) and Sportak (10 %), the rest of the treatments presented incidences of 26,7 to 40 %. However, after 17 days of storage, the lowest incidence (13,3 and 32,4 %) was only achieved in the treatments with Sportak and chitosan 1 %, the rest of the treatments with films recorded incidents of 57,8 to 81,5 %, but were equal to the control treatment that achieved 59,9 %.

The index of severity of anthracnose in papaya fruits stored at room temperature for all treatments was 1,3 to 2,0 without statistical differences. The fruits stored at a controlled temperature for 13 days presented a similar behavior, but with lower rates, which varied from 1,1 to 1,4, without statistical differences with the control. Finally, fruits stored at a controlled temperature for 17 days showed slightly higher rates, being the lowest for the Sportak (1,1) and chitosan 1 % (1,2) treatments, and the highest (2,0) for the treatment with chitosan, beeswax and clove essential oil; the severity index for the rest of the treatments varied from 1,7 to 1,9, but statistically they behaved equal to the control.

The films can have a protective effect probably due to the presence of chitosan, which prolongs the life and improves the quality of the fruits by presenting selective permeability to the gases, likewise it works as a protective barrier against the deterioration of the fruits, retarding the natural processes of maturation and restricting the growth of microorganisms (5). The incorporation of antimicrobial agents such as thyme essential oil presents a phenolic compound which can penetrate the cytoplasm membrane causing a destabilization, acting as a proton exchanger reducing the pH gradient along the membrane and this phenolic compound can be the responsible for the effect it has when applied to films, covers or packaging where it inhibits the development of fungi and bacteria (12). In papaya Maradol fruits inoculated with *C. gloeosporioides* and later treated with an edible coating formulated with mesquite gum and candelilla wax and thyme essential oil, the concentration at 0,15 % reduced the incidence by 0 % and the concentration of 0, 12 reduced by 50 % (13).

The total soluble solids, firmness and weight loss did not show significant differences in the days of storage, as they were passing the days of storage the disease was increasing without affecting the three variables of study, noting that the content of total soluble solids, firmness and weight loss were not affected by the application of the treatments. Weight loss in papaya fruits Maradol increased during storage and that this is due to the loss of water from the fruits as it depends both on physiological activity and external factors, particularly temperature and relative humidity (5).

The reduction in firmness during the days of storage is due to the loss of pectic substances and the hydrolytic changes of protopectin (5). The edible wax applied to Maradol papaya fruits stored for 12 days, did not cause apparent damage to the fruits or their physicochemical characteristics after nine days of storage, the quality parameters showed a normal trend expected with the ripening of the fruit, however, after 12 days the fruits were affected by pathological damage (5).

On the other hand, the color of the fruits did not show statistical differences between the treatments. The fruits of the different treatments maintained similar brightness values and increased their chromaticity, decreasing their hue angle, in comparison with the initial fruits that were not treated, presenting a chromaticity of C=34,8 and a hint angle hº = 87,5. According to the color exhibited with the CIELAB graph after 17 days of storage, the fruits kept a yellow color in most of the treatments, including the initial fruits with a hue angle (hº=71,7-87,5), with the exception of the treatments 1 % chitosan + 1 % oleic acid + 1 % clove essential oil, 1 % chitosan + 1 % oleic acid + 1 % cinnamon essential oil and 1 % chitosan + 0,1 % beeswax + essential oil 1 % nail that presented a yellow-orange color with a hue angle (hº = 70,5). The ripeness of Maradol papaya fruit consumption was reached between 13 and 15 days after harvest under storage conditions at 23 ± 1 °C and 75 % relative humidity and the hue angle of the shell was between 70 and 80°, the content of total soluble solids varied from 10 to 11,5 °Brix and the firmness of the pulp from 4,7 to 6,9 N, allowing to differentiate two states of maturity of consumption (14,15). These characteristics can be proposed as objective indices to describe the ripeness of Maradol papaya consumption (14). In this study the shelf life was 19 days.

CONCLUSIONS

Biodegradable films based on chitosan, beeswax, oleic acid and essential oils of thyme, clove and cinnamon at the 1 % concentration, inhibited the development of *R. stolonifer*, *C. gloeosporioides, F. oxysporum, A. alternata* and *P. digitatum in vitro*, and allowed to observe a better effect in the control of the disease caused by *C. gloeosporioides* when the fruits were kept at a controlled temperature (14 ± 2 °C), without affecting the physiological parameters of the treated fruits, which allowed the useful life and quality of the papaya fruits to be extended up to 19 days.

BIBLIOGRAPHY

1. FAO. Stadistical Production and Marketing of Papaya [Internet]. FAOSTAT. 2010. Disponible en: http://www.fao.org/faostat/es/#home
2. Bautista-Baños S, Sivakumar D, Bello-Pérez A, Villanueva-Arce R, Hernández-López M. A review of the management alternatives for controlling fungi on papaya fruit during the postharvest supply chain. Crop Protection. 2013;49(12):8-20. doi:10.1016/j.cropro.2013.02.011
3. Rojo-BáEz I, GarcíA-Estrada RS, SañUdo-Barajas JA, LeóN-FéLix J, Allende-Molar R. Proceso de infección de antracnosis por *Colletotrichum truncatum* en papaya maradol. Revista Brasileira de Fruticultura [Internet]. 2017 [citado 8 de febrero de 2018];39(spe). doi:10.1590/0100-29452017379
4. Dotto GL, Vieira MLG, Pinto LAA. Use of chitosan solutions for the microbiological shelf life extension of papaya fruits during storage at room temperature. LWT - Food Science and Technology. 2015;64(1):126-30. doi:10.1016/j.lwt.2015.05.042
5. Petit-Jiménez D, Terán Y, Rojas B, Salinas-Hernández R, García-Robles J, Báez-Sañudo R. Efecto de las ceras comestibles sobre la calidad en frutos de papaya. Revista Iberoamericana de Tecnología Postcosecha. 2010;11(1):37-42.
6. Ong MK, Ali A. Antifungal action of ozone against *Colletotrichum gloeosporioides* and control of papaya anthracnose. Postharvest Biology and Technology. 2015;100(2):113-9. doi:10.1016/j.postharvbio.2014.09.023
7. Hamzah HM, Osman A, Tan CP, Mohamad Ghazali F. Carrageenan as an alternative coating for papaya (*Carica papaya* L. cv. Eksotika). Postharvest Biology and Technology. 2013;75(1):142-6. doi:10.1016/j.postharvbio.2012.08.012
8. López-Mata MA, Ruiz-Cruz S, Navarro-Preciado C, Ornelas-Paz J de J, Estrada-Alvarado MI, Gassos-Ortega LE, *et al*. Efecto de recubrimientos comestibles de quitosano en la reducción microbiana y conservación de la calidad de fresas. Biotecnia. 2012;14(1):33-43. doi:10.18633/bt.v14i1.113
9. Jiménez A, Fabra MJ, Talens P, Chiralt A. Edible and Biodegradable Starch Films: A Review. Food and Bioprocess Technology. 2012;5(6):2058-76. doi:10.1007/s11947-012-0835-4
10. Ramos-García M, Bosquez-Molina E, Hernández-Romano J, Zavala-Padilla G, Terrés-Rojas E, Alia-Tejacal I, *et al*. Use of chitosan-based edible coatings in combination with other natural compounds, to control *Rhizopus stolonifer* and *Escherichia coli* DH5α in fresh tomatoes. Crop Protection. 2012;38(8):1-6. doi:10.1016/j.cropro.2012.02.016
11. Barrera-Necha LL, Bautista-Banos S, Flores-Moctezuma HE, Estudillo AR. Efficacy of essential oils on the conidial germination, growth of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc and control of postharvest diseases in papaya (*Carica papaya* L.). Plant Pathology Journal. 2008;7(2):174-8. doi:10.3923/ppj.2008.174.178
12. Cagri A, Ustunol Z, Ryser ET. Antimicrobial edible films and coatings. Journal of Food Protection. 2004;67(4):833-48. doi:10.4315/0362-028X-67.4.833

13. Bosquez-Molina E, Jesús ER, Bautista-Baños S, Verde-Calvo JR, Morales-López J. Inhibitory effect of essential oils against *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* in stored papaya fruit and their possible application in coatings. Postharvest Biology and Technology. 2010;57(2):132-7. doi:10.1016/j.postharvbio.2010.03.008

14. Santamaría Basulto F, Díaz Plaza R, Sauri Duch E, Espadas y Gil F, Fernández S, Manuel J, *et al*. Características de calidad de frutos de papaya Maradol en la madurez de consumo. Agricultura técnica en México. 2009;35(3):347-53.

15. Acosta Ramos M, Nieto-Ángel D, Domínguez-Álvarez JL, Delgadillo-Sánchez F. Calidad y tolerancia en frutos de papaya (*Carica papaya* L.) a la inoculación del hongo *Colletotrichum gloeosporioides* Penz en postcosecha. Revista Chapingo Serie Horticultura 7(1): 119-130, 2001.

Received: March 14th, 2017
Accepted: October 17th, 2017