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Identification of Postharvest Chayote (*Sechium edule)* Diseases in México

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Abstract. *One of the most important problems during transport, storage, and marketing of smooth green chayote (Sechium edule) export fruits is rot through fungi. Few studies on the diseases attacking this fruit during the postharvest phase have been reported; therefore, the present research had the objective to identify the fungi causing chayote diseases in Mexico. Five different diseases were singled out: blister caused by Colletotrichum gloeosporioides, anthracnose caused by C. orbiculare, reddish-purple mould provoked by Fusarium oxysporum, white mould provoked by Phytophthora capsici, and acid rot caused by Geotrichum sp. It was also detected that an important source of fungi dissemination was triggered through the manipulation of selection and packing personnel. Finally, a previous study showed that hot water treatment (50 ± 1 ºC) and use of chlorine (1.5 %) for 30 seconds inhibits the development of the principal fungi identified in this study.*

Resumen. *Uno de los problemas más importantes durante el transporte, almacenamiento y comercialización de los frutos chayote (Sechium edule) verde liso es la pudrición por hongos. Pocos estudios se han reportado acerca de las enfermedades que atacan a este fruto durante la etapa postcosecha, por lo cual la presente investigación tuvo el objetivo de identificar los hongos causantes de las enfermedades de chayote en México. Se identificaron cinco diferentes enfermedades: la vejiga o ampolla causada por Colletotrichum gloeosporiodes, antracnosis causada por C. orbiculare, moho púrpura-rojizo causado por Fusarium oxysporum; moho blanco provocada por Phytophthora capsici y la pudrición ácida provocada por Geotrichum sp. También se detectó que una fuente importante de diseminación de los hongos se realiza por la manipulación del personal de selección y empaque. Finalmente un estudio previo mostró que el uso del tratamiento con agua caliente (50 ±1 ºC) con cloro (1.5 %) por 30 seg, inhibe el desarrollo de los principales hongos identificados en este estudio.*

Key words*: Rot, hydrotreatment, anthracnose, inoculum.*
Palabras clave: pudrición, hidrotratamiento, antracnosis, inóculo

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Sechium edule fruit (Cucurbitaceae) is consumed as vegetable at horticultural maturity. Its success on export markets has represented up to 8 % of annual growth (Anonymous, 2003). Mexico is the second world-wide exporter after Costa Rica, with a production estimated in 281,346 tons from approximately 3634 ha, 2500 ha of which are contributed by the State of Veracruz (Cadena, 2005). One of the main limiting factors in the marketing of this vegetable is the losses through fungi diseases, which reach from 15 % (Valverde *et al.*, 1989) to 25 % (Narayanasamy, 2006).

Chayote fruits deteriorate significantly at postharvest due to phytopathological problems; Saenz (1985) determined that the fruit diseases during storage are severer during the rainy seasons, since the splashing of rain is the medium of pathogen dissemination. Saenz and Valverde (1986) and Vargas (1987) mention *Mycovellosiella cucurbicola, M. lantanae, Ascochyta phaseolorum, and Macrophomina* sp. as principal
pathogens of chayote fruit in Costa Rica; whereas the plant is attacked by *Fusarium* sp., *Cladosporium* sp., *Macrophomina* sp., and *Colletotrichum* sp. In Mexico there are no reports on postharvest pathology of *S. edule* fruit; therefore, the objective of this study was to identify the principal fungus diseases of smooth green chayote *var.virens levis* for export, as well as their source of infection and dissemination.

Materials and Methods

Plant Material

S. edule var.virens levis (smooth green) export fruits were used, originating from commercial plantations of the Huatusco-Coscomatepec-Tuxpanguillo producer region, located in the central region of Veracruz, Mexico, in the months of June to December 2004 and October to November 2005. The fruits were harvested at horticultural maturity at 18 ± 2 days, equatorial width of 8-10 cm, 12-15 cm length, and mean weight of 322 g, following the standards of Mexican Official Regulation NMXFF-047-SCFJ-2003, equivalent to Codex-Stan-83-933 (Anonymous, 2003).

Two experiments were carried out; the first was divided in two phases; in the first, symptom description, isolation, and identification of the disease causal agent were made, and in the second phase, pathogenicity tests were conducted. In the second experiment, an assessment was performed in order to detect the possible source of fungi dissemination as well as the application of a hydrothermal treatment to control pathogen development.

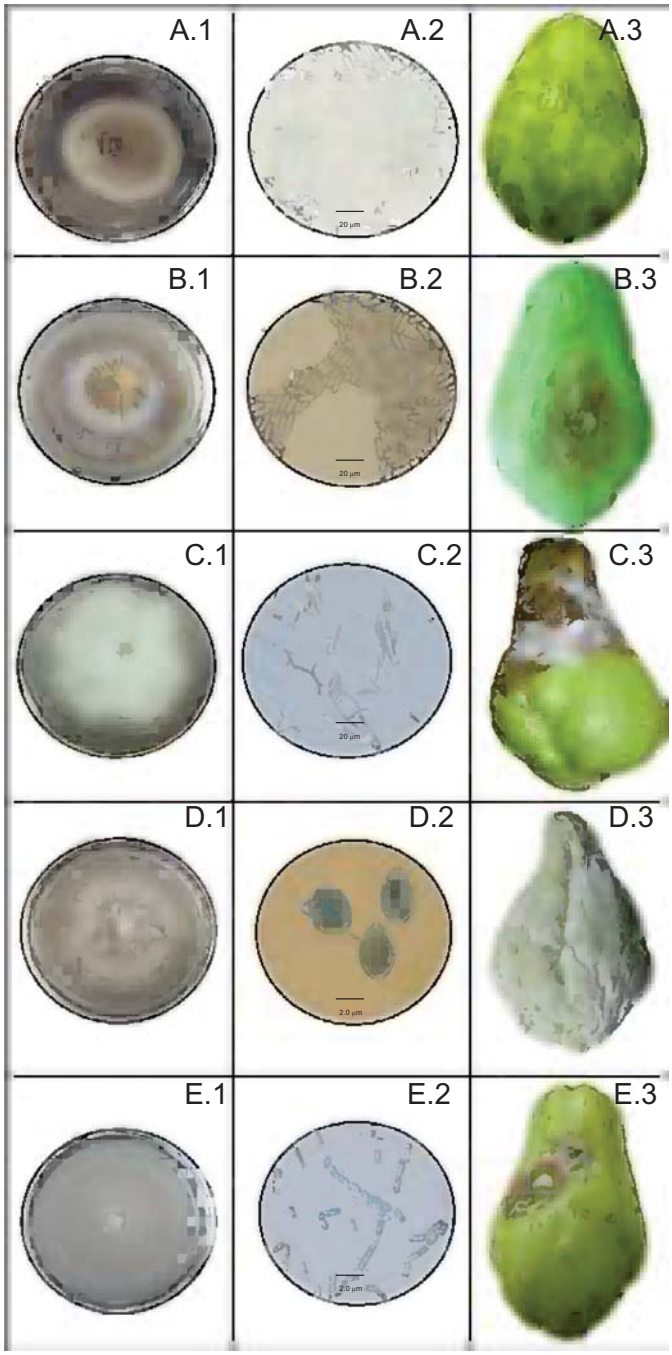

Figure 1. Mycelial growth (1), morphological structures (2) and symptoms of postharvest diseases in chayote fruits (3). A: Bladder or blister (*Colletotrichum gloeosporioides*); B: Anthracnose (*C. orbiculare*); C: Reddish-purple mould (*Fusarium oxysporum*); D: White mold (*Phytophthora capsici*) and E: Acid rot (*Geotrichum* sp).

Symptom Description, Isolation, and Identification of Phytopathogens

The symptoms were characterized from affected fruits as watery pustules with yellowish-green relief (bladder or blister) cleft brown spots with salmon-pink to black a acervuli (anthracnose), soft rots with spongy purple-white mycelium (reddish mould), soft rots with spongy whitish mycelium (white mould), and soft rots with creamy white surface mycelium (acid rot). Fifty cuts of each symptom with one healthy and one sick part of 1×0.5 cm were disinfected with sodium hypochlorite at 1.5% during 1.5 min; they were rinsed three times with distilled water and sown in Potato-Dextrose-Agar (PDA) medium. They were incubated during 72 h at white light, at 25 ± 2 ºC in Petri dishes. Isolation frequency of each symptom was evaluated eight days after sowing, being pre-identified by shape and color of the colonies. Subsequently, the final identification of the fungi was made through

symptomatology in the fruits, mycelial growth and morphology, utilizing the keys of Waterhouse (1963), Booth (1971) and Barnett and Hunter (1998).

Pathogenicity Tests (Koch's Postulates)

Once the characteristics of the possible pathogen causing the diseases isolated and described, the chayotes of the same variety and age were inoculated in the following way:

Concentration of inoculum, inoculation methods, and incubation conditions: A drop of polyoxyethylene sorbitan monostearate (Tween 20™) was added to each of the isolations, in order to distribute the propagules homogeneously, 1mL was taken to make the count using a hemacytometer or Neubauer camera under a compound microscope with a 40× objective, making three replications calculating with the following formula: $\Sigma Ci \times 2000$ = number of conidia mL^{-1}, where Ci = number of conidia in the i-eth quadrant (corners and center of the hemacytometer) (Frech and Hebert, 1980). From the mother solutions, solutions at concentrations of 5×10^5 conidia mL^{-1} were prepared for all the isolated fungi.

Visibly healthy fruits at horticultural maturity, harvested and manipulated with surgical gloves, were submerged in sodium hypochlorite at 1.5 % for three minutes, subsequently, 5 inoculation types were rinsed with distilled water and evaluated.

Deposition with lesion (Dp-h) and without lesion (Dp-s): Lesions of 1mm diameter and 2 mm depth were made in the equatorial parts of the fruits, and a conidial solution at 0.1 mL, containing 500,000 conidia, propagules, and/or fragmental spores $mL^{-1}$of each fungus was applied to separate fruits.

Slices of culture medium with mycelial growth with lesion (RM-h) and without lesion (Rm-s): The fruits were perforated at a diameter of 3 and 5 mm at the equatorial part, where a slice of culture medium with mycelial growth of the same dimensions as the perforation was placed and incubated in a moist chamber. For Rm-s, inoculation was without perforation, it was only put into contact with fruit epidermis.

Contact of sick with healthy fruits (Cs-e): Healthy fruits were put in contact with fruits diseased of each of the isolated fungi, simulating direct contact, which occurs during transport or storage, and they were incubated under moist chamber conditions (24 ± 3 ºC and 95 % HR).

Fungi purification and inoculum increase: With the obtained isolations dilutions were made to promote monoconidial, mono-angiospore, and mono-fragmospore cultures, using 10 mL of distilled water and one drop of Tween 20™, adding the conidia mother solution. One mL of this solution was diluted in 9 mL of sterilized distilled water; this dilution was made three times until obtaining a dilution of dispersed conidia. From this dilution three drops with dispersed conidia, angiospores, or fragmospores were sown in Petri dishes with PDA and incubated at 24 ºC during 24 hours, and before the mycelial growth areas of each conidium were joined, they were re-isolated in PDA, thus, pure cultures being obtained (Morales, 1996); subsequently, they were kept during 15 d under white light at 24 ± 2 ºC to increase the inoculum.

Evaluated Variables

Isolation frequency: Evaluated through the number of samples that presented the same growth of the fungus, with respect to the total number of samples sown for each symptom, expressed in percentage.

Incubation period: Time passed from inoculation until appearance of the first symptoms.

Incidence of diseases: Assessed, taking into account the number of fruits showing the symptoms of each of the fungi with respect to the total number of inoculated fruits, in field and in laboratory every 24 h, at 24 ± 3 ºC and 95 % HR.

Experimental Design

A completely randomized factorial design of 5 × 5 (five ways of inoculation, five fungi, and one control) was used, with three replications and two fruits as experimental unit.

Inoculum detection in the field and dissemination in packing

With the purpose of identifying probable fungi dissemination during the work of selection and packing, 100 fruits were washed with water in the field as well as the hands of the personnel carrying out fruit selection and packing. From both sources of washing, three water samples of 100 mL each were taken and 750 µL were deposited in Petri dishes with PDA, using five replicates per sample for a period of five days under laboratory conditions (24 ± 3 ºC and white light). The colonies per dish were counted and described by color and form. From these colonies semi-permanent preparations were made for the identification of the fungi found in those samples.

Hydrothermal treatment and use chlorinated water

A control treatment of fungal disease incidence was evaluated, which consisted in submerging 100 chayote fruits, inoculated with anthracnose and bladder, fungi of greatest economic importance, in chlorinated water (sodium hypochlorite at 1.5 % for 30

seconds), and subsequently in water at 50 ± 1 ºC during 10 seconds; they were incubated under moist chamber conditions (24 ± 3 ºC and 95 % HR), evaluating every five days during a total period of twenty.

Results and Discussion

Isolation Identification

The symptoms of the collected fruits with fungal diseases were classified as bladder or blister, anthracnose, reddish-purple mould, white mould, and acid rot, which were identified as *Colletotrichum gloeosporioides, C. orbiculare, Fusarium oxysporum, Phytophthora capsici,* and *Geotrichum* sp., respectively. It is worth pointing out that the common name of "bladder", which in Mexico is given to the symptom caused by *C. gloeosporioides,* does not correspond to "bladder" caused by *Mycovellosiella cucurbicola* and *M. lantanae* reported by Saenz and Valverde (1986) in Costa Rica. In none of the isolates made, origin, or collection period, any structure related with those fungi was registered. The detected fungi and their symptomatology are described below.

Table 1. Isolation frequency of fungi obtained from different symptoms of fungal diseases in postharvest chayote (*Sechium edule*).

Symptoms	Isolated Fungus	Isolation Frequency (%)
Bladder or blister: watery pustules, with relief and of yellowish green color	*Colletotrichum gloeosporioides*	84
Anthracnose: brown cleft spots with presence of salmon-pink to black accumulations	*Colletotrichum orbiculare*	100
Reddish-purple mould; soft rot with spongy reddish-white mycelium	*Fusarium oxysporum*	80
White mould; soft rot with whitish mycelium	*Phytophthora capsici*	100
Acid rot; soft rot with creamy white surface mycelium	*Geotrichum* sp	80

Isolation frequency was calculated based on 50 samples sown in Potato-Dextrose-Agar (PDA) culture medium for each of the diseases.

The percentage of isolation suggests that there was a high level of association between the isolated organism and the observed symptoms (Table 1), which as well in the management of fruits of economic importance has been translated into considerable losses. In Castilla blackberry (*Rubus glaucus*) Cedeño and Palacios-Prü (1992) reported up to 75 % of fruits infected by inoculum of *Glomerella cingulata*, whose anamorphic phase was identified as *Colletotrichum gloeosporioides* (Penz.) Penz.& Sacc. In other fruits of economic importance such as melon (*Cucumis melo*) and papaya (*Carica papaya* L.), Albornett and Sanabria de Albarracín (1994) identified *C. gloeosporioides, Fusarium oxysporum,* and *Cladosporium cucumerinum* as the principal causes of postharvest diseases, whereas the main cause of high postharvest losses in pepper (*Capsicum annuum*) is attributed to the infection of *P. capsici.* With relation to *Geotrichum* sp., it has been determined that *G. candidum* species is severely parasitizing citrus fruits (Suprapta *et al.*, 1995).

Symptoms description and identification of phytopathogens

Bladder or *blister:* The symptom was the presence of watery pustules with relief of yellowish-green color, of 0.5-1.0 cm diameter (Figure A.3), being identified, according to Sutton (1980), as *Colletotrichum gloeosporioides,* whose morphological characteristics are: elliptic- cylindrical hyaline conidia with rounded apices, unicellular, 9 to12 µm and 3 to 4 µm wide (Figure A.2). Furthermore, small accumulations with septa, typical of the species, were observed. The colonies sown in PDA culture medium were grayish white with pink to orange tonalities in the center, and dark gray or black at the back (Figure A.1) (Holliday, 1984; Bailey and Jeger, 1992). Likewise, Vargas (1987) in Costa Rica, describes the bladder symptom as large protuberances, of 2-7 mm diameter and watery appearance, slightly lifted, irregularly distributed on the fruit. This author indicates *Mycovellosiella* as causal agent of the disease, which forms grayish colonies of little aerial growth and tight hyphae (1.5 -3.5 µm), producing abundant primary conidiophores, simple or ramified, dark and of variable length (12-35 µm), some up to 80 µm. Nevertheless, due to the symptomatology of bladder found in this study, it is caused by *C. gloeosporioides.* This fungus attacks the fruits in the field. When the spores germinate, they form *appresorium,* but the subcuticular hypha does not develop until the fruit advances in its

maturity. It has been mentioned that germination, formation, and adhesion of *appresorium* in *C. gloeosporioides* on the fruit surface might be directly related to the nature of epicuticular waxes of the host fruit (Podila *et al.*, 1993)

Anthracnose: The symptom on the fruit was sunken brown spots with salmon-pink to black little accumulations, *C. orbiculare* being isolated (Figure B.3). Their conidia are hyaline without septa, generally oblong and 14 µm long by 4.5 µm wide (Figure B.2) (Sutton, 1980). The colonies having grown on PDA were of grayish white color with yellowish tonalities in the center and dark gray or black at the back (Figure B.1). The development of the fungus is favored by high relative humidity and temperatures between 19 and 24 ºC; the conidia are disseminated by water as well as by the contact of the fruit with infected vegetal residues and by certain insects (Blancard *et al.*, 2000).

Reddish-Purple Mould: F. oxysporum was identified (Figure C.3), the colonies were of fast growth in PDA culture medium; the mycelium, white at the beginning and later on changing to pink or reddish, was of spongy consistence (Figure C.1) (Nelson,1927; Booth, 1971). The *F. oxysporum* colonies presented sickle-shaped macro-conidia with bottle-shaped *"monofialides"* and fusiform micro-conidia. The conidiophores hold the *monofialides* sideways, the conidia (micro-,and macro-conidia) originate from the apex; micro-conidia were of ellipsoid-oval form, sized 6-15 × 3-3.5 µm, the hyaline macro-conidia had three to five elongated transverse septa with the pointed apex and the base in form of a foot with thin walls, measuring 34-60 × 3-5 µm (Figure C.2) (Booth, 1971). This fungus is a saprotroph of soil and organic matter, which has the capacity to survive in the soil and among the different plant cycles, being able to survive as mycelium or in any of their different types of spores, dispersing through leaves and fruits and contaminated equipment as well as by the wind (Agrios, 2002).

White Mould: The symptoms were soft rot with whitish mycelium, identifying *P. capsici* (Figure D.3); the mycelium is c*enocitic"* with abundant straight and swollen ramifications, ovoid sporangium of 1.7 µm length by width of indeterminate growth, with conspicuous papilla, twisted and occasionally with two papillae with long petiole (Figure D.2) (Waterhouse, 1963). Likewise, the colonies presented whitish mycelium finely radiate, star-shaped (in the form of a star) (Figure D.1). Initial infection of *Phytophthora* usually occurs in plants growing in zones of high humidity, the fungus survives as resistant spores (co-spores) in the soil and as mycelia in tissue of the infected plant. These fungi, once in the soil, are capable of remaining in the humid soil without a host up to 15 months (Agrios, 2002).

Acid or Bitter Rot: The symptom caused by *Geotrichum* sp.was soft rot with creamy white surface mycelium (Figure E.3).The mycelium is septate with conidia (fragmospores) in cane form, with truncated ends and formed by mycelium fragmentation (Figure E.2) (Barnett and Hunter, 1998). Furthermore, the colonies presented creamy-white surface mycelium (Figure E.1). This fungus may develop under conditions of poor ventilation, when the air is hot, humid, and low in oxygen; the infection occurs in the field but develops very well during postharvest stage (Snowdon, 1991).

Pathogenicity Tests

It was only *P. capsici* among the assessed types of inoculation (Dp-h, Dp-s, Rm-h, Rm-s, Cs-e) with the different fungi species, that induced symptoms at being inoculated by direct contact of sick fruits with healthy ones (Cs-e), which became evident at 72 h with an incidence of 100 %. Under field conditions, inoculation type Dp-h showed an incidence of 40 % in the first 48 h, and of 100 %, at nine days after inoculation. The fact that only one fungus (*P. capsici*) and two inoculation types (contact and lesion) provoked the development of the symptom of one single disease (white mould) in the pathogenicity tests made with artificial inoculation, suggests that the symptoms of the fungi *C. gloeosporioides, C. orbiculare, F. oxysporum*, and *Geotrichum* sp., becoming evident afterwards, might be due to latent infections, occurring at a susceptible fruit stage in the field, and which finally, at postharvest, manifest themselves in a natural way. It is probable that subsequent fungi germination is facilitated by conditions of humidity generated by the fruit itself in plastic packing during transport and/or commercial storage, because of the high transpiration rate of chayote fruit (Cadena *et al.*, 2006).

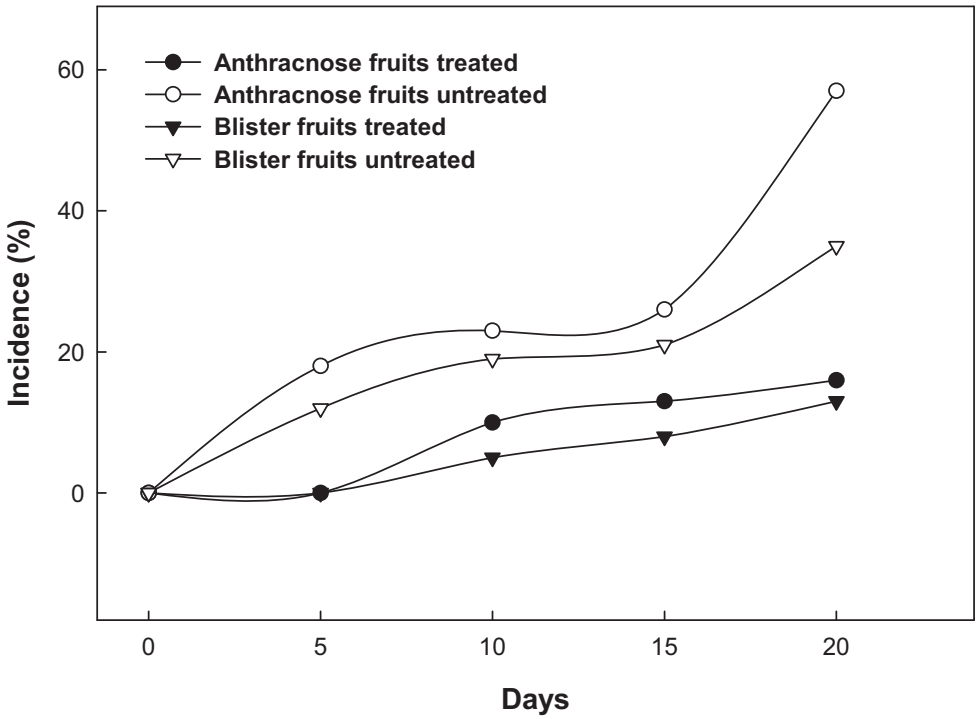

Figure 2. Incidence of anthracnose and blister in chayote fruits treated with water (50 ± 1°C, 10 sec) and storage during 20 days at 24 ± 3°C and 90 % RH.

Detection of Inoculum in the Field and Dissemination in Packing

Among the samples of fruit washing obtained in the field, *Geotrichum* sp. was identified with an average of 40 colonies per box, with creamy white mycelium, septate, with conidia (fragmospores) in the form of a walking stick, with truncated ends and formed by mycelium fragmentation; whereas from the samples obtained by the packing staff's hand washing, *Geotrichum* sp., *F. oxysporum,* and *Cladosporium cucumerinum* were identified with an average of 45, 32, and 1 colonies per box, respectively. *Geotrichum* sp. colonies showed septate, creamy-white mycelium, with cane-shaped fragmospores with truncated ends (Barnett and Hunter, 1998); whereas those of *F. oxysporum* had spongy pinkish-white mycelium. Besides, they presented micro-conidia of ellipsoid-oval shape, hyaline macro-conidia of three to five septa, elongated with pointed apices and foot-shaped base (Booth, 1971). The *C. cucumerinum* colonies showed velvety green mycelium, long ramified conidiophores, grouped or solitary, straight or flexible, tight at the base and widening towards the apex, with one-sided nodules like short light brown or olive-brown branches; dark conidia (blastospores) of variable form and size, ovoid, cylindrical, or irregular of 4.6 – 5.7 × 16.4 - 22.5 µm (Zitter *et al.*, 1996).

The results suggest that spore dissemination is present to a higher degree during fruit handling at harvesting and packing, due to the selection and packing staff's manipulation, since not any disinfection treatment is carried out in order to prevent postharvest diseases.

The fact that *Colletotrichum* sp. conidia have not been detected in any of the samplings, in spite of being one of the fungi that most frequently appear in form of bladder and anthracnose, might be due to the sampling being carried out in field in the morning (9:00 – 10:00 a.m.), when conidia had not yet been released, as well as the sampled fruits were visibly healthy. Nevertheless, the damage caused by these diseases and the presence of conidia was observed after 10 and 15 days of harvest, which indicates that the infection occurs in the field at an early and susceptible stage of the fruit, and it becomes evident subsequently, at postharvest. With regard to the absence of *P. capsici,* it may be attributed to the fact that all the fruits were harvested manually, and zoospores had not yet been released, since for this, temperatures between 20 and 40 ºC are required, and none had contact with the soil, this fungus being one of its natural dwellers (Galindo 1960).

Hydrothermal Treatment and Chlorinated Water

Damages, caused by handling and natural openings of the fruit surface (lenticels and stomata), a frequent route to infection, are the factors, which influence the infection of harvested fruits, especially, if it has been handled or rinsed with water after harvesting, and comes from the field, infected. Regarding this, hydrothermal treatment has proved to be effective in preventing the development of rotting, due partly to temporary thermal inhibition of the pathogen (germination and growth), permitting the infected fruit to increase the response to resistance. This resistance mechanism of the vegetal tissue consists in the fast production

of lignins at the inoculation site, followed by accumulation of phytoalexins. Furthermore, the hot water provokes a redistribution of the epicuticular wax layer and a significant reduction of cuticular breaking, increasing the physical barriers against pathogen penetration. Besides, heat may inhibit the pathogen dispersion by inducing defense mechanisms in the external layers of the epicarp (Fallik, 2004). On the other hand, it has been proved that the immersion of the fruits in chlorinated water reduces postharvest damages (Barkai and Phillips, 1991). Albornett and Sanabria de Albarracín (1994) disinfected diseased fruits in papaya and melon with sodium hypochlorite at 3 % (NaClO) during 3 minutes, in order to eliminate several fungi likd *Phomopsis* sp.,*Colletotrichum gloeosporioides, Colletotrichum dematium, Glomerella cingulata, Fusarium* sp., *Rhizopus stolonifer, Penicillium implicatum* among others with success. Likewise Barbosa *et al.,* (2000) proved that chlorine and iodine are effective germicides against *F. oxysporum, Lasiodiplodia theobromae,* and *Pestalopiopsis mangiferae.*

In this study, hydrothermal treatment and chlorinated water reduced anthracnose and bladder incidence in the fruits by 64 and 71 %, respectively. The treated fruits delayed the incidence of both diseases by five days. Infection by anthracnose after 20 days of storage was 13 % in the fruits subjected to hydro-treatment; whereas in the control fruits, incidence was by 36 %. For bladder, incidence was 16 % in treated fruits, and for control fruits 57 % (Figure 2). The aforementioned suggests that rinsing with chlorinated water and hydrothermal treatment may be a good option to minimize the losses in postharvest chayote. Alvarado *et al.,*(1998) reported a type of physical control of bladder subjecting chayote fruits to a temperature of 50 ºC; these thermal treatments have also been effective in reducing incidence of *Penicillum* spp. and *Colletotrichum* sp. in citrus fruits, avocado, and other fruits (Fallik, 2004; Couey, 1989).

Conclusions

The symptoms observed in commercial fruits of *S. edule* registered a high level of association related to isolation frequency of the fungi *Colletotrichum gloeosporioides, C. orbiculare, Fusarium oxysporum, Phytophthora capsici* and *Geotrichum* sp.. It is probable that the infection of the fruits begins in the field during the early growth stages, and that the growth of these fungi is favored by the conditions of high relative humidity during their subsequent management. It has been observed that handling in the packing department promotes the dispersion of the pathogens such as *Geotrichum* sp., *Fusarium oxysporum* and *Cladosporium cucumerinum.* The treatments with hot and chlorinated water seem to be a good option in reducing the incidence of diseases, for which it is considered important to develop more intense research with regard to this, which would contribute to reducing the losses by rot in chayote fruits.

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