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USE OF *Penicillium*, *Colletotrichum*, AND *Rhizopus* SPECIES IN ORGANIC WASTE TREATMENT

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Abstract

As population grows, and with it the quantity of waste produced, it becomes more important to find ways to recycle waste or to shorten degradation time of organic waste materials. This study was conducted to evaluate the capacity of *Colletotrichum* and *Rhizopus* species to degrade 'Paraiso' mango fruits and of *Rhizopus* and *Penicillium* species to degrade 'Valencia' orange fruits. In both cases, intact and chopped fruits were inoculated with the fungi by spraying (3.5 x 10^6 spores/mL). Three fruits were an experimental unit, with three replicates per treatment; seven treatments were done per fruit. Weight loss, pH and volume of leachates, incidence and severity of damage caused by the fungi were recorded daily for 33 days. Results showed greater weight loss and leachates production in both types of chopped fruits inoculated with fungi. Incidence and severity was greater in mango inoculated with *Colletotrichum*. It is concluded that degradation of mango and orange fruits was greater when inoculated with a mixture of *Colletotrichum* and *Rhizopus;* and *Penicillium* and *Rhizopus*, respectively.

Key words: Fungi, postharvest losses, leachates, fruit degradation.

INTRODUCTION

Inadequate management of solid waste leads to soil, air and water pollution. Rational use of municipal solid waste (MSW) can reduce damage to the environment. Mexico's City population generates approximately 12000 t of solid residues every day; 28% is organic matter from households, commerce and industry. Nearly 22% of the organic solid waste (OSW) come from the Central de Abastos (central wholesale market), that is, 21000 t of OSW per month. Treating the OSW would help to prevent their disposal in city landfills and prolong the useful life of these. Treatment such as composting, on one hand, reduces pollution and, on the other, permits recycling wastes to convert them into organic fertilizer. However, most of the organic waste from Mexico's City Central de Abastos is deposited in landfill and is not used in composting; its high water content creates anaerobic conditions that limit the composting process.

Composting transforms organic waste into compost that can be used to improve soils for agriculture. However the quality of the compost is directly related to its potential use. In the composting process biodegradable waste (left over food, fruits and vegetables, branches and leaves of trees, wood in general, grass, straw, animal excrement, and paper) can be treated. However, if the thermophilic phase of the process is not adequate, pathogens harmful to humans and plants may survive (Johnson, 1996), and when the compost is applied to fields undesirable microorganisms will be dispersed; among these may be plant pathogens that can cause epidemics in crops.

Year after year millions of dollars are lost in crops that are susceptible to a wide range of pathogenic microorganisms such as fungi, bacteria, insects and nematodes. These can damage, or even cause the death of seeds, stems, leaves, fruits and roots. In the last 40 or 50 years synthetic pesticides have been used to control these agents (EPA, 1998). However, most of the commonly used pesticides have been prohibited or severely restricted during the last 20 years (Quarles and Grossman, 1995), motivating the search for alternatives, among the biological control. Some of the available alternatives for natural control that have great potential are composting and compost, which have been extensively studied (EPA, 1998).

Mango is affected by a large number of diseases caused by bacteria, fungi and nematodes. However, the most important of these is anthracnose, which affects fruits in pre- and post-harvest. Anthracnose, a disease caused by the fungus *Colletotrichum gloeosporioides*, is the most important mango disease in all of the producer regions of the world and is associated with conditions of high relative humidity (Fitzel and Peak, 1984; Jeffries *et al*., 1990; Dodd *et al*., 1992). This disease affects young leaves and flower panicles; the fungus causes quiescent infections in developing fruits and grow when the fruit is ripe. However, the degree of damage depends on several factors, such as orchard management, weather conditions during fruit development, storage conditions in postharvest and mango variety (Dodd *et al*., 1989).

The growth of microorganisms causing rotting is the main problem in loss of quality during mango fruits marketing. Besides anthracnose, rotting is caused by *Botriodiplodia theobromae* is another important fruit disease, which develops as the fruit ripens, making it difficult to detect symptoms in immature fruits. The process of inoculation occurs in leaves, stems and flowers, and pre-harvest treatments are important for reducing post-harvest losses (Ledger, 1991).

Most studies are focused on damage assessment in mango fruits with the objective of reducing this damage to give fruits longer shelf-life. Mena *et al*. (1996) stated that the incidence of damage caused by *Colletotrichum* in 'Manila' mango fruits stored at 10ºC for 18 days reaches 67%, which is reduced to 7.7% with a hydrothermal treatment at 46.1 ºC for 90 min. Regarding severity of the disease, Gutiérrez *et al*. (2003) found damage of up to 94% in fruits with lesions and 9.2% in fruits without lesions inoculated with isolates of *C. gloeosporioides* after a week of storage in plastic bags at 24 ºC.

Rotting caused by *Penicillium digitatum* in 'Persa' limes covered individually with plastic film and stored for 30 days with a gradual decrease in temperature (up to 1.5 ºC) had an incidence of 24% (Saucedo and Mena, 1995).

No studies were found in mango or orange that evaluates damage caused by these fungi to complete degradation since this degree of rotting would not be included in the study of post-harvest management or plant pathology, areas that focus on quality conservation or reduction of damage. It is, however, of interest in waste management.

According to Kashmanian (1993), some countries have developed or proposed legislation to control the use of soil amendments produced by composting of different types of organic material. Thermophilic conditions and the intense microbial competition during composting kills or inactivates almost all of the microorganisms that cause diseases in plants, animals or humans (Farrell, 1993; Bollen and Volker, 1996; Avgelis and Manios, 1992). One exception to this is the Tobacco Mosaic Virus, which can survive composting. Once the infested crop wastes have been composted, the material is no longer infective and may be applied safely to field crops without provoking disease. In contrast, non-composted wastes can be a source of inoculum. The composting process has been proved to be effective in the destruction of nematodes, bacteria, viruses and pathogenic fungi (Bollen and Volker, 1996; López-Real and Foster, 1985; Bollen, 1985).

Akbar *et al*. (2001) analyzed the thermophilic and mesophilic phases during the composting process of OSW of the municipality of Esfahan, Iran, for up to 28 days and over the four seasons of the year. They found that after 20 days at temperatures of 60-68ºC mesophilic fungi did not survive, but on day 15 a large quantity of heat tolerant fungi such as *Cladiosporium*, *Aspergillus*, *Mucor rhizopus* and *Absidiae* species were observed. They indicated that, according to their results, to obtain a quality product during the cold seasons, the composting process requires a longer period and must be turned more often. Quality control of composts is, therefore, of utmost importance in the biological control of plant pathogens area (Litterick *et al*., 2004). Bollen *et al*. (1989) consider heat produced during composting the most important factor in eliminating pathogens, they have reported that the temperature of 50-70 ºC for six days, or a thermophilic phase above 40 ºC for 2 to 3 weeks plus a maturation phase of less than 40 ºC for 5 months, will eliminate most of the pathogens (including 17 fungi identified) in crop residues. Only *Olpidium brasicae* and one specie of *Fusarium oxysporum* survived composting, although the inoculum was drastically reduced. At the present work, the objective was to study the degrading capacity of *Colletotrichum* and *Rhizopus* species in mango fruits and *Penicilium* and *Rhizopus* species in orange fruits; in order to evaluate their use in a composting process preteatment.

MATERIALS AND METHODS

Plant material. ‘Paraiso’ mango and ‘Valencia’ orange fruits were obtained from the Mexico’s City Central de Abastos. The fruits were selected according to its size and ripeness, washed under running water and disinfested with a solution of 1.5% sodium hypochlorite for 3 min.

Fungi (inoculum). The fungi (*Colletotrichum* sp. and *Penicillium* sp.) used in this study were isolated and purified from mango and orange fruits with symptoms of anthracnose and green mold, respectively. *Rhizopus* sp. were obtained from tomato rhizopus rot symptom.

Degradation tests. Intact and chopped mango fruits were inoculated by spraying with a solution of equal parts of pure (monosporic) cultures of *Colletotrichum* sp. and *Rhyzopus* sp. (3.5 x 10^6 spores/mL). As the same, orange fruits were inoculated with *Penicillium* sp. and *Rhyzopus* sp. The inoculated fruits were placed in 3 kg capacity plastic trays and incubated at 25 ºC and 95-100% relative humidity (RH) for 33 days. The experimental unit was three fruits, and three replicates per treatment. The treatments in mango and orange were distributed in the following manner:

a) Mango fruits

Intact mango fruits (IM)

Chopped mango fruits (ChM)

Intact mango fruits inoculated with *Colletotrichum* sp. (IM+C)

Chopped mango fruits inoculated with *Colletotrichum* sp. (ChM+C)

Intact mango fruits inoculated with *Rhizopus* sp. (IM+Rh)

Chopped mango fruits inoculated with *Rhizopus* sp. (ChM+Rh)

Intact mango fruits inoculated with *Rhizopus* sp. + *Colletotrichum* sp. (IM+RhC)

Chopped mango fruits inoculated with *Rhizopus* sp. + *Colletotrichum* sp. (ChM+RhC)

b) Orange fruits

Intact oranges (IO)

Chopped oranges (ChO)

Intact oranges inoculated with *Penicillium* sp. (IO+P)

Chopped oranges inoculated with *Penicillium* sp. (ChO+P)

Intact oranges inoculated with *Rhizopus* sp. (IO+Rh)

Chopped oranges inoculated with *Rhizopus* sp. (ChO+Rh)

Intact oranges inoculated with *Penicillium* sp. + *Rhizopus* sp. (IO+PRh)

Chopped oranges inoculated with *Penicillium* sp. + *Rhizopus* sp. (ChO+PRh)

Evaluated variables

The variables that were evaluated daily up to 33 days were the following:

a) Weight loss

A 2.5 kg capacity digital balance was used. The fruits were weighed daily and weight loss was obtained with the following equation:

$$WL = \frac{Wi - Wf}{Wi}$$

where WL=weight loss (g)

Wi=initial weight (g)

Wf=final weight (g)

b) Production of leachates

The volume of leachates produced daily was determined with a 100 mL graduated glass test tube. For accumulated leachates, volume was summed consecutively.

c) Leachates pH

pH was determined daily with a digital pH-meter.

d) Data analysis

A completely random design was used. The data were analyzed with an Analysis of Variance and the Tukey comparison of means (α=0.05) method. The SAS System for Windows v. 6.12 software was used.

RESULTS AND DISCUSSION

a) Mango fruits

Fig. 1a shows that inoculated treatments with the mixture of pathogens (IM+RhC) and those inoculated with *Rhizopus* (IM+Rh) had the greatest weight losses: 22 and 25%, respectively, 33 days after inoculation. In contrast, the control, intact mangos (IM) and fruits inoculated with *Colletotrichum* (IM+C) had values of 10 and 13%, respectively, over the same time.

Weight losses for treatments of chopped mango are found in Fig. 2a. All of the treatments surpassed 30% on day 33 after inoculation. Chopped fruits inoculated with the mixture of pathogens (ChM+RhC) were those for which the greatest weight losses (35 %) were recorded.

In general, the intact fruit treatments (Fig. 1a) had the lowest weight losses compared with the chopped fruit treatments (Fig. 2a).

Leachates production in the treatments of intact mango fruits (Fig. 1 b) was less than the obtained in the treatments with chopped fruit (Fig. 2b).

Fig. 1b shows the leachates production in treatments with intact mango fruits. The inoculated treatments with *Rhizopus* (IM+Rh) and the mixture of pathogens (IM+RhC) produced 45 and 60 mL of leachates respectively, 20 days after inoculation, while the control (IM) and the inoculated with *Colletotrichum* (IM+C) produced 7 and 12 mL.

Fig. 2b shows the leachates production in treatments with chopped mango. Treatments ChM+RhC and ChM+C had the highest production of leachates from day 4 to day 23, when leachates production decreased in all of the treatments.

The highest production of leachates from the treatments with intact fruit occurred between days 10 and 23 (Fig. 1b), while in chopped fruit (Fig. 2 b) it occurred from day 4 to day 23. This is due that in chopped fruit the cells damage is greater when fruits are chopped into smaller pieces and the surface in contact with the pathogen is larger, causing greater degradation and consequently greater damage and higher production of leachates.

Accumulated production of leachates from treatments with intact mango fruits is found in Fig. 1c and again, the IM+RhC and IM+C treatments are those that had the highest production of accumulated leachates: 160 mL and 175 mL, respectively, 33 days after inoculation. IM and IM+C treatments produced only 55 mL and 65 mL over the same time period.

Fig. 2c presents the production of accumulated leachates from treatments with chopped mango fruits. Treatments ChM+RhC and ChM+C had a production of nearly 250 mL, while treatments ChM and ChM+Rh did not surpass 200 mL.

The importance of inoculation with pathogens in the production of leachates is evidenced. The mixture of pathogens produced more leachates, in intact and chopped fruits, and production was greater in the latter.

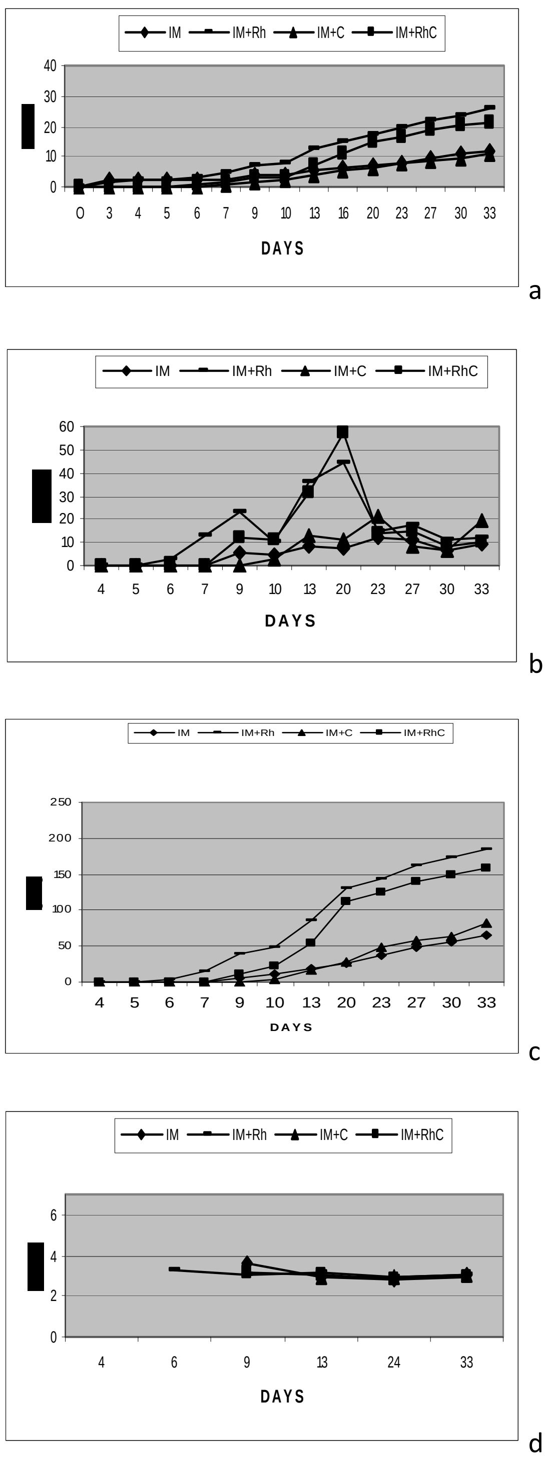
Fig. 1d and 2d show the pH value of leachates obtained in mango fruits. In these figures it can be observed that generally the pH range is between 2.6 and 3.6. It can also be observed in the control with intact fruit (IM), the first value of pH recorded does not appear until day 9, the day leachates production began in this treatment.

b) Orange fruits

Orange fruits used in the evaluation of weight loss resulted similar to those obtained with mango fruits: chopped oranges (Fig. 4a) had higher weight loss than intact fruits (Fig. 3a).

It can also be observed that intact fruits of the control treatment (IO) were those that lost the least weight (Fig. 3a). The oranges remained intact even after 33 days, while chopped fruits of the control treatment (ChO) had weight losses above 50% (Fig. 4a). The treatment with the mixture of pathogens (ChO+PRh) in chopped fruits followed as the treatment with greatest weight loss.

Intact mango fruits



Chopped mango fruits

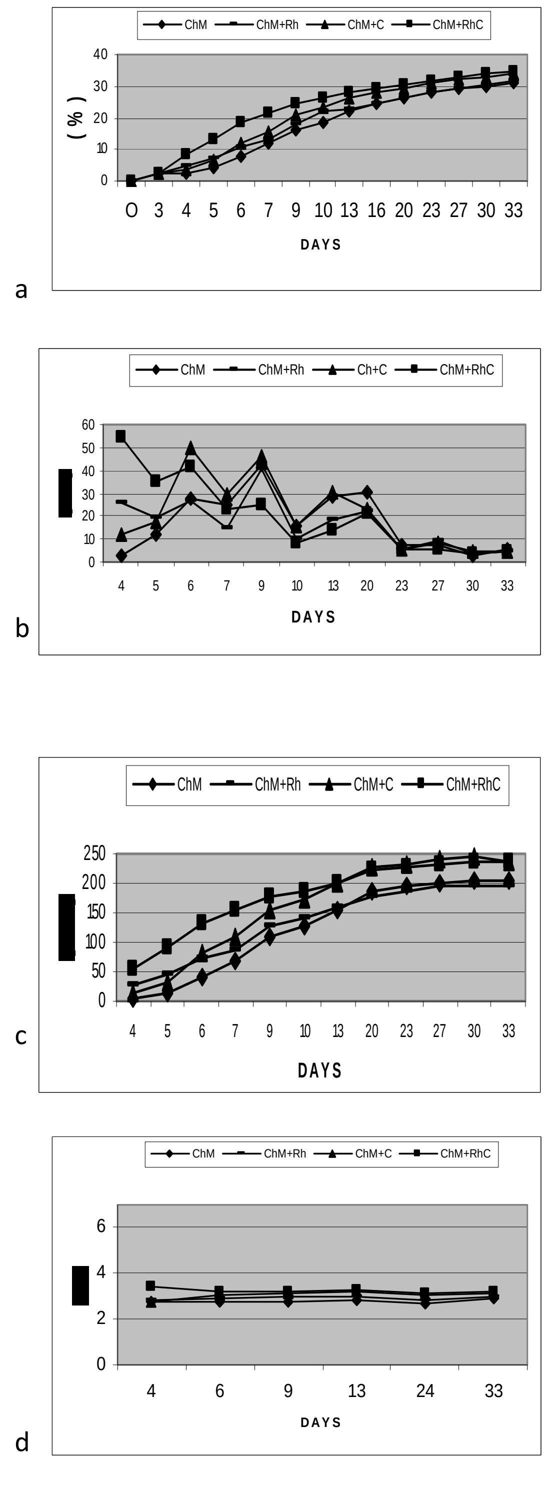


Fig. 1. Changes in weight loss (a), leachates production (b), accumulated (c), and pH (d) of leachates in intact mango fruits.

Fig. 2. Changes in weight loss (a), leachates production (b), accumulated (c), and pH (d) of leachates in chopped mango fruits.

Intact orange fruits

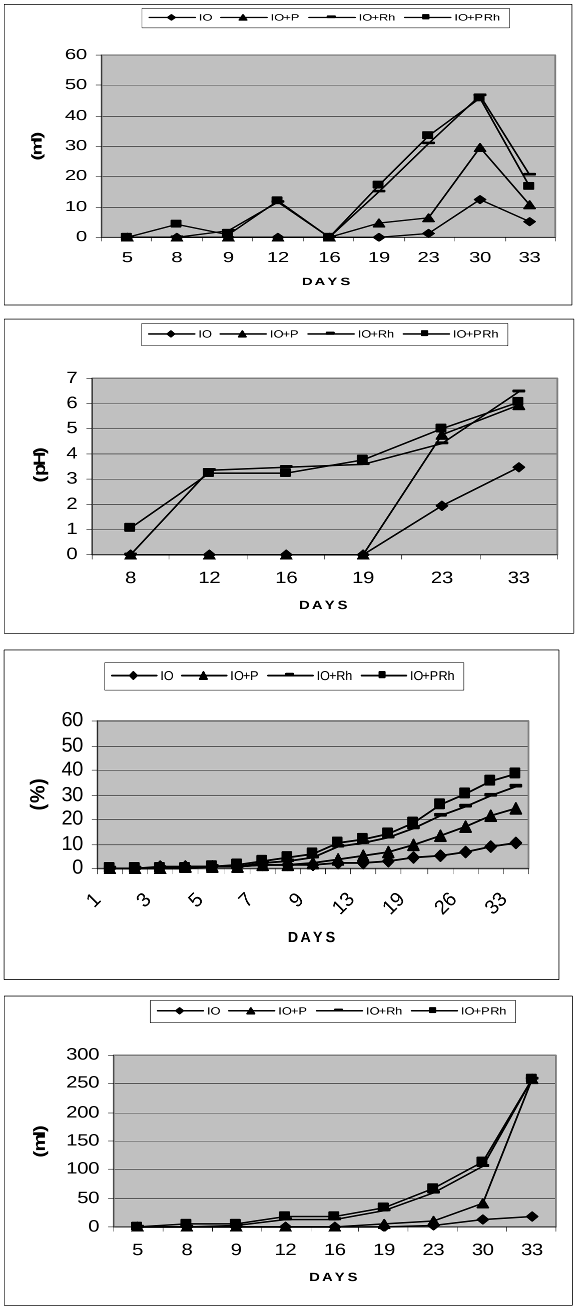


Fig. 3. Changes in weight loss (a), leachates production (b), accumulated (c), and pH (d) of leachates in intact orange fruits.

Chopped orange fruits

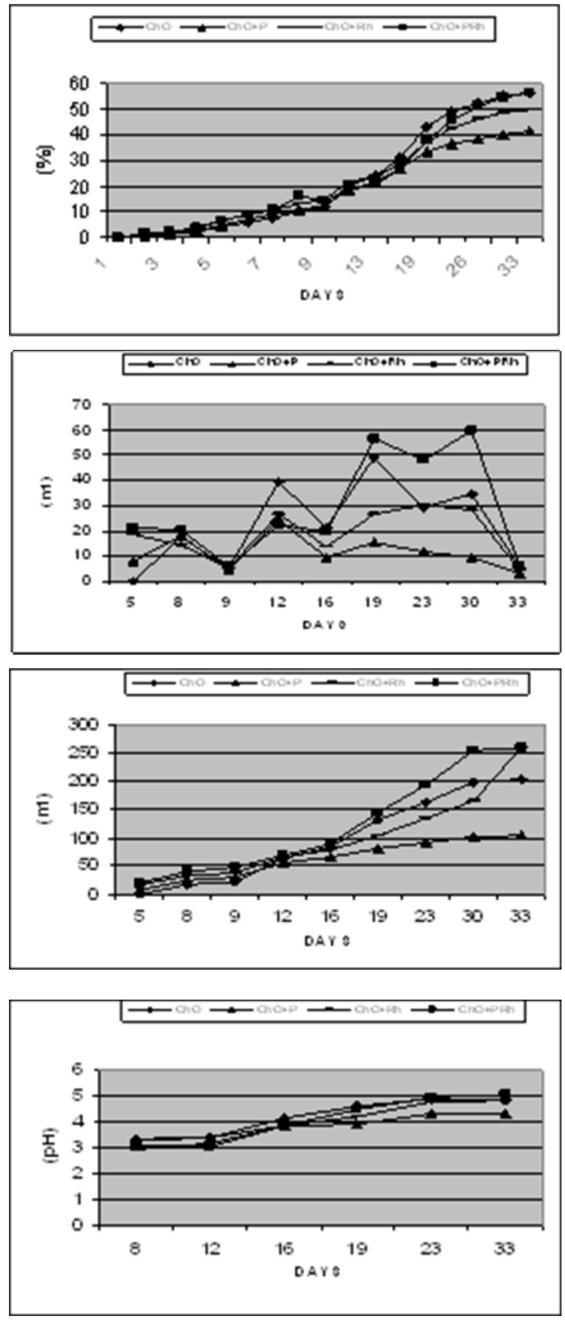


Fig. 4. Changes in weight loss (a), leachates production (b), accumulated (c), and pH (d) of leachates in chopped orange fruits.

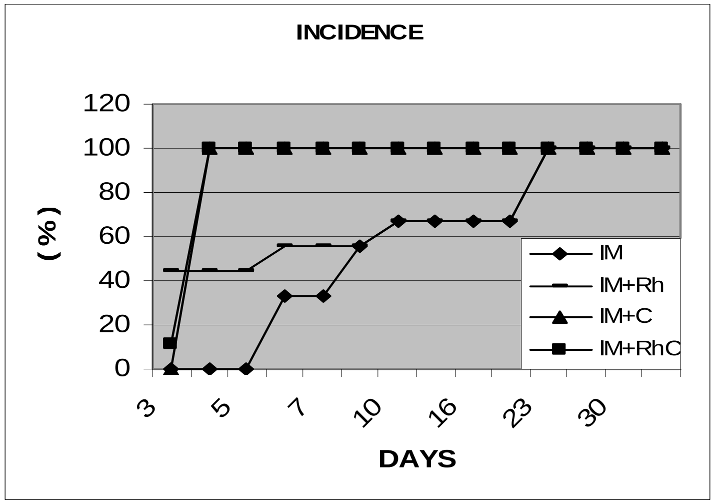


Fig. 5. Incidence of damage in mango fruits inoculated with *Rhyzopus* and *Colletotrichum*.

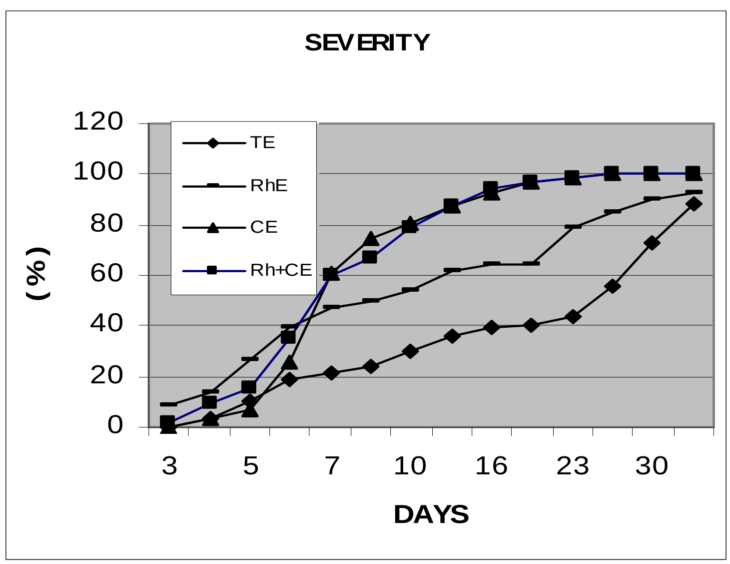


Fig. 6. Severity of damage in mango fruits inoculated with *Rhyzopus* and *Colletotrichum*.

During the first 15 days of the experiment, oranges produced a lower volume of leachates than mangoes. The treatments with oranges that had the highest production of leachates did not reach 100 mL (Figs. 3a, 4a), while the treatments with mango produced more than 200 mL (Fig. 2c) of leachates. Mangoes contain more sugars than oranges making mangoes a better nutrient culture medium for the development of fungi. Consequently, the fungi are able to cause greater degradation and release more leachates.

It can also be observed that the treatments with chopped oranges (Fig. 4c) had a higher leachates production than the treatments with intact fruits (Fig. 3c). Leachates production on day 30 was 250 mL, 200 mL and 100 mL for ChO+PRh, ChO and ChO+P, respectively, while for intact fruits over the same time was 100 mL, 15 mL and 40 mL for IO+PRh, IO and IO+P respectively (Fig. 3c).

For oranges, leachates pH is more acid (1-3 on day 8) (Fig. 3d) than that obtained with mango fruits (2.7-3.2) (Fig. 1d).

On day 33 the resulting pH in treatments with oranges was above 3.5 in all of the treatments (Fig. 3d), while in mangoes pH value did not reach 3.5 (Fig. 1d and 2d). This shows a metabolism different from that of mangoes, a factor that should be considered if discarded fruits are to be used in compost piles because it will have some influence on the process.

It can also be observed that the oranges used in treatment IO (Fig. 3d) had the smallest change in the pH of its leachates and production began after day 20.

CONCLUSIONS

Plant pathogenic fungi play an important role in the degradation of organic matter. The affected fruit part is different depending on the type of fruit and species or genus of fungus each of which has a distinct mechanism of infection and source of energy. Hence, it can be said, in general, that: *Colletotrichum* basically damages the fruit peel, causing little damage to the flesh, *Rhizopus* attacks the flesh of both fruits (orange and mango) more severely than the other fungies. *Penicillium* attacks both fruit flesh and peel (oranges). The presence of *Colletotrichum, Penicillium and Rhizopus* can accelerate the composting process. A higher degree of degradation occurred in chopped fruits than in intact fruits, while a greater degradation was observed in inoculated treatments with a mixture of fungi, resulting in a higher production of leachates. Weight losses in fruits reached up to 35% in mangoes and more than 50% in oranges, by the other hand, fruit degradation was faster in mangoes than in oranges.

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