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Full Length Research Paper

Degradation capacity of fungi (*Colletotrichum* sp., *Penicillium* sp. and *Rhizopus* sp.) on mangoes and oranges

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Most fruits, flowers and vegetables show diseases caused by specific microorganisms that generate large postharvest losses. The degradation capacity of some phytopathogenic fungi could be useful to treat organic wastes, for instance in a pre-composting process. The objective of this study was to evaluate the capacity of *Colletotrichum* sp., *Rhizopus* sp. and *Colletotrichum* sp. + *Rhizopus* sp. to degrade 'Paraíso' mangoes, and *Rhizopus* sp., *Penicillium* sp. and *Rhizopus* sp. + *Penicillium* sp. to degrade 'Valencia' oranges. In both cases eight treatments were prepared, where six treatments of whole and chopped fruits were inoculated by spraying a solution of fungi (3.5 x 10^6 spores mL^{-1}). All treatments were prepared by triplicate; the experimental unit consisted of three fruits. Five parameters were recorded daily during 33 days (weight loss, pH of leachates, leachate production, incidence and severity of damage). Results showed the greatest weight loss and highest leachate production in chopped mangoes and oranges inoculated with a mixture of fungi. Incidence and severity were greatest in mangoes inoculated with *Colletotrichum* + *Rhizopus*. It was concluded that degradation was greater in mangoes and oranges inoculated with a mixture of *Colletotrichum* and *Rhizopus,* and *Penicillium* and *Rhizopus,* respectively. Thus, mixtures of fungi appear to have great potential for use in the pre-composting process.

Key words: Fruit degradation, postharvest losses, leachates, solid waste.

INTRODUCTION

The main causes of postharvest losses in fruits and vegetables are problems during maturation, development of different diseases and damage caused by severe cold exposure (Capellini et al., 1988). Fruits, flowers and vegetables are attacked by diseases caused by specific microorganisms that generate large postharvest losses (FAO, 1993). For instance, fungus causes postharvest losses in avocado (Vidales, 1997) and reduces the shelf life of citrus fruits up to 49%. It can also cause postharvest losses of 15 to 50% in mango (Becerra-Leor, 1995) and 30% in papaya (Anonymous, 1991). The most common and devastating rots in citrus fruits are caused by three *Penicillium* species, which are responsible for more than 90% of postharvest losses (Hong-Yin et al., 2004). *Rhizopus stolonifer* is responsible for soft rot in most fruits and vegetables and generates important economic losses (Velázquez del Valle et al., 2008). For the mango fruit, Mena et al. (1996), report the incidence of damage caused by *Colletotrichum* spp in 'Manila' mango stored at 10°C for 18 days reached 67%. *Colletotrichum gloeosporioides* causes important damage and symptoms in tropical and subtropical fruits in Mexico; it may attack different plant parts at different phenological stages of the crop (Gutiérrez et al., 2001). The

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Table 1. Characteristics of treatments.

Treatment	Condition of fruit	Inoculum
	Mangoes	
WM	Whole	--------
WM+Rh		*Rhizopus* sp
WM+C		*Colletotrichum* sp
WM+RhC		*Rhizopus* sp + *Colletotrichum* sp
ChM	Chopped	--------
ChM+Rh		*Rhizopus* sp
ChM+C		*Colletotrichum* sp
ChM+RhC		*Rhizopus* sp + *Colletotrichum* sp
	Oranges	
WO	Whole	--------
WO+Rh		*Rhizopus* sp
WO+P		*Penicillium* sp
WO+RhP		*Rhizopus* sp + *Penicullium* sp
ChO	Chopped	--------
ChO+Rh		*Rhizopus* sp
ChO+P		*Penicillium* sp
ChO+RhP		*Rhizopus* sp + *Penicillium* sp

horticultural products damaged are rejected as municipal wastes in big markets in the cities. Mexico's City population generates approximately 13 000 tons of municipal solid waste everyday (SMA, 2010), of which more than 600 tons are horticultural products from households, commerce and industry. Nearly 22% of solid organic waste (SOW) comes from Central de Abastos (the city's main wholesale market), which is equivalent to 17 550 tons of SOW per month. These wastes are dumped in a landfill with no previous treatment.

Fruits, flowers and vegetable wastes could be treated by composting. During compost process, thermophilic conditions and the intense microbial competition inactivate almost all microorganisms that cause diseases in plants, animals and humans (Farrell, 1993; Gerba et al., 1995; Suárez-Estrella et al., 2003; Erickson et al., 2009).

Furthermore, composting has proven effective for eliminating nematodes, bacteria, viruses, and pathogenic fungi (Bollen and Volker, 1996). No studies were found in the literature that evaluate damages caused by fungi in oranges or mangoes to the point of complete degradation, since this degree of rotting is not contemplated in postharvest management or plant pathology studies, which focus on quality conservation or damage control.

It is, however, of interest in waste management. The objective of this research was to study the degradation capacity of *Colletotrichum* sp. and *Rhizopus* sp. in mangoes, and of *Penicilium* sp. and *Rhizopus* sp. in oranges in order to evaluate their use in a pre-composting process.

MATERIALS AND METHODS

Plant material

'Paraiso' mangoes and 'Valencia' oranges purchased at the Central de Abastos in Mexico City were selected based on size and ripeness. They were washed under running water and disinfested with a 1.5% sodium hypochlorite solution for 3 min.

Fungi (inoculum)

Fungi (*Colletotrichum* sp. and *Penicillium* sp.) used in this study were isolated and purified from mangoes and oranges showing symptoms of anthracnose or green mold. *Rhizopus* sp. was obtained from tomatoes presenting symptoms of Rhizopus rot.

Degradation tests

Whole and chopped mangoes were inoculated by spraying with a solution of equal parts of pure (monosporic) cultures of *Colletotrichum* sp., *Rhizopus* sp. and a mixture of *Colletotrichum* sp + *Rhizopus* sp. (3.5 x 10^6 spores/mL). Similarly, whole and chopped oranges were inoculated with *Penicillium* sp., *Rhizopus* sp. and a mixture of *Penicillium* sp. and *Rhizopus* sp. The inoculated fruits were placed in plastic trays (3 kg capacity) and incubated at 21 to 23°C and 95 to 100% relative humidity (RH) for 33 days. Each experimental unit consisted of three fruits, with three replicates per treatment. The treatments are shown in Table 1. The following variables were evaluated daily for up to 33 days.

Weight loss

The fruits were weighed daily using a 2.5 kg-capacity digital scale.

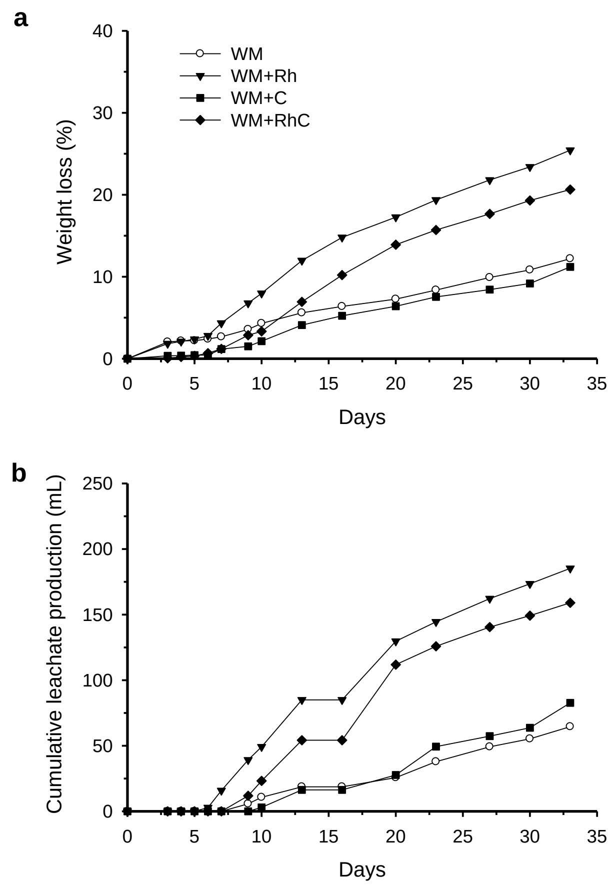

Figure 1. Changes in weight loss (a) and cumulative leachate production (b) in whole mangoes.

Weight loss was determined based on the fruits initial and final weights and presented as a percentage of the initial weight.

Leachate production

The fruit was put in a box strainer, and then it was placed over a plastic container where the leachate was collected. The volume of leachates produced daily was determined with a 100 mL graduated glass test tube. To determine cumulative leachate production, the daily volume was added consecutively.

pH of leachates: A digital pH meter was used to determine pH on a daily basis.

Incidence: Incidence was evaluated as the percentage of infected fruits relative to total fruits used in this study.

Severity: Severity was evaluated by the Horsfall and Barratt (1945) method, using a diagrammatic scale with eight levels (0=0%,1=1.44%, 2=2.77%, 3=5.29%, 4=9.85%, 5= 17.61%, 6=29.49% and 7=45%) to assess the damage on the surface on one side of the fruit, although both sides of the fruit were evaluated.

Data analysis

A complete randomized design was used. The data were analyzed with a Multivariate Analysis of Variance (MANOVA) and a *posteriori* test of Duncan (α=0.05). The SPSS software for Windows v. 12 was used.

RESULTS AND DISCUSSION

Mango

Weight loss

When fruits are stored without injuries or infected by microrganisms, the weight loss is lower; for instance, Acosta et al. (2003) found weight losses of 5.99 to 6.42% in mangoes stored at 23°C for 20 days. However, when microorganisms infect fruits, organic matter is degraded and high weight losses are observed. In this work, the weight loss is due to organic matter degradation and loss of water (leachates).

Figure 1a shows that treatments of whole mangoes inoculated with a fungal mixture (WM+RhC) and mangoes inoculated only with *Rhizopus* (WM+Rh) had the best results in terms of weight loss (20 and 25%, respectively) 33 days after inoculation. In contrast, the control, whole mangoes (WM), and whole mangoes inoculated with *Colletotrichum* (WM+C) had values of 11 and 12%, respectively, over the same period, with no

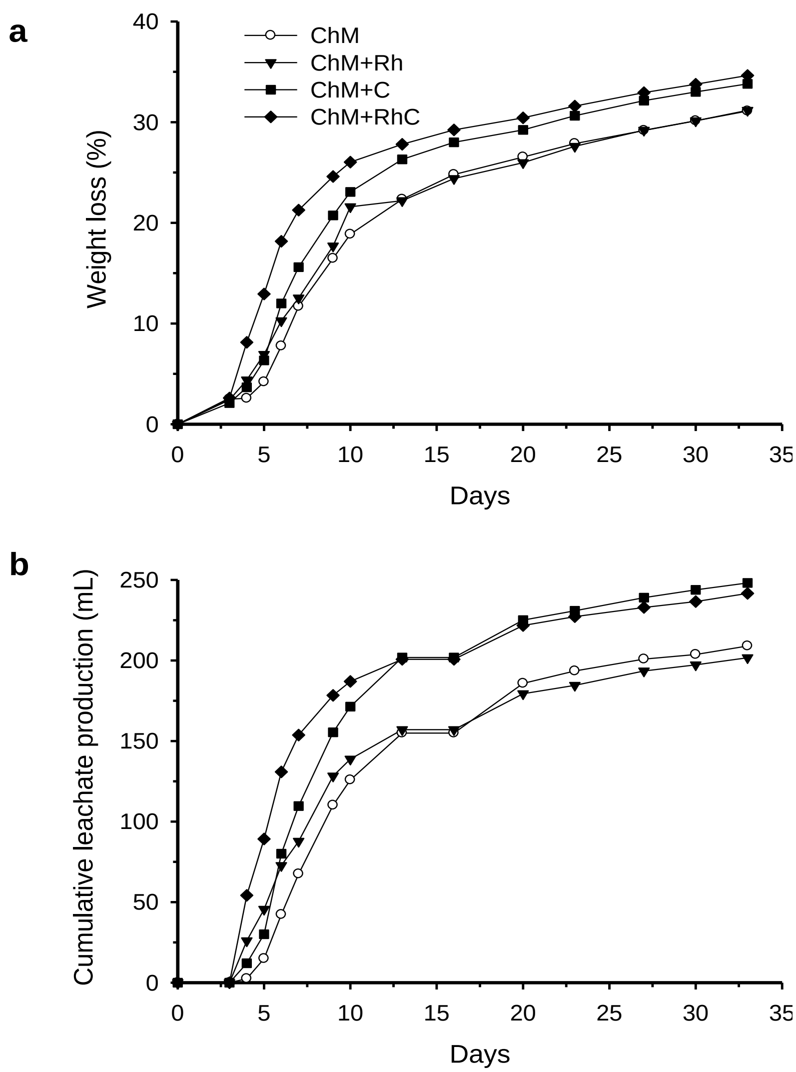

Figure 2. Changes in weight loss (a) and cumulative leachate production (b) in chopped mangoes.

statistically significant differences between the results of treatments.

Weight loss values for chopped mango treatments are found in Figure 2a. All treatments showed weight losses of more than 30% on day 33 after inoculation. The greatest weight loss (35%) was recorded for chopped mango inoculated with the mixture of fungi (ChM+RhC). There were no statistically significant differences between the results of the treatments, but significant differences were found ($F_{(1,16)}$=90.9, p=0.0001) when the results of treatments using whole fruit (Figure 1a) were compared to those using chopped fruit (Figure 2a).

Cumulative leachate production

Cumulative leachate production in treatments using whole mangoes is given in Figure 1b. The WM+RhC and WM+Rh treatments showed the greatest cumulative leachate production (160 and 175 mL, respectively) 33 days after inoculation. The WM and WM+C treatments produced only 55 and 65 mL, respectively, over the same period. There were no significant differences between treatment results. Statistically significant differences were found only when the results of whole fruit were compared to those of chopped fruit, that is, the WM treatment showed a significant difference ($F_{(1,4)}$=34.8, p=0.004), as did the WM+C treatment ($F_{(1,4)}$=43.56, p=0.003). However, no significant differences were found in the WM+Rh and WM+RhC treatments, perhaps due to pectolitic enzyme production by *Rhizopus.* This enzyme also causes damage to the middle lamella (Barkai, 2001), which may be responsible for the speedy degradation of both whole and chopped fruit inoculated. The essential nature of polygalacturonase enzymes (PG) and pectin methyl esterase (PME) in a strain of *R. stolonifer* has

Table 2. pH of leachates of whole and chopped mangoes.

Treatment	Days						
	4	6	9	13	23	33	$\bar{x}$
			Whole mangoes				
WM	-----	-----	3.59±0.74	2.91±0.09	2.80±0.12	3.00±0.24	
WM+C	-----	-----	-----	3.00±0.08	2.93±0.49	3.00±0.78	
WM+Rh		3.26±0.05	3.07±0.14	3.12±0.09	2.93±0.52	3.00±0.17	
WM+RhC	-----	-----	3.1±0.02	3.05±0.07	2.73±0.45	2.90±0.00	
			Chopped mangoes				
ChM	2.72±0.02	2.73±0.03	2.77±0.03	2.85±0.03	2.72±0.04	2.90±0.00	2.78^a
ChM+C	2.85±0.05	3.00±0.00	2.96±0.03	3.00±0.00	2.80±0.00	2.96±0.03	2.93^b
ChM+Rh	2.78±0.06	3.03±0.03	3.10±0.06	3.17±0.03	3.03±0.03	3.13±0.03	3.04^c
ChM+RhC	3.10±0.12	3.23±0.09	3.27±0.12	3.27±0.12	3.13±0.09	3.22±0.09	3.2^c

Note: Values represent the Mean±SEM (Standard Error of the Mean), n=3. The treatments marked with $^{(a, b, c)}$ show that there were significant statistical differences, post hoc Duncan (P<0.05).

been demonstrated (Blandino et al., 2001). Similar results were obtained by Velazquez del Valle et al. (2008), who found that soft rot occurred 24 h after inoculating tomatoes (*Lycopersicum esculentum* Mill.) with *R. stolonifer*, and cellular fluids (leachate) were produced 96 h later. Figure 2b shows the cumulative leachate production in chopped mango treatments. Treatments ChM+RhC and ChM+C produced nearly 250 mL, while treatments ChM and ChM+Rh did not produce more than 200 mL; however there were no significant differences between results of these treatments. Fungal inoculation proved effective for the production of leachates. The fungal mixture produced more leachates in whole and chopped fruits.

pH values of leachates

The pH values of leachates obtained from mangoes ranged between 2.6 and 3.6 (Table 2). Statistically significant differences were found in the results of chopped fruits ($F_{(3,26)}$ = 10.07, p = 0.004), showing three groups: in the first one the ChM showed the lowest value (2.78); in the second one, ChM+Rh was medium value (2.93), and in the third one, ChM+C (3.04) and ChM+RhC (3.20) were the highest. In the whole fruit control (WM), the first pH value was not recorded until day 9, when leachate production began in this treatment (Table 2).

Severity

Severity was greater in mangoes inoculated with a mixture of fungi (Figure 3a, Figure 7). It can be said in general that *Colletotrichum* damages the skin but causes little damage to the flesh, while *Rhizopus* attacks the flesh more severely than the other fungi, due to its pectolitic enzymes PG and PME. The *Colletotrichum* and *Rhizopus* mixture accelerated the degradation process. The WM+C and WM+RhC treatments reached 100% severity in 23 days, in contrast to the WM treatment, which reached just 42% over the same period (Figure 3a). Other authors have found low severity values, because as previously mentioned, their experiments aimed at conserving fruit with minimal damage. Saborio et al. (2000) reported an incidence of 11 to 23% for anthracnose in papayas, 12 days after harvesting, while Acosta et al. (2003) reported severe damage (61%) in the control and only 15% damage in fruit cultivated under integrated pest management. The fruits were stored at 13°C for 20 days.

Incidence

One hundred percent (100%) incidence was reached in only five days in WM+C and WM+RhC treatments, in contrast to WM and WM+Rh, which reached the same value in 25 days (Figure 3b). Statistically significant differences were found between treatments. Other authors have found low levels of incidence; for example, Arias and Carrizales (2007) found 18 to 55% anthracnose incidence in mangoes stored at 16°C for 25 days, while Acosta et al. (2003) reported anthracnose incidence of 80% in the control and 20% in fruit cultivated under integrated pest management; in their study fruits were stored at 13°C for 20 days. It is clear that temperature has an important effect, because fruits mature slowly at lower temperatures, and fungal development is also slow.

Oranges

Weight loss

Oranges used to evaluate weight loss produced results

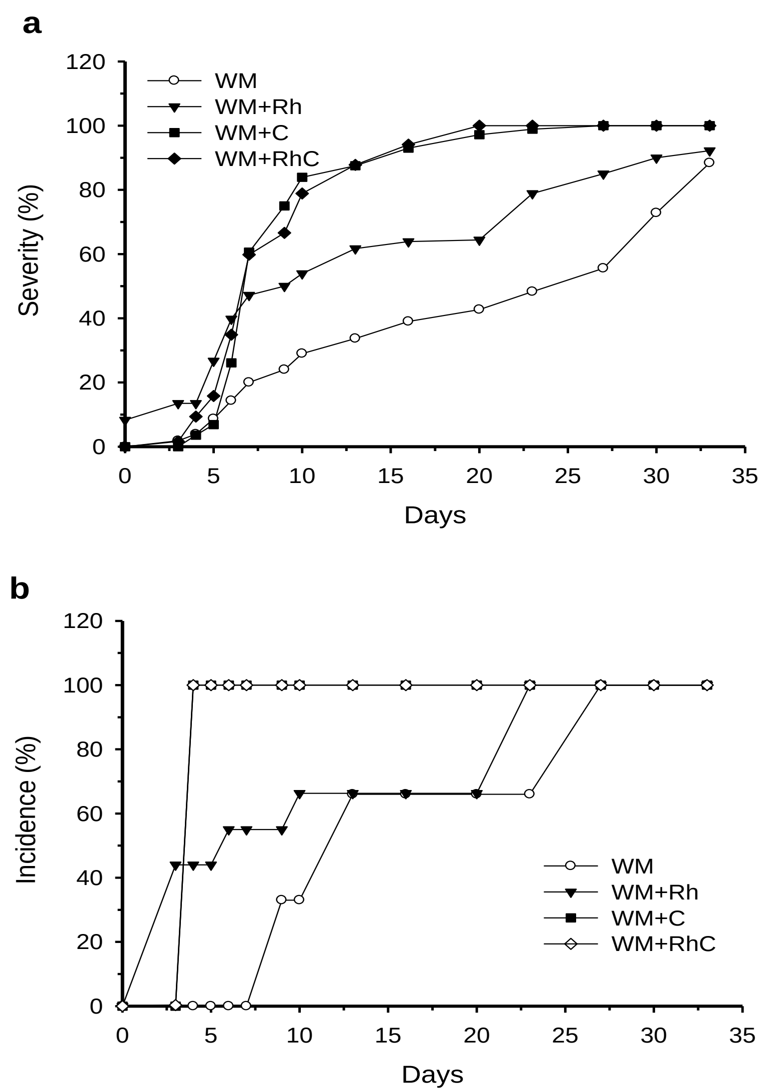

Figure 3. Severity (a) and Incidence (b) of damage in whole mangoes inoculated with *Rhizopus* and *Colletotrichum*.

similar to those obtained with mangoes: whole fruits (Figure 4a) had lower weight loss than chopped oranges (Figure 5a). The statistical analysis indicated a significant difference ($F_{(1,4)}$=276.9, p<0.0001) between whole orange and chopped orange treatments. Whole oranges in the control treatment (WO) had the lowest weight loss (Figure 4a). The treatment with whole oranges showed no significant change until day 33, while chopped oranges in the control treatment (ChO) had over 50% weight loss (Figure 5a). Treatment with the mixture of fungi (ChO+RhP) in chopped oranges produced the greatest weight loss. Statistical analysis found a significant difference ($F_{(3,8)}$ = 41.8, p<0.0001).

Duncan's test indicated two groups were formed: (1) the first group included treatments inoculated with *Rhizopus* and treatments that were inoculated with the mixture of fungi (WO+RhP) and showed major weight loss, and (2) the second group included treatments with small weight loss, that is, the control (WO) and the *Penicillium* treatment (WO+P). Treatments using whole oranges inoculated with *Rhizopus* (WO+Rh) and chopped oranges and the mixture of fungi (ChO+RhP) showed the greatest weight loss, with no significant differences between treatments.

Cumulative leachate production

Cumulative leachates in whole oranges are shown in Figure 4b. On day 33, the lowest leachate production was recorded for WO, but there were no significant differences between treatment results. However, there was a

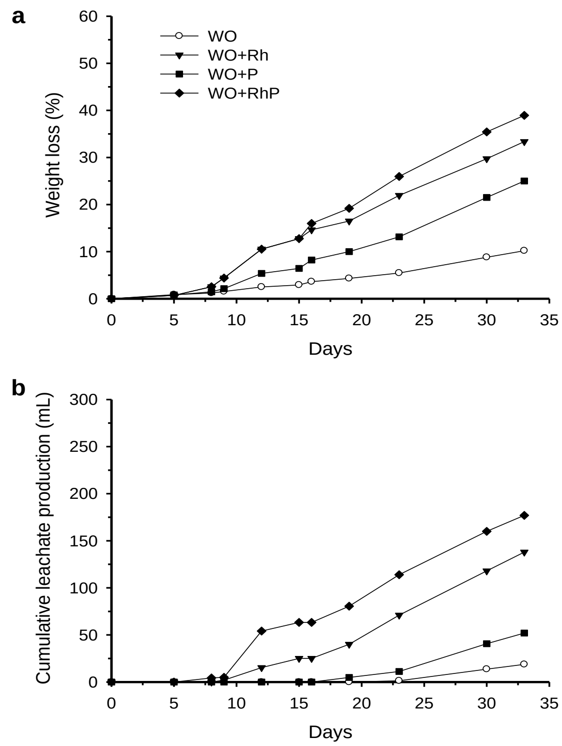

Figure 4. Changes in weight loss (a) and cumulative leachate production (b) in whole oranges.

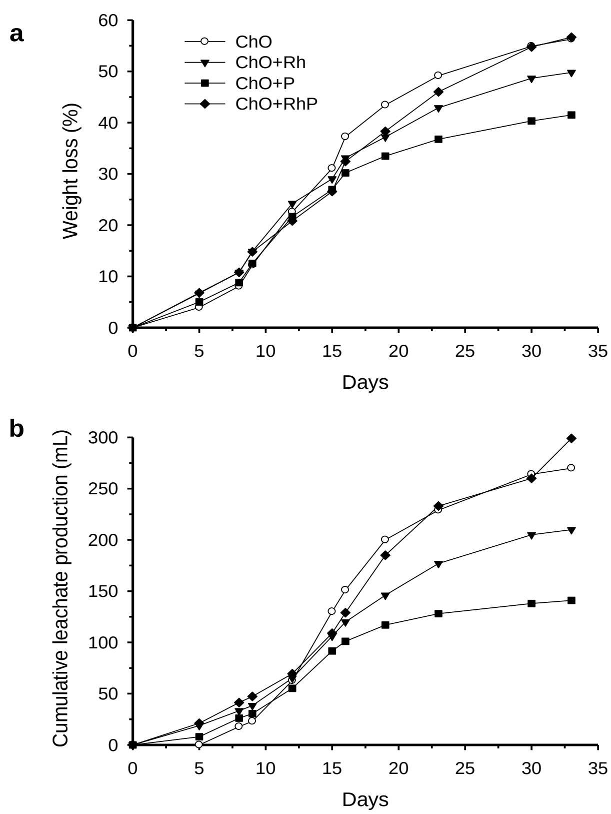

Figure 5. Changes in weight loss (a) and cumulative leachate production (b) in chopped oranges.

Table 3. pH of leachates of whole and chopped oranges.

Treatment	Days					
	4	6	9	13	23	33
				Whole oranges		
WO	-----	-----	-----	-----	5.84 ±0.02	5.95 ±0.01
WO+P	-----	-----	-----	-----	4.79 ±0.82	4.84 ±0.80
WO+Rh	-----	-----	6.00 ±0.01	3.37 ±0.11	4.44 ±0.40	4.53 ±0.40
WO+RhP	-----	-----	1.75 ±0.94	3.25 ±0.07	5.01 ±0.72	5.06±0.72
				Chopped oranges		
ChO	-----	-----	5.17 ±0.38	3.38 ±0.14	4.89 ±0.18	5.03 ±0.14
ChO+P	-----	-----	4.4 ±1.14	3.42 ±0.08	4.30 ±0.22	4.47 ±0.18
ChO+Rh	-----	-----	5.13 ±1.54	3.16 ±0.05	4.79 ±0.09	4.82 ±0.09
ChO+RhP	-----	-----	6.00 ±0.11	3.06 ±0.03	4.94 ±0.26	5.02 ±0.24

Note: Values represent the Mean±SEM (Standard Error of the Mean), n=3.

significant difference ($F_{(3,8)}$ = 7.58, p=0.010) in the case of chopped oranges. The ChO+P treatment presented the lowest value (76.13), in contrast to all other evaluated treatments (ChO+Rh, ChO+RhP, and ChO; Figure 5b).

It is very important to mention that the treatment with chopped oranges inoculated with *Penicillium* (ChO+P) produced less leachate than the control. This is because as soon as *Penicillium* infects the fruit, it damages the skin; then it begins to sporulate, covering the fruit completely with a layer of film that blocks leachate loss. Consequently, this treatment produces a smaller amount of leachates in comparison to the other treatments.

pH of leachates

The pH values of leachates obtained from whole and chopped oranges ranged between 1.75 and 6.00 (Table 3), ANOVAs test did not found significant differences between the results of treatments using whole and chopped oranges. In the WO and WO+P treatment, the first pH value was not recorded until day 23, when leachate production began in this treatment (Table 3).

Severity

Severity was greater in oranges inoculated with a fungi and mixture of fungi (Figure 6a, Figure 8). The WO+P, WO+Rh and WO+RhP treatments reached 100% severity in 14 days, in contrast to the WO treatment, which reached just 33% in 33 days (Figure 6a). Statistically significant differences were found between treatments ($F_{(3,8)}$ = 1271.70, P=0.0001).

Incidence

One hundred percent (100%) incidence was reached in only eleven days in WO+P, WO+Rh and WO+RhP treatments, in contrast to WO, which reached the 33% in 33 days (Figure 6b). Statistically significant differences were found between treatments ($F_{(3,8)}$=703.45, P=0.0001).

Conclusion

Fungal inoculation did not have a significant effect on weight loss and cumulative leachate production in whole mangoes, but significant differences were found in the pH of leachates, incidence, and severity of damages with this treatment. However, when a statistical analysis was performed to compare the results of treatments using whole fruit against treatments using chopped fruit, it revealed very significant differences, indicating that degradation is faster in chopped fruit treatments. Fungal inoculation in whole oranges did not affect cumulative leachate production, but there were significant differences in weight loss. In the case of chopped oranges, there were differences in cumulative leachate production. Plant pathogenic fungi play an important role in the degradation of organic matter. Which plant part a fungus damages depends on the type of fruit and the species or genus of the fungus, each of which has a distinct infection mechanism and energy source. Hence, it can be said, in general, that *Penicillium* attacks both flesh and skin in oranges, *Colletotrichum* basically damages the skin but causes little damage to the flesh of mangoes while *Rhizopus* attacks the flesh of oranges and mangoes more severely than *Penicillium* and Colletotrichum respectively. The presence of *Colletotrichum, Penicillium* and *Rhizopus* may accelerate the composting process. A higher degree of degradation occurred in chopped fruit than in whole fruit, and greater degradation was also observed in treatments inoculated with a mixture of fungi, which caused higher leachate production. Weight loss reached up to 35% in chopped mangoes inoculated with

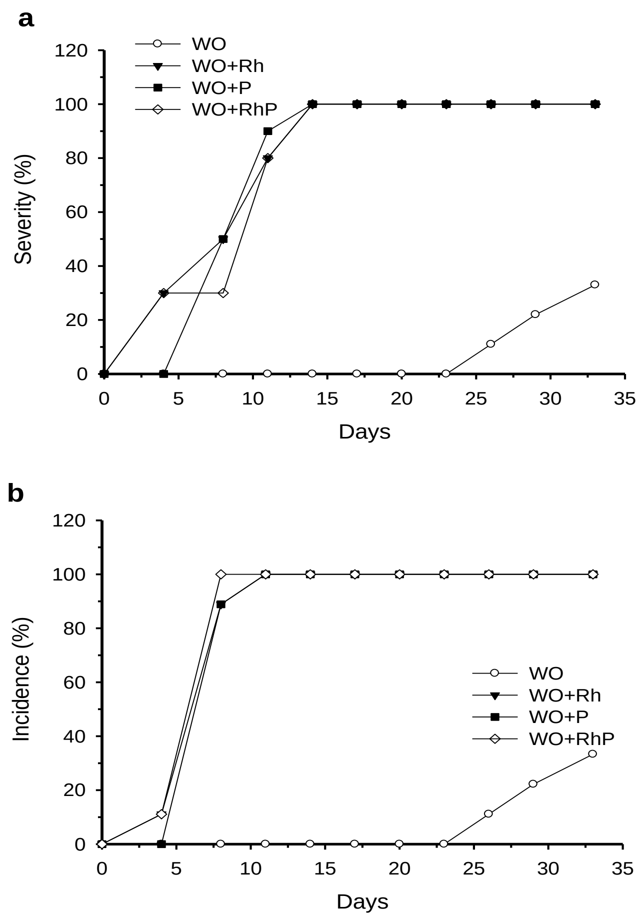

Figure 6. Severity (a) and Incidence (b) of damage in whole oranges inoculated with *Rhizopus* and *Penicillium.*

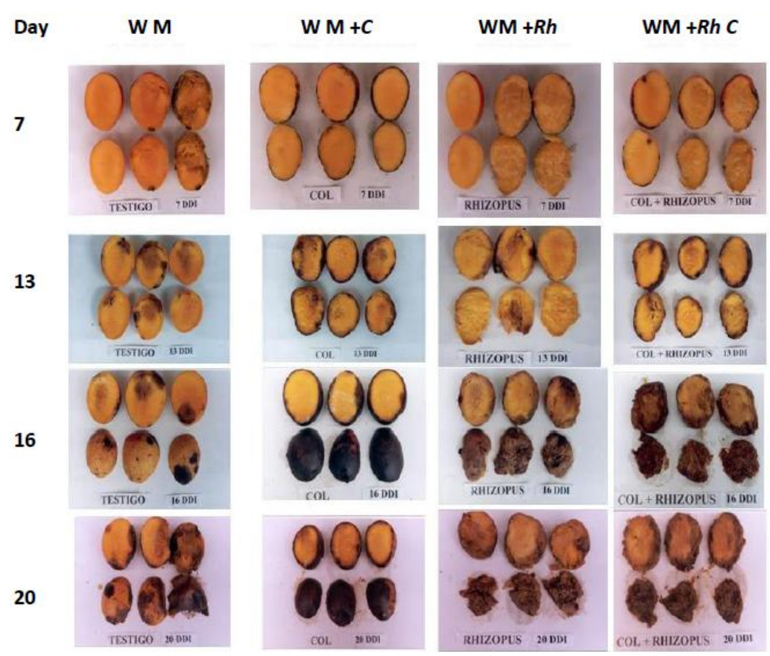

Figure 7. Degradation of whole mangoes inoculated with *Rhizopus* and *Colletotrichum.*

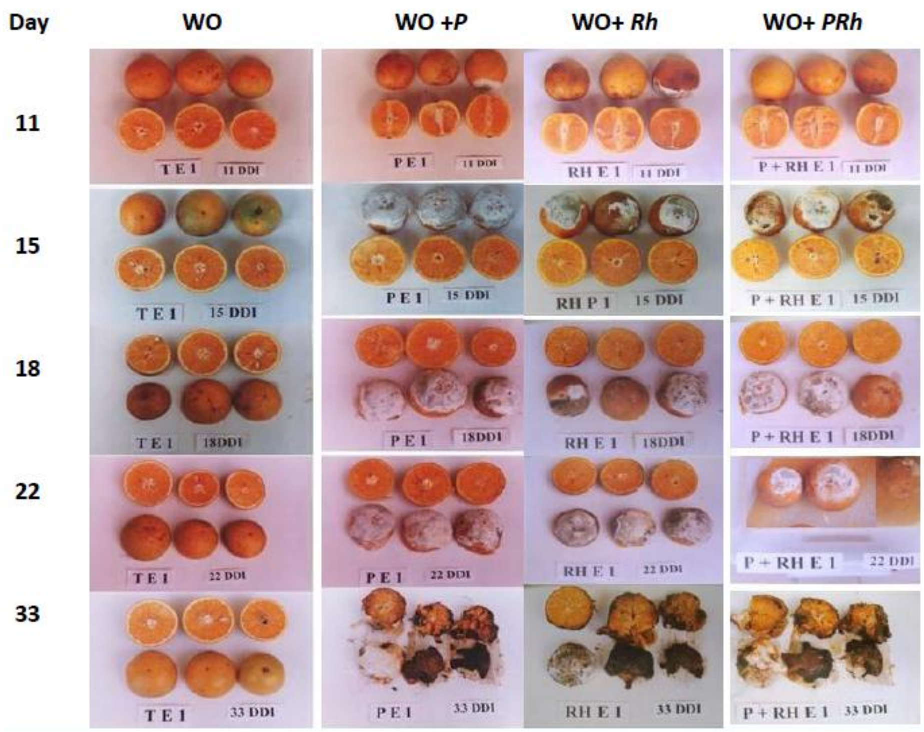

Figure 8. Degradation of whole oranges inoculated with *Penicillium* and *Rhizopus.*

the mixture of fungi and more than 50% in chopped oranges inoculated with the mixture of fungi; on the other hand, degradation was faster in mangoes than in oranges. In conclusion, mixtures of fungi (*Colletotrichum* + *Rhizopus* in mangoes and *Penicillium* + *Rhizopus* in oranges) appear to have high potential for use in the pre-composting process.

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