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Effect of Alternative Products against Green Mold in Postharvest of Oranges

(*Citrus sinensis* (L.) Osbeck**)**

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Abstract: Considering the viability and the antimicrobial potential of secondary metabolites synthetized by plants, this work aimed at evaluating the effect of natural vegetal products on orange green mold. Fruits of orange, naturally infected with *P. digitatum*, were submitted to fumigation with 2mL of essential oil of *Citrus aurantium* ssp. *bergamia* and *Citrus aurantium* ssp. *sinensis* and also to immersion in citric extracts at a concentration of 2% and 4% (fruits immersed in distilled water constituted the control treatment). The treated fruits remained in room conditions (25 ± 2ºC and 85 ± 5% R.H.) for a period of 12 days. By analyzing the results, we observed lower incidence of the pathogen over the fruits that were submitted to treatments with natural products, being that treatments with citric extract at 2% and 4% and the treatment with essential oil of *C. aurantium* ssp. *sinensis* did not differ significantly among them, but there was a significant different among them and the treatment with essential oil of *C. aurantium* ssp. *bergamia*, which differed from the control treatment, with relation to the incidence (83.33%) and severity (30.20%) of the disease. All treatments caused a high percentage of control (higher than 94.41%), differing significantly from the control treatment (59.06%).

Indexation terms: *Penicillium digitatum*, alternative control, plant extracts, essential oils

1. Introduction

The cultivation of citrus is of great importance to the Brazilian and world agriculture, considering that Brazil is the biggest producer of oranges and largest exporter of concentrated juice. Out of the produced volume, Brazil exported only 0.5% of fresh oranges. These percentages can be explained by the fact that most parts of orchards are destined to juice extraction, where the quality control is less rigorous. However, this low rate of exportation is also credited to the lower quality of the fruits. The increase in exportation rates is mostly dependent on the improvement of fruit quality. Therefore, it is said that orange is handled, prepared and packed in a way that is old and obsolete for the market. There are many causes for this technological delay: the power of market for concentrated juice, the lack of incentive to the producer in order to improve the fruit quality, the low income of population and the internal commercialization chain that did not use to demand quality (Chimenti, 2012) .

Fruits comprehend an area of the market with particularities, once they are highly perishable and are susceptible to mechanical damage, physiological disturbances and putrefaction. It is estimated to be losses from 20% to 95% after harvest, for a production of 22.040 tons of citrus in developing countries (FAO, 1983). Diseases that happen after harvest diminish the quality of the fruit and lead to considerable losses, being that those ones caused by fungi are more common in humid tropical areas, because the high temperature and high relative humidity enhance the growth of microorganisms.

In postharvest, there are several types of mold, but green mold is the most frequent in the main producing areas of Brazil. This disease caused by

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Penicilliumdigitatum causes huge damages to the product, especially the products that are destined to exportation, being that all varieties and cultivars of fruits are susceptible to it. *P. digitatum*, through injuries, even those that involve only some oil glandules, is enough to induce infection. The fungus survives in fields and remaining cultures in soil and produce spores that infect the injured fruits, both in soil and in trees (Kimati and Galli, 1980; Brown and Eckert, 1988; Brown, 1994).

At first, a soft and aqueous area of putrefaction develops on any part of the fruit surface, which grows quickly and surrounds the organ completely. The affected tissue is covered by white mycelia and olive-green fungus fructification, surrounded by a relatively wide area of white growth. In a dry environment, the affected fruit remains complete, but it wilts and becomes mummified. In a humid environment, it disintegrates completely (Kimati and Galli, 1980; Franco, 1999).

As mentioned before, the agent that causes green mold is the mitosporic fungus *Penicillium digitatum* Saccardo, which shows inter and intracellular mycelium with enzymes that are able to solve the middle lamella of the fruit tissues. They produce a huge amount of conidia and, thus, the fungus survives saprophytically every year, over several kinds of organic substrates. The infections arise from conidia carried by the wind and reach the surface of the injured fruit. The cycle can repeat many times throughout the year, thus, increasing exponentially the fungus propagula, both in orchards and in warehouse where the fruits are packed and stored. The ideal temperature for the development of the fungus is around 24ºC, being that in temperatures above 30ºC and under 10ºC its development is quite slow. Even though they are favored by relatively high temperatures, the fungus proceeds with its activities slowly, at temperatures close to freeze (Kimati and Galli, 1980; Franco, 1999).

Alternatives that aim at reducing the use of fungicides have been studied and have shown promising results on the control of several plant pathogens. A particular focus is given to biological control and to the use of plant extracts, foodstuff, food additive, residues from food production and food conservatives in the control of plant diseases, in general (Sholberg and Gaunce, 1995).

Smid et al. (2013) evaluates fifteen compounds of essential oils in order to verify the antifungal activity over *Penicillium hirsutum* and *P. allii*. Carvone, cuminaldehyde, perillaldehyde, cinnamaldehyde, salicylaldehyde and benzaldehyde were selected as the most potent growth inhibitors in vitro. However, growth inhibition by means of carvone was reversible. The exposition to cinnamaldehyde resulted in an irreversible growth inhibition for both fungi. Franco (1999) verified that alternative products such as Sodium Bicarbonate, Sodium Carbonate, Boric Acid, Potassium Sorbate, Sodium Metabisulfite and essential oil of *Cymbopogon citratus* were promising in the control of *Penicillium digitatum* over bitter orange in postharvest.

Considering the viability and the antimicrobial potential of secondary metabolites synthetized by plants, this work aimed at evaluating the effect of natural plant products over the green mold of orange, which is caused by *Penicillium digitatum.*

2. Material and methods

Aiming at evaluating alternative plant products, we carried out an experiment in the Laboratory of Medicinal Plants of the Agronomy Department of State University of Maringá, Maringá, Paraná, Brazil.

Fruits of orange (*Citrus sinensis* (L.) Osbeck) were obtained in the Unit CEASA-Maringá/PR. This variety belongs to Group I, Class 63, in which the fruits have longitudinal diameter longer than 63 and shorter than 70 millimeters. When we acquired the fruits, they had been conditioned to propylene bags and presented color C1 that corresponds to a uniform green color and average weight of 150.31 grams (Brasil, 2012; Ceagesp, 2012).

We carried out a pre-selection of the fruits, aiming at homogeneity of format, coloration and absence of mechanic injuries (visible to naked eye). Due to the natural occurrence of *Penicillium digitatum*, it was not necessary to inoculate this pathogen artificially. After due weighing in a semi-analytic scale and due verification of longitudinal and transversal diameters, the fruits were superficially disinfected by immersing them in a Sodium Hypochlorite solution, at 0.5% (v/v), for 3 minutes and washed twice in running water. After drying the fruits, they were numerically identified and submitted to the following treatments: T1: Fumigation with 2mL of essential oil of *Citrus aurantium* ssp. *bergamia*/ 1 hour; T2: Fumigation with 2mL of essential oil of C*itrus aurantium* ssp. *sinensis* / 1 hour; T3: Immersion in a solution at 2.0% (v/v) of citric extract (Ecolife) / 3 minutes/ T4: Immersion in a solution at 4.0% (v/v) of citric extract (Ecolife) / 3 minutes; T5: Immersion in distilled water / 3 minutes (control).

The treated fruits were conditioned in plastic cubes wrapped in polyethylene bags, remaining in room conditions with temperature monitoring (25 ± 2ºC) and relative humidity (85 ± 5% R.H.) aiming at simulating the commercialization conditions, for a period of 12 days, when the control fruits (T5. Control) had more than 50% of incidence of green mold. By this time, we carried out the final physical and phytosanitary assessments.

We assessed: a) reduction of fruit mass (%); b) reduction of transversal and longitudinal diameters (%); c) color of the peel. According to the norms developed by the Center for Horticulture Quality of the Brazilian Program of Horticulture Quality, the grades were: C1: Green = 1; C2: Pale Green = 2; C3: greenish yellow = 3; C4: Yellow = 4; C5: Orange yellow = 5 (Brasil, 2012; Ceagesp, 2012).; d) yield of juice, calculated through the equation: %Juice = (weight of juice / weight of fruit) x 100/ e) incidence of disease, calculated through the formula: %Incidence = (No. of infected fruits / Total No. of fruits) x 100; f) severity of the disease, determined from the average diameter of injured in each treatment, through the formula: %Severity = (Treatment/Control Treatment) x 100 and g) disease control, calculated by using the formula: % Control = (Control Treatment - Treatment / Control Treatment) x 100.

We chose the Fully Randomized Design, with five treatments and four repetitions with six fruits as experimental unit, totalizing 120 fruits in each experiment. The results were submitted to analysis of variance and the averages of each variable were compared by Duncan Test at 5% of probability. The software SAEG was used to carry out the analysis of variance and the application of the test of average.

3. Results and discussion

The weight loss of fruits of orange submitted to treatments, via fumigation with essential oils, immersion in citric extracts and immersion in distilled water (Control) can be seen on Table 1. There was a significant difference between the control treatment and the other treatments, which did not differ among them. We observed the same situation for the diameter of fruits, because the control treatment also differed from the other treatments. This result can be justified by the fact that orange is a non-climacteric fruit, in which the production of ethylene is reduced and the energy provided remains in constant decline during the whole maturation process until senescence (Gomes, 1996; Chitarra and Chitarrra, 2005;). Moreover, we verified that the control of green mold was efficient.

In Table 2, it is possible to observe the data related to color alterations that occurred in the fruits submitted to different treatments and the yield of juice. The color alterations are due to degenerative processes and synthesis processes. Chlorophyll is gradually degraded and there is formation of carotenoids. Regarding fruits of orange, not always does color alteration, from green to yellow, indicate quality. Fruits in the control treatment did not differ significantly from the fruits treated with *Citrus aurantium* ssp. *bergamia*; the fruits treated with citric extract at 2% did not differ from the treatment with essential oil of *Citrus aurantium* ssp. *sinensis*. The treatment with citric extract at 4% provided pale green fruits, which, most likely, will have longer shelf life, due to the maintenance of green color that will probably avoid a high percentage of infection.

There was a significant difference in relation with juice yield: fruits submitted to the treatment with citric extract at 4% provided more juice than the others.

Regarding the incidence of disease, the treatments with citric extract at 2% and 4% and the treatment with essential oil of *C. aurantium* ssp. *sinensis,* did not differ significantly from each other, but there was a significant difference between them and the treatments with essential oil of *C. aurantium* ssp. *bergamia* and the control treatment (Table 3). The fruits in the control treatment showed 83.33% of incidence, being that the severity was 30.20%, and differed significantly from the other treatments, at the level of 5% of probability, by Duncan test. All treatments showed a high percentage of control, being different from the control treatment. Franco (1999) proved the effect of several alternative products on the control of *Penicillium digitatum* in citrus in postharvest, highlighting the use of essential oil of *Cymbopogon citratus*.

Essential oils that are present in the flavedo of citric fruits in high quantities may exercise an important bio-regulating action over the spores of the most aggressive fungal pathogens of citric fruits: *Penicillium digitatum* (Pers.) Sacc. And *Penicillium italicum* Wehm. As part of a research to investigate the bio-regulating action of essential oils of citric fruits, in order to determine the activity of compounds of essential oils of citric fruits (nonanal, citral, alpha-pinene, myrcene, alpha-phellandrene and d-limonene) a study in vitro was carried out to evaluate the germination of spores de *P. digitatum* and *P. italicum.* The tests were conducted adding several doses (1000, 500, 132, 250 and 125 ppm) of various compounds in agar and sucrose. The most active compound, in relation to the method and for the lineage of fungi, was citral, which at 250ppm, proved to be able to exercise a fungistatic action over both pathogens (Caccioni et al., 1995).

The efficiency of the citric extract was evaluated in other studies and the essential oils of *Citrus* had acetaldehyde, acetic acid, alpha-phellandrene, benzoic acid, caryophyllene, cinnamic acid, cis-ocimene, citral, citronelal, citronelol, cumarine, formaldehyde, geraniol, linalool, myrcene, naringenin, nobiletin, p-cymene, phenol, seseline, synensetine, terpinolene, thymol and umbelliferone with proven fungicide activity (USDA, 2003). The citric extract product (Ecolife®40) is provided with citric bioflavonoids, citric phytoalexins, ascorbic acid (Vitamin C), citric acid and lactic acid in its composition. Among the compounds of this citric extract, it is known that phytoalexins (antimicrobial natural compounds) are low-weight organic compounds that accumulate in the plant, after the infection by a pathogen or some environmental stress. For a long time it is known the antimicrobial activity of some foods and nowadays it is known that this activity is mainly due to bioflavonoids that, together with ascorbic acid, are organic elicitors, as well as phytoalexins. Lactic acid and citric acid have antimicrobial activity (Pszczola, 2002; IST, 2013).

Fortes et al. (2012) evaluated in vitro the efficiency of a product based on citric biomass (Lonlife) in relation to the fungicide Cerconil PM, over the inhibition of mycelial growth of fungi *Colletotrichum lindemuthianum* and *Rhizoctonia solani*. The authors applied Cerconil PM (20mg.L^{-1}) and Lonlife (15; 20; 25 and 30 Mg.L^{-1}) and, as results, they observed that *C. lindemuthianum* was more sensitive to doses of Lonlife and that *R. solani* was more resistant to Lonlife. The concentration of 25 mg.L^{-1} was the most efficient dose to inhibit the mycelial growth of *C. lindemuthianum*, when compared to the recommended dose of Cerconil PM.

Considering the properties of products derived from citric species, it is possible to justify the lower percentages of incidence and severity and the highest control of green mold in oranges.

4. Conclusions

All treatments with alternative products, derived from plants, differed significantly from the control treatment. The reductions in weight and longitudinal and transversal diameters were lower when the fruits were submitted to treatments with plant products. The green color of the peel does not indicate higher yield of juice. Products derived from species of genus *Citrus* show potential to be used as antifungal products in postharvest treatments, aiming at the control of green mold in oranges, considering that the latter was superior to 94.41%.

5. References

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TABLES

Table 1. Average percentages of reductions in weight and longitudinal and transversal diameters of fruits of orange, naturally infected with *Penicillium digitatum*, submitted to distinct treatments, after 12 days (from April 5th to April 17th, 2003) under room conditions (25ºC ± 2ºC, 85 ± 5% R.H.).

Treatments	**Reduction (%)**		
	Weight	Diameters	
		Longitudinal	Transversal
Control	10.40 a*	5.50 a	5.03 a
E.O.** *Citrus aurantium* ssp. *bergamia*	6.40 b	3.05 b	1.83 b
Citric Extract (2%)	5.76 b	2.34 bc	1.40 bc
E.O. *Citrus aurantium* ssp. *sinensis*	5.49 b	2.07 c	1.28 bc
Citric extract (4%)	3.36 b	1.52 c	0.80 c
CV (%)	31.11	21.34	23.59

* Averages followed by the same letter in the column do not differ by Duncan Test at the level of 5% of probability. Average of 4 repetitions.
** E.O. Essential oil.

Table 2. Average values of peel coloration and juice yield of fruits of orange naturally infected with *Penicillium digitatum*, submitted to different treatments, on time 0 (zero = April 5th, 2003) and after 12 days (April 17th, 2003) under room conditions (25ºC ± 2ºC, 85 ± 5% R.H).

Treatments	Grades[x]		Juice Yield (%)	
	Initial Color	Final Color	Initial[y]	Final
Control	1	5.00 a*	50.52	42.99b
E.O.** *Citrus aurantium* ssp. *bergamia*	1	4.50	50.52	43.05b
Ecolife (2%)	1	3.25 b	50.52	45.58 ab
E.O. *Citrus aurantium* ssp. *sinensis*	1	3.50 b	50.52	46.85 ab
Ecolife (4%)	1	2.25 c	50.52	48.50 a
CV(%)		16.37		5.60

* Averages followed by the same letter in the column do not differ by Duncan Test at the level of 5% of probability. Average of 4 repetitions.
** E.O. Essential oil.
[x]Grades: Coloration (peel): C1 - green = 1; C2 - pale green = 2; C3 - greenish yellow = 3; C4 - yellow = 4; C5 - Orange yellow = 5.
[y]Average of 120 fruits.

Table 3. Average values of incidence, injury diameter, severity and control of green mold in fruits of orange, naturally infected with *Penicillium digitatum*, submitted to different treatments, after 12 days (from April 5th to April 17th, 2003) under room conditions (25ºC ± 2ºC, 90 ± 5% R.H.)

Treatments	Incidence[v] (%)	Injury[x] (cm)	Severity[y] (%)	Control[z] (%)
Control	83.33 a*	2.14	30.20 a	59.06 b
E.O.** *Citrus aurantium* ssp. *bergamia*	41.66 b	1.08	7.77 b	94.41 a
Ecolife (2%)	16.67 c	0.45	7.17 c	99.74 a
E.O. *Citrus aurantium* ssp. *sinensis*	20.84 c	0.38	4.13 c	99.74 a
Ecolife (4%)	12.51 c	0.13	3.53 c	99.78 a
CV (%)	25.23		29.15	5.35

* Averages followed by the same letter in the column do not differ by Duncan Test at the level of 5% of probability. Average of 4 repetitions.
** E.O. Essential oil.
[v]24 fruits in each treatment; [x] Average diameter of injury in each treatment; [y] Severity (%) = (Treatment/Control treatment) x 100/ [z] Disease Control (%) = (Control Treatment - Treatment/Control Treatment) x 100.