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Research Article

Control of phytopathogenic microorganisms of post-harvest in tomato (*Lycopersicon esculentum* Mill.) with the use of citrus extract

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Abstract

Diseases are a major cause of post-harvest losses depending on season, region and management practices. Chemical control is the most used but with serious consequences for human health and the environment. This forces us to carry out more exhaustive studies on botanical products. The general objective of the present study was to evaluate the effect of citrus extracts for the control of pathogens that cause post-harvest diseases in tomato fruit. The product to be evaluated is of botanical origin from citrus extracts. Doses were evaluated (0, 666, 1000, 2000, 4000, 8000 ppm). The treatments were located at a temperature of 25°C±2 and 45% relative humidity (rH). The design used corresponded to a completely random design. The least significant difference was estimated by Tukey Multiple Range test at *P*=0.05. The statistical tests were performed through the SAS computer program. The results indicate that the pathogens detected and identified correspond to *Alternaria tenuissima; Botrytis cinerea; Cladosporium fulvum; Colletotrichum coccodes; Fusarium oxysporum; Geotrichum candidum; Rhizopus stolonifer* and *Stemphylium macrosporoideum*. Our conclusion is that the efficient doses correspond to 666, 2000 and 8000 ppm. With the application of citrus extracts, the damage percentage of tomato fruit was reduced in relation to the control treatments. Based on the results with the application of citrus extracts, the shelf life of the tomato was lengthened.

Introduction

Post-harvest handling is a series of stages that are carried out with the aim to protect the organoleptic and nutritional quality of different foods; in vegetables such as tomatoes, quality is linked to a set of attributes such as shape, color and appearance. The technology and management practices in the post-harvest period (post-harvest) are aimed at preserving the quality obtained in the field and reducing possible losses during the marketing and distribution process until final consumption. This is not an easy task to carry out, especially in cases where the tomato must go to markets generally more distant than the national ones and where the competition factor and the marketing strategies make necessary a good quality and a prolonged period of useful life in the fruit (longer shelf life) [1].

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Diseases are a major cause of post-harvest losses depending on season, region and management practices. Generally, roting and lesions on the surface are caused by phytopathogenic fungi such as *Alternaria* spp. (black rot), *Botrytis* spp. (gray mold rot), *Geotrichum* spp. (acid rot) and *Rhizopus* spp. (cotton rot) [2]. Bacterial soft rot caused by *Erwinia* spp. It can become a serious problem, particularly when the harvest is not properly done and the packer's health is not taken care of. The treatments with hot air or hot water immersion (55°C for 0.5- 1.0 min), have been effective to prevent the development of fungi on the surface, but have not been widely used in commercial treatments [3]. The controlled atmosphere can be effective to delay fungal growth in the peduncle scar and on the surface of the fruit. Greenhouse tomatoes marketed in clusters are very susceptible to *Botrytis* spp. (gray mold), especially when placed on trays and wrapped with plastic films [4].

The above information forces us to carry out more exhaustive studies on products of botanical origin, such as citrus extracts that we will use in the present work, in order to control some postharves diseases and increase shelf life. In addition to using these products in post-harvest, we have the alternative of integrating them into a pest and disease management program in crops of nutritional importance [5-8].

The objective of this study was to evaluate the effects of differents concentrations (0, 666, 1000, 2000, 4000 and 8000 ppm) of citrus extracts on surfaces of post-harvest tomatoes in vitro conditions, identifying the diseases and to determine the percentage of damage caused by the pathogens in the period of evaluation.

Materials and Methods

Experimental site

The research work was carried out in the Agriculture Department of the University of Sonora, located at coordinates 29º00'48 "North Latitude and 111º08'07" West Length at a height of 150 mals and 21 kilometers west of the city of Hermosillo, Sonora, Mexico. Saladette tomato fruit were donated by the commercial house "Super del Norte located in the city of Hermosillo, Sonora, Mexico. The product to be evaluated is from botanical origin of citrus extracts called for terms of the present investigation as "CitryAntiBio". The study consisted in evaluating six treatments with four repetitions each; handling different doses, as explained in table 1. The tomatoes were transferred to the Laboratory of the Agriculture Department. Immediately the different doses were prepared in plastic containers with capacity for 20 liters each and using only 10 liters of water for each repetition. After that, the tomatoes were submerged for 5 minutes and then they were placed in plastic containers (1.4 kg / repetition) divided into four cells each and arranged in a completely random design. The treatments were placed at a temperature of 25°C±2 inside the Laboratory, for 21 days. Subsequently, daily monitoring was done to observe and evaluate the appearance of symptoms and signs of diseases in all fruit. The tomatoes that were damaged by a pathogen were immediately weighed to determine the weight and the percentage of damage caused by the pathogen.

The identification of the causal agent of the disease that was causing damage to the fruit, was carried out by direct seeding of infected tissue in humid chambers to later

Table 1: Doses of extracts used in the different treatments expressed in parts of product by parts of water and in parts per million (ppm).

Treatmen	Dose (Product: water)	Type	Dose (ppm)
T1	0:0	Control	0
T2	1:1,500	Low dose	666
T3	1:1000	Low dose	1000
T4	1:500	Medium dose	2000
T5	1:250	High dose	4000
T6	1:125	High dose	8000

isolate the pathogen and identify it by means of assemblies in glass slides seen through the compound microscope. The pathogens directly taken from the infected fruit were also assembled. For their identification, was using the support of the keys for fungal identification of Gilman [9], Romero [10], Barnett and Hunter [11], and Abad [12].

The causal agent was also identified by the symptomatology on the fruit according to Romero [10]. For the data collection it was supported by an annotation table taking into account the previously mentioned parameters, such as the weight of the fruit, average percentage of tomato loss for each treatment and repetition due to the effect of pathogen damage in tomato as a whole and in this way proceed to its statistical analysis.

For the analysis of results, the average percentage of damage was obtained, causing pathogens for each of these and as a whole, as well as the general average percentage of tomato loss according to their weight. This was done for each of the treatments and repetitions. The design used corresponded to a completely random design. The least significant difference was estimated by Tukey Multiple Range test at P=0.05. The statistical tests were performed through the SAS computer program.

Results and Discussion

Based on the methodology proposed, the results indicate that once the symptoms were observed in tomato fruit and microorganisms were were analyzed under microscopy, the following fungal species were identified as: *Alternaria tenuissima, Botrytis cinerea, Cladosporium fulvum, Colletotrichum coccodes, Fusarium oxysporum, Geotrichum candidum, Rhizopus Stolonifer* and *Stemphylium macrosporoideum.* The previous results agree with some of the isolates of Salazar [13], who when developing a study with *Candelilla wax* (*Euphorbia antisyphilitica*) organic in different concentrations, in different storage forms and with the factor of a cold chamber and in the environment, caused the poor condition in litchi fruit (*Litchi chinensis*). It should be noted that the same ones identified have been previously reported by Ureña *et al*. [14], and León and Arosemena [15], as the main problems of post-harvest fruit and vegetables, including tomatoes and mango [16,17].

It is important to note that, the fungi detected in the present study were found to affect only the peduncle area, and the entire back of the tomato was intact, healthy, practically undamaged, with exception of those fruit which where affected by *Rhizopus* and *Geotrichum* which caused a soft rot in the fruit almost completely damaging them. This area of the peduncle, is a site of cell reproduction and enlargement that causes a breakdown of the epidermis, causing fungal spores that become sporulated and generate an infection in the fruit [18,19]. In relation to the average variable of tomato damage due to the effect of pathogen damage for each treatment and repetition, it is shown in table 2. When the data were entered into the statistical program SAS (2001), an Analysis of Variance was run and the test of multiple means comparison by the Tukey method. The results show that no significant difference was found as shown in table 3. Furthermore, when performing the means test, no response was found for the treatments, since all the means managed to be within the same statistical group, which means that all the treatments behaved the same as can be seen in table 4.

In table 4 the average percentage of loss for each treatment is shown, being treatment 1, which corresponds to the control, which presented a greater loss. On the other hand, treatments 2, 4, and 6 were those that numerically reflected the lowest average values or treatments that were less affected by post-harvest pathogens. It should be noted that treatment 6, which corresponds to the highest dose, showed the lowest loss compared to the lowest dose treatments, with a difference of 2 to 3% compared with treatments using doses of "CitryAntiBio". However, when treatment 6 was compared with control treatment, the diference was showed in 6%. The results of the present study agree with [20], obtaining differences among treatments with higher doses compared to the control, up to 10%.

Table 2: Average percentage of tomato loss (*Lycopersicon esculentum* Mill.) for each treatment and repetition due to the effect of tomato pathogen damage in tomato as a whole.

	Repetitions			
Treatments	I	II	III	IV
T1	36.9216	40.203	39.2566	39.2565
T2	33.1811	29.3473	32.3995	33.3545
T3	33.6858	40.6058	28.9665	33.1057
T4	33.145	26.869	40.9371	42.7098
T5	31.3464	37.1449	41.828	32.8417
T6	42.3085	27.3377	40.0027	20.3469

Table 3: Analysis of variance for the answer variable "Percentage of loss" of tomato (*Lycopersicon esculentum* Mill.) by effect of the damage of store pathogens in a completely randomized design with different number of repetitions.

FV	GL	SC	CM	F	F Tabla	
Tratamientos	5	269.0293	52.4036	0.5611 **NS**	0.05	2.61
Error	17	1593.0000	89.7201		0.01	3.12
Total	22	1866.0293				
C.V. =25.36%						

Table 4: Results of the multiple comparison of means for the response variable "Percentage of loss" of tomato (*Lycopersicon esculentum* Mill.) due to the effect of storage pathogen damage, by the Tukey method for a completely randomized design with different number of repetitions.

Treatment	Media	Group
1	38.905	A
4	32.065	A
5	35.78	A
3	34.08	A
6	32.497	A
2	32.065	A

The previous analyzes show us that it is important to extend the dose range, so that the results can be noted a significant inhibitory effect of citrus extracts against pathogens. These results agree with Valencia [20], when carrying out an evaluation in post-harvest fruit with the use of plant extracts. Although all the treatments behaved statistically equal to the percentage of loss, most of the tomatoes that were not damaged arrived at the end of the experiment, being able to realize that some of those fruit of tomatoes, were kept completely healthy until six days after the end of the experiment. The above agrees with Casas *et al.* [21], Who tells us that the tomato can last up to a week in storage at room temperature and from 14 to 21 days at 12°C. The present study in the same way, agrees with Valencia [20] and Oliveira [22], who when carrying out a study with extracts of citrus plants, managed to fulfill the main objective of their study, by lengthening the shelf life of the fruit until for two weeks, after 12 days of evaluation.

It was also observed that the losses caused by the pathogens separately are minimal, but when adding these percentages it is obtained that the pathogens as a whole cause shrinkage in the fruit to be evaluated. In this sense, the temperature and humidity conditions are a fundamental factor, for a better conservation of the fruit that allow less losses during the period of transport and commercialization. This last result was obtained by evaluating the effect of three environments on the quality of life of post-harvest of four varieties of extra firm tomato and long shelf life with peduncle [23,24].

Acording to figure 1, it can be seen how the percentages of losses are expressed for each treatment, due to the effect of damage of the pathogens as a whole in the different sampling dates. It can be seen that in the first six days the losses due to the attack of the pathogens are moderate, and they increase from the ninth day, presenting the highest percentage of losses on days 13 until the end of the study, in the control treatment. However, the treatments with the different doses evaluated, did not exceed 80%, highlighting those treatments with low doses.

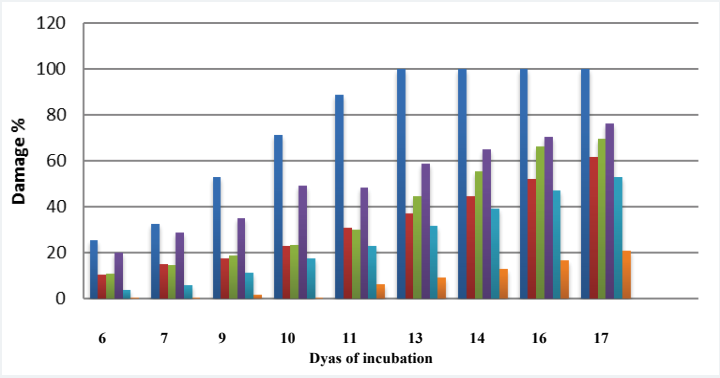

Figure 1: Percentage of losses of tomato (Lycopersicon esculentum Mill.) by the effect of damage of the warehouse pathogens as a whole in each treatment in the different dates in which they were presented. The dark blue column is treatment 1 (control=0); the red column is treatment 2 (666 ppm); the green column is treatment 3 (1000 ppm); the purple column is treatment 4 (2000 ppm); the sky blue column is the treatment 5 (4000 ppm); and the orange column is treatment 6 (8000 ppm).

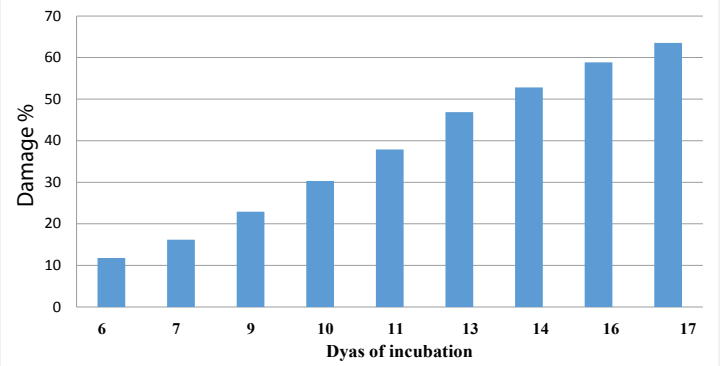

Figure 2: Percentage of total losses of tomato (Lycopersicon esculentum Mill.) due to the effect of damage of storage pathogens in the different sampling dates.

In figure 2 shows the percentage of loss of the total of the experiment due to damage of store pathogens for each sampling date. It can be seen that the same trend as in the previous figure is followed, with percentages above 50% up to 14 days.

It is important to indicate that of the pathogens identified, they were presented in the following order: in treatment 1, six days after the start of the study, the fungus *Fusarium oxysporum*. Subsequently Geotrichum spp. Two days later; at 9 days, the presence of *Alternaria tenuissima, Fusarium oxysporum* and *Cladosporium fulvum.* On the 11th day, the presence of *Botrytis cinerea, Colletotrichum coccodes* and *Stemphylium macrosporoideum*. On the other hand treatments 2 and 6, showed six days after the start of the study, the genus *Rhizopus Stolonifer*; on the 9th day the fungi *Geotrichum candidum*; *Alternaria tenuissima* and *Stemphylium macrosporoideum* on the 19th day; *Colletotrichum coccodes* was shown on the 20th day.

Treatments 3, 4 and 5 showed on the sixth day the presence of *Rhizopus Stolonifer*, *Alternaria tenuissima*; On the 9th day, the fungi *Fusarium oxysporum* and Geotrichum spp. On the 11th day, the fungi *Cladosporium fulvum* and *Colletotrichum coccodes*. Finally, after 14 days, the presence of *Fusarium oxysporum* and *Stemphylium macrosporoideum*. These results agree with Yli-Mattila [25], when identifying ssp of *Fusarium* in a morphological-molecular study in chili fruit.

Conclusions

The pathogens detected and identified correspond to *Alternaria tenuissima, Botrytis cinerea, Cladosporium fulvum, Colletotrichum coccodes, Fusarium oxysporum,*

Geotrichum candidum, Rhizopus stolonifer and *Stemphylium macrosporoideum*. The range of doses of citrus extracts is reduced, causing the same effect in all treatments in relation to the "Percentage of damage". Notwithstanding the above, it is concluded that the doses numerically efficient correspond to those of 666, 2000 and 8000 ppm. The highest percentage of damage tomato fruit occurs up to 14 days with the application of citrus extracts not more than 50%, while the control was affected in an 80% due to pathogen. Based on the above, with the application of citrus extracts, the shelf life of the tomato was lengthened.

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