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Perdones Montero, A.; Tur, N.; Chiralt A.; Vargas, M. (2016). Effect on tomato plant and fruit of the application of biopolymer  $\zeta$  oregano essential oil coatings. *Journal of the Science of Food and Agriculture*. 96(13):4505-4513. doi:10.1002/jsfa.7666



The final publication is available at

<http://doi.org/10.1002/jsfa.7666>

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Additional Information

# Effect on tomato plant and fruit of the application of biopolymer-oregano essential oil coatings

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## Abstract

**BACKGROUND:** Oregano essential oil (EO) was incorporated into film-forming-dispersions (FFDs) based on biopolymers (chitosan and/or methylcellulose) at two different concentrations. The effect of the application of the FFDs was evaluated on tomato plants (cultivar Micro-Tom) at three different stages of development, and on pre-harvest and postharvest applications on tomato fruit.

**RESULTS:** The application of the FFDs at “3 Leaves” stage caused phytotoxic problems, which were lethal when the EO was applied without biopolymers. Even though plant growth and development were delayed, the total biomass and the crop yield were not affected by biopolymer-EO treatments. When the FFDs were applied in the “Fruit” stage the pre-harvest application of FFDs had no negative effects. All FFDs containing EO significantly reduced the respiration rate of tomato fruit and diminished weight loss during storage. Moreover, biopolymer-EO FFDs led to a decrease in the fungal decay of tomato fruit inoculated with *Rhizopus stolonifer* spores, as compared with non-treated tomato fruit and those coated with FFDs without EO.

**CONCLUSION:** The application of biopolymer-oregano essential oil coatings has been proven to be an effective treatment to control *R. stolonifer* in tomato fruit.

Keywords: Coating, pre-harvest, postharvest, chitosan, methylcellulose, decay.

## INTRODUCTION

Tomato fruit (*Solanum lycopersicum*) is one of the most important crops in the world due its economic and nutritional value. Tomato is a climacteric fruit and has a short postharvest life, which is limited by several factors like transpiration and postharvest diseases. *Rhizopus stolonifer* is the most predominant fungus of tomato fruit and is also acquired during harvest, handling and transportation<sup>1</sup>.

Among the plethora of tomato genotypes, Micro-Tom cultivar has being extensively used as model plant based on the following characteristics: short life cycle (70-90 days), small plant size (10-20 cm), and small fruit size (2 cm in diameter). This cultivar was developed by crossing Florida Baket and Ohio 4013-3 cultivars<sup>2</sup>. Several studies suggested that Micro-Tom cultivar exhibits the above mentioned characteristics due to several mutations<sup>3,4,5</sup>

Natural ingredients such as antimicrobials obtained from plant sources are becoming of great interest both in crop protection and food preservation due to worldwide awareness of environmental and food safety concerns associated with non-natural additives. The general use of synthetic fungicides in plant protection has resulted in the development of resistance, toxicity to non-target organisms and adverse effects on the environment<sup>6</sup>. Essential oils are natural antioxidants and antimicrobial hydrophobic substances extracted from fruits and vegetables with an intense aroma<sup>7</sup>. The most common components of essential oils are terpens and terpenoids<sup>8</sup>. The composition of essential oils can vary depending on agronomic factors and characteristics of the raw material<sup>9,10</sup>. The main components of oregano essential oil (*Origanum compactum* L.) are carvacrol, thymol,  $\gamma$ -terpinene and p-cimene. Essential oils are highly volatile and thus high doses are often required to achieve the desired antifungal effect, which in turn can entail phytotoxicity problems and high application costs. Previous studies have shown the

effectiveness of oregano essential oil vapours to control fungal decay in tomato plant<sup>11</sup> in greenhouses as well as in postharvest treated tomato fruit<sup>12</sup>. Nevertheless, previous studies have pointed out the necessity of controlling the application dose in greenhouses in order to prevent the occurrence of signs of phytotoxicity in tomato plant and fruit<sup>13</sup>.

An interesting strategy for reducing dose of EO and decrease their potential adverse effects, is to incorporate them in biopolymer-based film-forming dispersions, such as chitosan and methylcellulose<sup>14</sup>. Chitosan is a biodegradable cationic hydrocolloid with antimicrobial and antioxidant activity that shows excellent film-forming ability<sup>15,16</sup>. Chitosan-based films can modify the internal atmosphere of coated products thus delaying fruit ripening and decreasing the respiration rate<sup>17</sup>. Methylcellulose is a biopolymer with good film-forming characteristics, which is colorless, odorless and resistant to fats<sup>18</sup>. Methylcellulose is compatible with chitosan, yielding composite films with lower water vapour permeability than pure chitosan films<sup>19</sup>.

Several studies have reported the effects of the application of chitosan-based edible coatings on different fruit<sup>20</sup>. Chitosan-based coatings inhibited the growth of *Botrytis cinerea* and improved resistance of tomato fruit against gray mold during storage<sup>21</sup>. Chitosan-beeswax-lime EO coatings inhibited the growth of *Rhizopus stolonifer* in tomato fruit at three different maturity stages throughout storage<sup>1</sup>. However, to the best of our knowledge, there are no published studies on the application of chitosan-methylcellulose based films enriched with oregano essential oil on tomato fruit.

The aim of this study was to evaluate in tomato plant and in tomato fruit the effect of pre-harvest and postharvest applications of film-forming dispersions based on chitosan and/or methylcellulose containing oregano essential oil.

## **EXPERIMENTAL**

## Materials

High molecular weight chitosan (CH) (deacetylation degree of 24%, viscosity in glacial acetic acid solution in 1% = 1.3 Pa.s. Sigma-Aldrich, USA), Methylcellulose (MC) (VWR, Barcelona, Spain), glacial acetic acid (Panreac, Barcelona, Spain), Tween85 (Sigma-Aldrich, USA), liquid silicone defoamer (Panreac, Barcelona, Spain) and oregano (*Origanum compactum* L.) essential oil (EO) (Herbes del Molí, Alicante, Spain) with a composition of 46.1% carvacrol, 25.6% thymol, 10.5%  $\gamma$ -terpinene and 7.5% p-cymene as the major elements were used to prepare the FFDs.

For the *in vivo* assays, tomato plants cv. Micro-Tom cultivated under controlled temperature and humidity in greenhouse (ETSEAMN, Universitat Politècnica de València) were used.

Stock culture of *Rhizopus stolonifer* (CECT 2344, Burjasot, Spain) was used for the *in vitro* and *in vivo* assays. As an enrichment medium for the microbiological analyses buffered peptone water (BPW) (Scharlau, Barcelona, Spain) was used. *R. stolonifer* was cultured on potato dextrose broth (PDB) (Scharlau, Barcelona, Spain) and bacteriological agar (BA) (Scharlau, Barcelona, Spain). For the counting of the fungi in the *in vivo* assay Sabouraud Chloramphenicol (Scharlau, Barcelona, Spain) agar was used.

*R. stolonifer* spores, which were used for the antifungal tests, were collected with a sterile inoculating loop from a 7-days *R. stolonifer* culture. The obtained spores were suspended in physiological water solution with Tween 20 (0.1% w/w) to obtain a final spore concentration of  $10^6$  spores/mL or  $10^5$  spores/mL, for the *in vitro* and *in vivo* tests, respectively. The cell density of the spore suspensions was estimated by direct cell count using a Thoma camera.

## Preparation of the film-forming dispersions and stand-alone films

Chitosan (CH) (1% w/w) was dispersed in an aqueous solution of glacial acetic acid (AA) (1% v/w) and kept under continuous stirring for 12h. At the same time, Methylcellulose (MC) (1% w/w) was dispersed and heated up to 80°C for 10 minutes. Once dissolved methylcellulose was cooled to 5-10°C for 20 minutes. Both dispersions were mixed in the right proportions to obtain the film-forming dispersions (FFDs). To prepare CH/MC-essential oil (EO) FFDs, EO was incorporated at 0.25 or 0.5% (w/w) concentration. Tween 85 (0.01% v/v) and silicone (0.01% v/v) were added in each FFD to favor their extensibility on the plat tissues and to avoid bubble formation, respectively. The formulations were named as follows: MC<sub>0.5</sub>, MC<sub>0.5</sub>-CH<sub>0.5</sub>, MC<sub>0.5</sub>-EO<sub>0.25</sub>, MC<sub>0.5</sub>-EO<sub>0.5</sub>, MC<sub>0.5</sub>-CH<sub>0.5</sub>-EO<sub>0.25</sub>, MC<sub>0.5</sub>-CH<sub>0.5</sub>-EO<sub>0.5</sub> where the subscript stands for the concentration (wt %) of the ingredient in the FFDs. For *in vivo* assays two more treatments were used: Aqueous dispersions of EO at 0.25% (w/w), named as EO<sub>0.25</sub>, and EO at 0.5% (w/w), named as EO<sub>0.5</sub>. Tween 85 and silicone were also added at 0.01% v/v.

All FFDs and EO dispersions were homogenized with a rotor-stator (Ultra TurraxT-25 digital, IKA, Alemania) at 13,400 rpm for 4 minutes. The final emulsions were degased using a vacuum pump (Diaphragm VacuumPump MZ2CNT, Vacuubrand, Alemania).

Stand-alone coatings were obtained by casting, pouring an amount of FFD enough for provide a solids surface density of 56 g/m<sup>2</sup> as described by Vargas et al<sup>22</sup>. All FFDs were poured into polystyrene Petri dishes with 90mm diameter. The samples were dried for 7 days at room temperature (about 25°C) and 60% relative humidity (RH). Dry films were peeled off from the Petri dishes and used for the *in vitro* assays.

### **Antifungal activity *in vitro* assays**

PDA medium was sterilized and aseptically poured into sterile Petri dishes with 90 mm of diameter (20 g per Petri dish). After the culture medium solidified, a 100 µl aliquot from a 10<sup>6</sup> spores/mL suspension was inoculated on the surface of each Petri dish. The different films were placed on the inoculated surfaces, following the methodology reported by Perdonés et al<sup>23</sup>. Inoculated and non-coated samples without film were used as control samples. Petri dishes were sealed with Parafilm to avoid dehydration and incubated at 25 °C (optimal growth temperature of *R. stolonifer*). At different times of storage, Petri dishes content was removed aseptically and placed in a sterile plastic bag with 90 mL of BPW. The bags were homogenized with a Stomacher (Interscience BagMixer Stomacher 400 W Homogenizer, France). Serial dilutions were made and then poured into SCA Petri dishes. Samples were incubated at 25°C for 24 hours before colonies were counted. All the tests were run in triplicate.

### **Application of the treatments on tomato plants and fruits**

The application of all treatments on the tomato plants was made on three different moments according to their stage of development according to the solanaceae BBCH extended scale reference<sup>24,25</sup>: 1 13 stage (3 Leaves), 6 stage (Flowers) and 7 stage (Fruit). All applications were made with a pre-pressure sprayer (Menan Agrícola, Algemesí, Valencia) under controlled conditions of amount of product spilled and nozzle-plant distance. In the first stage, 10 plants per treatment and 10 for control were selected (90 plants). Prior to the application in stage 6 (Flowers), 5 flowers per plant were labeled. After the application of the different treatments, the following parameters were monitored in tomato plants: physiological damage, fruit set ratio (fruits/flowers), fruit size, plant yield and physiological state. In the applications at the stage 7 of development (Fruits), 56 plants with green fruits were sprayed (8 plants per treatment)

and the fruits were harvested one week after the application of the different treatments. For the postharvest application, green fruit around 2-2.5 cm in diameter were harvested and transported to the facilities of the Institute of Food Engineering for Development (Valencia, Spain) to apply the different treatments.

### **Respiration rate and weight loss of tomato fruits**

Tomato fruits (15 tomatoes per treatment) were introduced in 940 mL hermetic glass jar with a septum in the lid for sampling the gas in the headspace every two hours with a gas analyser (PBI Dansensor CheckMate 9900, Ringsted, Dinamarca). The jars were kept at 25 °C. Two replicates were performed per treatment. The respiration rate,  $RR_i$  ( $\text{mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ), of the samples in terms of  $\text{CO}_2$  generation and  $\text{O}_2$  consumption was determined from the slope of the fitted linear equation of the gas concentration in front of time as described by Vargas et al<sup>26</sup>. In order to record the variation on the sample weight during the storage, tomato fruit were weighed regularly with a balance (Sartorius Extend ED423S, Germany).

### **Colour of tomato fruits**

The color of the fruits was determined using a spectrophotometer (Minolta CM-3600d, Tokyo, Japan) previously calibrated. CIE-L\* a\* b\* coordinates, hue ( $h^*_{ab}$ ) (eq. 1) and chroma ( $C^*_{ab}$ ) (eq. 2), were obtained from the reflection spectra of the samples using D65 illuminant/10° observer<sup>26</sup>.

$$h^*_{ab} = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (1)$$

$$C^*_{ab} = \sqrt{a^{*2} + b^{*2}} \quad (2)$$



Five fruits per treatment were measured. To avoid the effects of heterogeneity on the raw material, measurements were always carried out in the same 5 previously marked fruit.

#### **Acidity, pH, soluble solids and maturity index of tomato fruits**

Acidity, pH and soluble solids were determined in triplicate as described by Perdonés et al.<sup>27</sup>. Acidity was analyzed following method AOAC 942.15 (AOAC, 1995), and was expressed as g of citric acid per 100 g of sample. In order to measure the pH, 3 g of each sample were weighed and added to 20 mL of distilled water. Measurement of pH was carried out by means of a pH-meter (GLP 21+, Crison, Barcelona, Spain). Soluble solids (Brix) were measured with a refractometer (Minolta, Japan). Maturity index (MI) was calculated as the quotient between Brix degrees and acidity.

#### **Fungal decay of tomato fruits**

Fully ripe non-treated tomato fruit were placed on plastic grilles. One group of samples (20 fruit per treatment) was inoculated with *R. stolonifer* spore suspension, and the treatments were applied by spraying (curative treatments). The other group of samples (20 tomatoes per treatment) was first treated with the different treatments by spraying and then inoculated (preventive treatment). Each tomato fruit was inoculated with 25 µl of a *R. stolonifer* suspension ( $10^5$  spores/mL), which was settled to the peduncle scar, since this is the most probable via of fungal infection. Results were expressed as the percentage of fruit with visible signs of fungal infection.

#### **Statistical analysis**

The results were submitted to an analysis of variance (ANOVA) by using STATGRAPHICS Centurion XVI (16.2.04 version) and taking into account the effect of the formulation (treatments). Comparisons were performed through LSD intervals at a 95% significance level.

## **RESULTS AND DISCUSSION**

### **In vitro assays**

Stand-alone films were transparent and slightly white as shown in Figure 1b. The films were deposited on the inoculated PDA plates and after 3 weeks of storage at 25 °C, all films containing oregano EO inhibited fungal sporulation (Figure 1b). The evolution of the *R. stolonifer* counts during storage at 25 °C is shown in Figure 2. At initial time the microbial counts of agar plates coated with films containing CH and/or EO were significant lower than the counts obtained in non-coated agar plates (control) and in the plates coated with pure polymer films. Similar inhibitory effect against *R. stolonifer* was obtained by Alvarado et al<sup>28</sup> by using CH-thyme essential oil films. It is important to point out that in the present study the plates coated with the films that contained EO showed no fungal growth during the whole storage period. MC films did not promote a significant reduction in fungal growth, as compared with control samples. When CH was added into the formulations, a significant reduction in the microbial counts was obtained during the first week of storage, in agreement with the antimicrobial effect of CH reported in previous *in vitro* studies performed with *R. stolonifer* isolated from tomato fruit<sup>29,30</sup>.

### **Applications to tomato plant**

The effect of the treatments on Micro-Tom plants in “3 Leaves” stage is shown in Figure 3a. The amount of EO applied per plant was higher for the formulations containing the highest EO concentration (0.022 g EO/plant) as compared to formulations with the lowest amount of EO (0.011 g/plant), in agreement with the composition of the FFDs. After each treatment application, at every development stage, the plant general physiological conditions were visually monitored (Figure 3c). Treatments without EO did not promote any visual damage on tomato plants. The application of MC<sub>0.5</sub>-EO<sub>0.25</sub> led to minor damages on tomato plants. The detected damages which were maintained throughout the whole monitoring period, with no detectable effects on the normal development of plants. The application of the highest dose of EO led to adverse effects on the physiological state of the tomato plants. In fact, all treatments with the highest EO concentration applied at “3 leaves” stage had lethal effects, being the percentage of death plants significantly lower when the EO was included in the FFDs (Figure 3a). Taking into account these results, EO<sub>0.5</sub> treatment was not used in the applications at “Flowers” and “Fruits” stages. The comparison of the obtained results after the application of EO<sub>0.5</sub>, with the results shown after the application of the same dose of EO incorporated throughout biopolymer-based FFDs suggests that chitosan and methylcellulose acted as encapsulating agent, promoting a controlled release of the EO and decreasing its potential toxicity. Previous studies shown the effectiveness of CH as an encapsulating agent, which allowed the controlled release of coriander and citronella essential oils<sup>33,34</sup>. It is important to point out that due to the physiological damages, plant development was delayed and so blooming. Moreover, some of the damaged plants were affected by other diseases due their weakened physiological state.

Figure 3b shows the effect of the treatments on tomato plants in the “Flowers” stage. The applications of the treatments were carried out when the first flowers appeared (22 days after starting the experiments at “3 Leaves” stage). The dose of EO per plant, using biopolymer based FFD, was similar to that applied in the experiments performed at “3 Leaves” stage. The treatments had no lethal effects. Non-treated (Control) and plants sprayed with MC<sub>0.5</sub> FFDs did not show any damage during the whole assay. All plants sprayed with EO<sub>0.25</sub> showed permanent damages. The 10% of plants treated with MC<sub>0.5</sub>-CH<sub>0.5</sub> showed slight damage and no differences between MC<sub>0.5</sub>-EO<sub>0.25</sub> and MC<sub>0.5</sub>-EO<sub>0.5</sub>, applications were detected; both FFDs promoting slight damages. The plants sprayed with MC<sub>0.5</sub>-CH<sub>0.5</sub>-EO<sub>0.25</sub> and MC<sub>0.5</sub>-CH<sub>0.5</sub>-EO<sub>0.5</sub> treatments showed medium damages (damage 2) although the level of damages was reduced two weeks after the treatment application.

The comparison between the level of damages detected in the plants after applications at “3 Leaves” stage and “Flowers” stage revealed that the application of FFDs containing EO had more negative effects on the physiological state of Micro-Tom plants when they were applied at an early stage of development, when the plants have a less developed cuticle.

Fruit set (number of fruits/number of flowers) and the total weight of yield was determined in plants submitted to the spray applications in the “flowers” stage. Fruit set ratio (%) and weight of fruit yield (g) for each treatment were: C (57%, 97.09 g), MC<sub>0.5</sub> (64%, 155.47 g), MC<sub>0.5</sub>-CH<sub>0.5</sub> (73%, 172.82 g), MC<sub>0.5</sub>-EO<sub>0.25</sub> (62%, 131.45 g), MC<sub>0.5</sub>-EO<sub>0.5</sub> (76%, 176.3 g), MC<sub>0.5</sub>-CH<sub>0.5</sub>-EO<sub>0.25</sub> (42%, 89.8 g), MC<sub>0.5</sub>-CH<sub>0.5</sub>-EO<sub>0.5</sub> (66%, 142.98 g) and EO<sub>0.25</sub> (61%, 127.07 g). The application of the different treatments did not have any negative impact over the fruit set. In fact, all FFDs, except MC<sub>0.5</sub>-CH<sub>0.5</sub>-EO<sub>0.25</sub>, led to higher fruit set ratio as compared with non-treated plants (C). The relation

between fruit set ratio and total fruit yield weight suggested that the treatments did not affect the total amount of fruit and that all of them reached a similar size.

Figure 4 shows the total fruit yield and the global weight of these fruits for each treatment, taking into account all plants treated at stage “3 Leaves”, “Flowers” and “Fruit” stages. There were no differences in total yield and weight among treatments and the incorporation of EO into the FFDs did not affect the global yield.

### **Pre-harvest and postharvest applications to tomato fruits.**

Figure 5 shows lightness, hue and chroma development of the fruit throughout the storage at 25°C after pre-harvest and postharvest applications. Lightness significantly decreased at the end of the storage, in line with weight loss and surface dehydration<sup>31</sup>. Colour significantly changed between 6 and 9 days of storage, due to the change from green to red, with no significant effects due to the treatment application. Hue decreased during storage until became red in all samples in line with the ripening progress. No significant effect of treatment application was detected. The incorporation of EO into FFDs had no significant effect on the change in the three colour parameters.

Table 1 shows the average and standard deviation of the values of acidity, pH, Brix and maturity index (MI) and weight loss of tomato fruit treated before harvesting at the end of the storage at 25°C. ANOVA showed significant differences ( $p < 0.05$ ) in acidity values among treatments. The application of oregano EO and its incorporation into FFDs led to a slight decrease on the acidity values of the samples. Brix and MI were significantly affected by treatment application ( $p < 0.05$ ). Nevertheless, no clear trends were observed when comparing the values of control samples with treated ones, and EO incorporation in the treatments did not affect fruit ripening.

Acidity, pH, Brix, MI and weight loss of the tomatoes treated in postharvest at the end of the storage at 25°C are shown in Table 2. All parameters (except weight loss) were significantly affected by treatment application ( $p < 0.05$ ), although important variations in the MI of samples were not detected. Weight loss of samples treated with formulations containing EO was reduced as compared to control and samples treated with pure biopolymer FFDs, although the differences were not significant. In general, coatings containing EO provoked a decrease in weight loss of tomato fruit during storage. This was notable in postharvest treatments where significant differences in weight loss with respect to the control sample and those coated with FFDs without EO were observed.

Figure 6 shows the development of respiration rate of the tomato fruit samples in terms of CO<sub>2</sub> production and O<sub>2</sub> consumption during storage. The kind of treatment, the storage time and the interaction between both factors had a significant effect in the respiration rate of the samples ( $p < 0.05$ ). In all samples, it was observed an increase in the respiration rate at the beginning, after 2 days of storage, due to the stress caused over the samples by the atmosphere changes<sup>32</sup>. In the pre-harvest application (Figure 6a), samples treated with EO<sub>0.25</sub> showed a complete different development than the others, especially in terms of oxygen consumption. This could be due to an effect of EO on the fruit metabolism when it was still in the plant, which was maintained during the postharvest storage.

For both moments of application, FFDs (with or without EO) led to a decrease in the respiration rate of the samples as compared with non-coated ones (control). Similar results were observed by El Ghaouth et al.<sup>33</sup> after the application of chitosan-based coatings on tomato fruit.

At the sixth day of storage, it was observed an increase in respiration rate values, followed by a respiration rate decrease. This is due to the increase in the fruit respiration activity just before the maturity stage (climaterium). This change is common in climacteric fruit such as tomato<sup>34</sup>. The respiratory quotient (RQ) was not affected by treatment application during storage, being all values near 1. RQ value for fresh fruit ranges from 0.7 to 1.3 for the aerobic respiration and it is much greater than one when anaerobic respiration takes place<sup>35</sup>.

### **Fungal decay**

Figure 7 shows the effect of the preventive and curative treatments performed with fully ripened tomato fruit samples inoculated with *R. stolonifer* spores. In preventive applications, all treatments delayed fungal decay as compared with inoculated control samples, although MC<sub>0.5</sub> formulation had no notable effect on the control of fungal decay and led to similar percentages of infection than those obtained in control tomatoes. Samples treated with MC<sub>0.5</sub>-CH<sub>0.5</sub> showed lower levels of infection than samples treated with MC<sub>0.5</sub>, due the effect of CH on *R. stolonifer* growth, which coincides with the results obtained in the *in vitro* assays. The percentage of infected fruits when samples were treated with formulations containing EO was lower, which indicates that EO incorporation improved the antifungal properties of the biopolymers-based FFDs, as observed in the *in vitro* assays. Samples treated with MC<sub>0.5</sub>-CH<sub>0.5</sub>-EO<sub>0.5</sub> showed the lowest percentage of fruit infection at 7 storage days. In general, the preventive applications seem to lead to better results than curative ones when FFDs contain EO. However, for MC<sub>0.5</sub>-CH<sub>0.5</sub> preventive treatments a higher fungal decay than in curative applications was obtained.

## CONCLUSION

Pre-harvest application of EO to tomato plants promoted phytotoxic effects, which were diminished when EO was incorporated at the same dose into chitosan-methylcellulose based FFDs. Plant growth and development were delayed due to EO application, although total biomass production, fruit set ratio and yield were not affected. Post-harvest application of biopolymer-oregano essential oil coatings decreased weight losses during storage and promoted a reduction in the respiration rate of tomato fruit, with no effect on the maturity index and pH of fruits. The incorporation of oregano EO into FFDs based on MC and CH has been proven to be an effective treatment to control *R. stolonifer* in tomato fruit both in postharvest curative and preventive applications.

## ACKNOWLEDGEMENTS

The authors acknowledge the support provided by Universitat Politècnica de València (SP20120518) and the Spanish Ministerio de Economía y Competitividad (AGL2013-42989-R-AR). Author A. Perdonés is grateful to Universitat Politècnica de Valencia for a FPI grant.

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## Tables

**Table 1.** Acidity, pH, soluble solids (Brix), maturity index (MI) and weigh loss of tomato fruit treated before harvest (pre-harvest application) at the end of the storage at 25°C. Average values and standard deviation in brackets.

Treatment	Acidity x10 <sup>-1</sup> (g ·kg <sup>-1</sup> )	pH	Brix	MI	Weight loss (%)
C	1.03 (0.05) <sup>c</sup>	4.128 (0.012) <sup>a</sup>	5.8 (0.09) <sup>d</sup>	5.6 (0.3) <sup>bc</sup>	10.9 (0.8) <sup>a</sup>
MC <sub>0.5</sub>	0.97 (0.05) <sup>bc</sup>	4.128 (0.012) <sup>a</sup>	5.02 (0.12) <sup>a</sup>	5.2 (0.2) <sup>ab</sup>	10.5 (1.4) <sup>a</sup>
MC <sub>0.5</sub> -CH <sub>0.5</sub>	0.93 (0.12) <sup>ab</sup>	4.1250 (0.0105) <sup>a</sup>	5.82 (0.08) <sup>d</sup>	6.4 (0.9) <sup>d</sup>	10.51 (0.05) <sup>a</sup>
MC <sub>0.5</sub> -EO <sub>0.25</sub>	1.04 (0.14) <sup>c</sup>	4.120 (0.014) <sup>a</sup>	5.00 (0.13) <sup>a</sup>	4.9 (0.7) <sup>a</sup>	14 (3) <sup>a</sup>
MC <sub>0.5</sub> -EO <sub>0.5</sub>	0.85 (0.04) <sup>a</sup>	4.16 (0.06) <sup>b</sup>	5.45 (0.08) <sup>bc</sup>	6.4 (0.3) <sup>d</sup>	9.9 (0.6) <sup>a</sup>
MC <sub>0.5</sub> -CH <sub>0.5</sub> -EO <sub>0.25</sub>	0.88 (0.06) <sup>ab</sup>	4.130 (0.009) <sup>a</sup>	5.5 (0.3) <sup>c</sup>	6.3 (0.5) <sup>d</sup>	9.90 (0.10) <sup>a</sup>
MC <sub>0.5</sub> -CH <sub>0.5</sub> -EO <sub>0.5</sub>	0.93 (0.08) <sup>ab</sup>	4.120 (0.014) <sup>a</sup>	5.13 (0.08) <sup>a</sup>	5.6 (0.5) <sup>bc</sup>	11.471 (0.103) <sup>a</sup>
EO <sub>0.25</sub>	0.87 (0.09) <sup>ab</sup>	4.135 (0.014) <sup>a</sup>	5.32 (0.12) <sup>b</sup>	6.1 (0.6) <sup>cd</sup>	13.4 (0.4) <sup>a</sup>

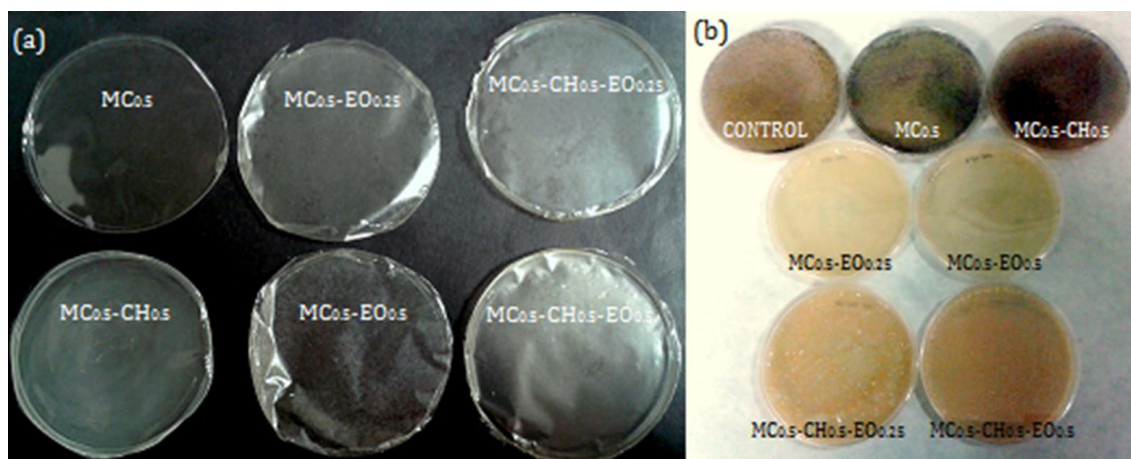
Different letters (a, b, c d) show significant differences (p<0.05).

**Table 2.** Acidity, pH, soluble solids (Brix) and maturity index (MI) of tomato fruit treated in post-harvest at the end of the storage at 25 °C. Average values and standard deviation in brackets.

Treatment	Acidity x10 <sup>-1</sup> (g ·kg <sup>-1</sup> )	pH	Brix	MI	Weight loss (%)
C	0.897 (0.113) <sup>a</sup>	4.13 (0.03) <sup>bc</sup>	5.25 (0.08) <sup>c</sup>	5.9 (0.8) <sup>ab</sup>	24 (7) <sup>c</sup>
MC <sub>0.5</sub>	0.81 (0.09) <sup>a</sup>	4.10 (0.02) <sup>b</sup>	5.17 (0.08) <sup>bc</sup>	6.4 (0.7) <sup>b</sup>	22 (2) <sup>bc</sup>
MC <sub>0.5</sub> -CH <sub>0.5</sub>	0.92 (0.12) <sup>ab</sup>	4.120 (0.009) <sup>bc</sup>	5.72 (0.08) <sup>e</sup>	6.3 (0.9) <sup>b</sup>	27 (6) <sup>c</sup>
MC <sub>0.5</sub> -EO <sub>0.25</sub>	0.80 (0.08) <sup>a</sup>	4.153 (0.012) <sup>c</sup>	5.20 (0.09) <sup>c</sup>	6.5 (0.7) <sup>b</sup>	24.2 (0.7) <sup>c</sup>
MC <sub>0.5</sub> - EO <sub>0.5</sub>	0.81 (0.08) <sup>a</sup>	4.115 (0.014) <sup>b</sup>	4.58 (0.12) <sup>a</sup>	5.7 (0.6) <sup>ab</sup>	5 (5) <sup>a</sup>
MC <sub>0.5</sub> -CH <sub>0.5</sub> -EO <sub>0.25</sub>	0.795 (0.095) <sup>a</sup>	4.15 (0.02) <sup>c</sup>	5.1 (0.2) <sup>b</sup>	6.4 (0.8) <sup>b</sup>	3.5 (0.6) <sup>a</sup>
MC <sub>0.5</sub> -CH <sub>0.5</sub> -EO <sub>0.5</sub>	1.0 (0.2) <sup>bc</sup>	4.15 (0.05) <sup>c</sup>	5.23 (0.05) <sup>c</sup>	5.14 (0.96) <sup>a</sup>	12 (6) <sup>ab</sup>
EO <sub>0.25</sub>	1.07 (0.09) <sup>c</sup>	4.01 (0.05) <sup>a</sup>	5.55 (0.05) <sup>d</sup>	5.2 (0.4) <sup>a</sup>	17 (2) <sup>bc</sup>

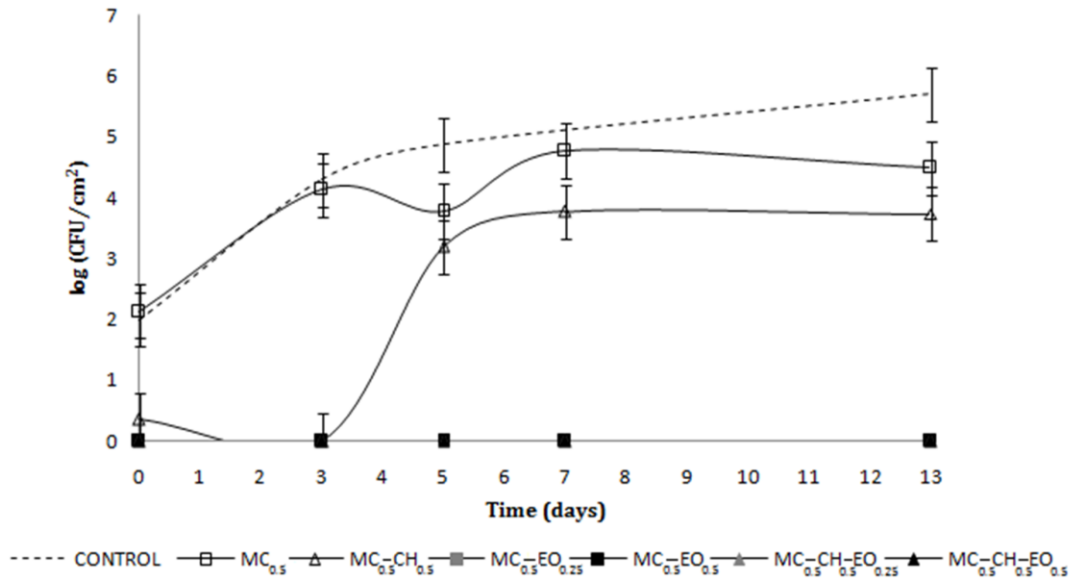
Different letters (a, b, c d) show significant differences (p<0.05).

## Figures

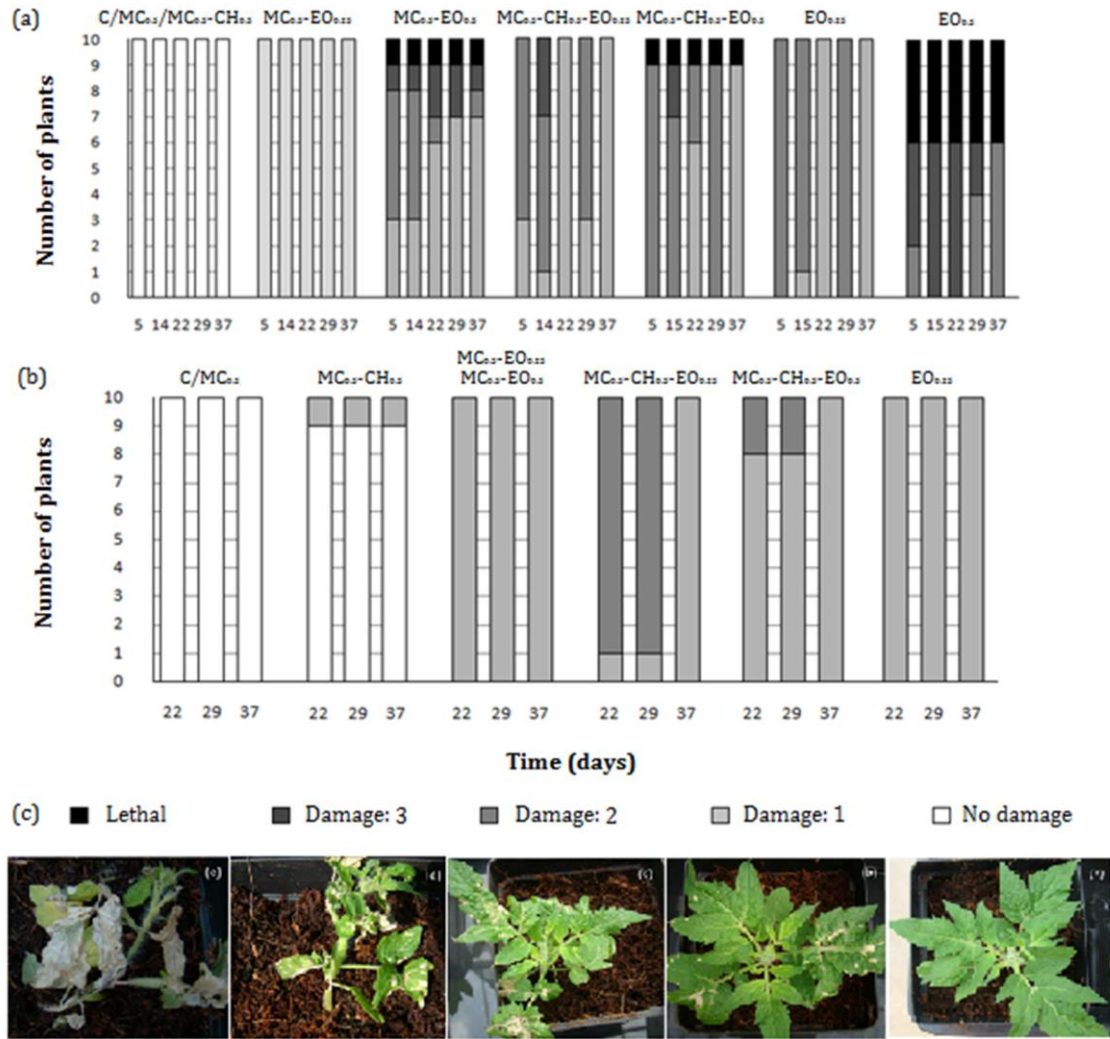


**Figure 1.** (a) Stand-alone films (b) Petri dishes inoculated with the spore suspension of *R. stolonifer* and coated with the films and non-coated (control).

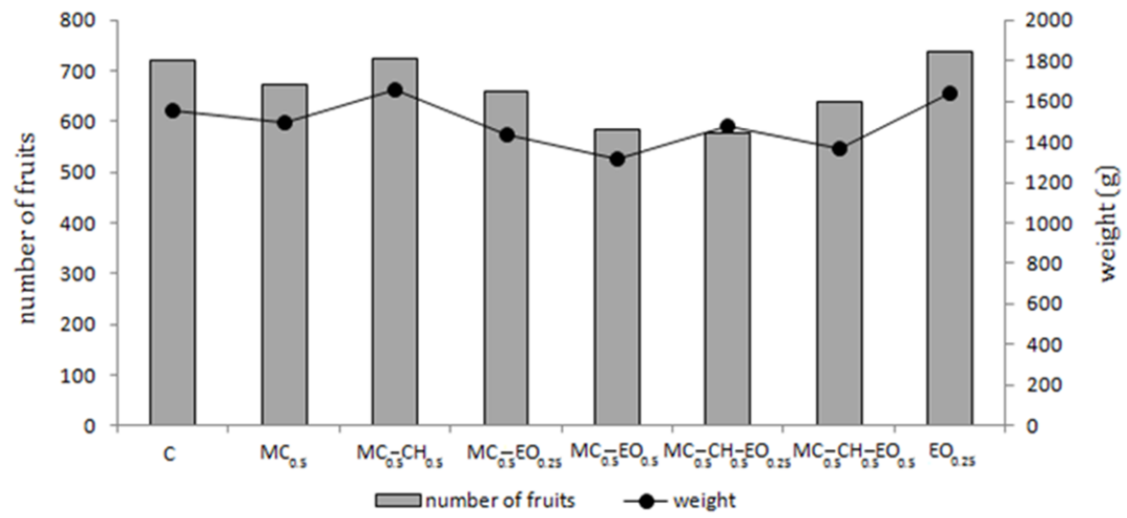




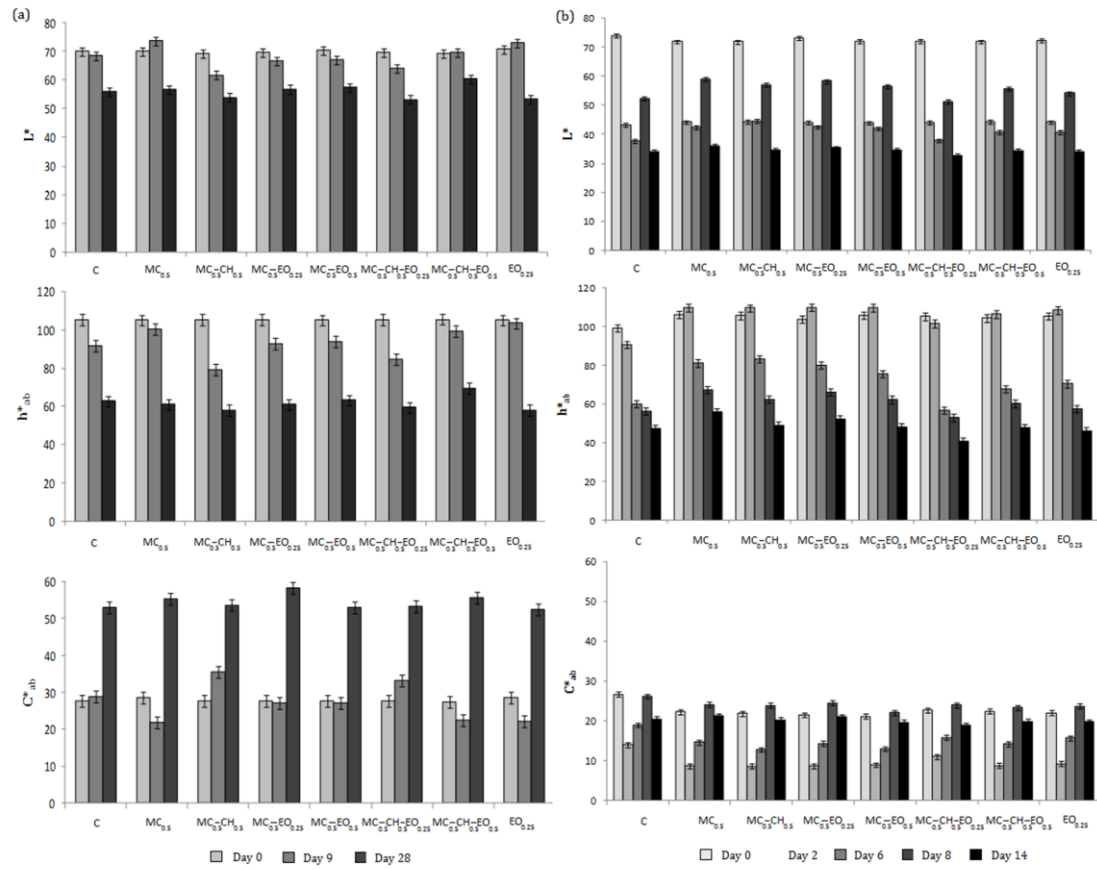
**Figure 2.** *R. stolonifer* counts during storage at 25 °C. Average values and 95% LSD intervals.

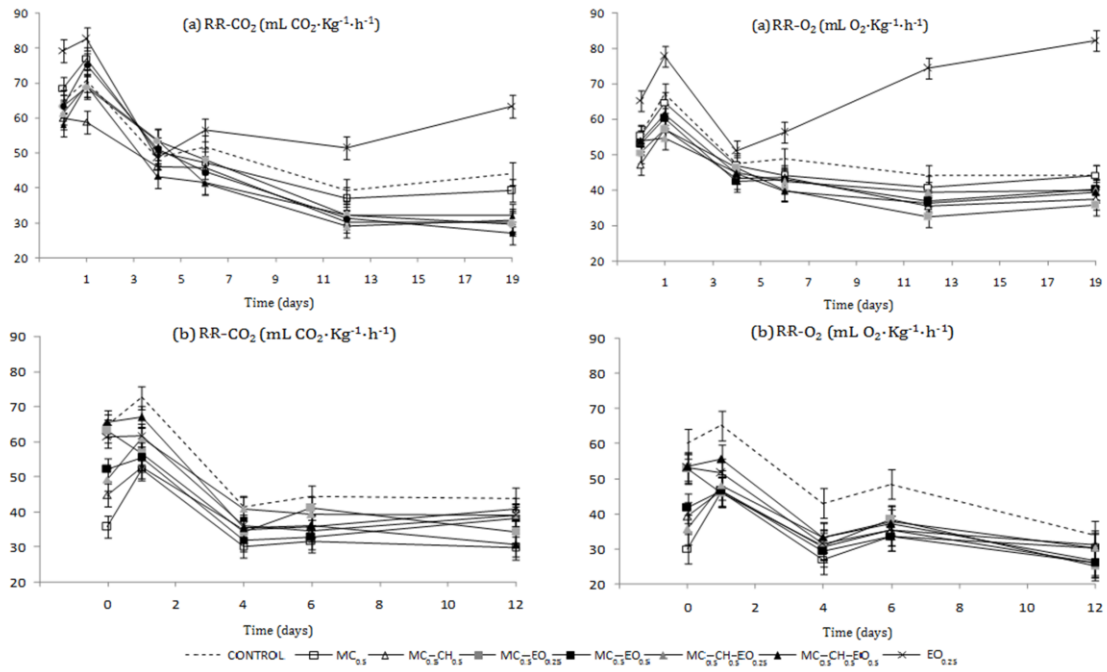


**Figure 3.** (a) Effect of the treatments on Micro-Tom plants in “3 Leaves” stage and (b) “Flowers” stage. (c) Visual rating scale ranges. Treatments with similar results are grouped.

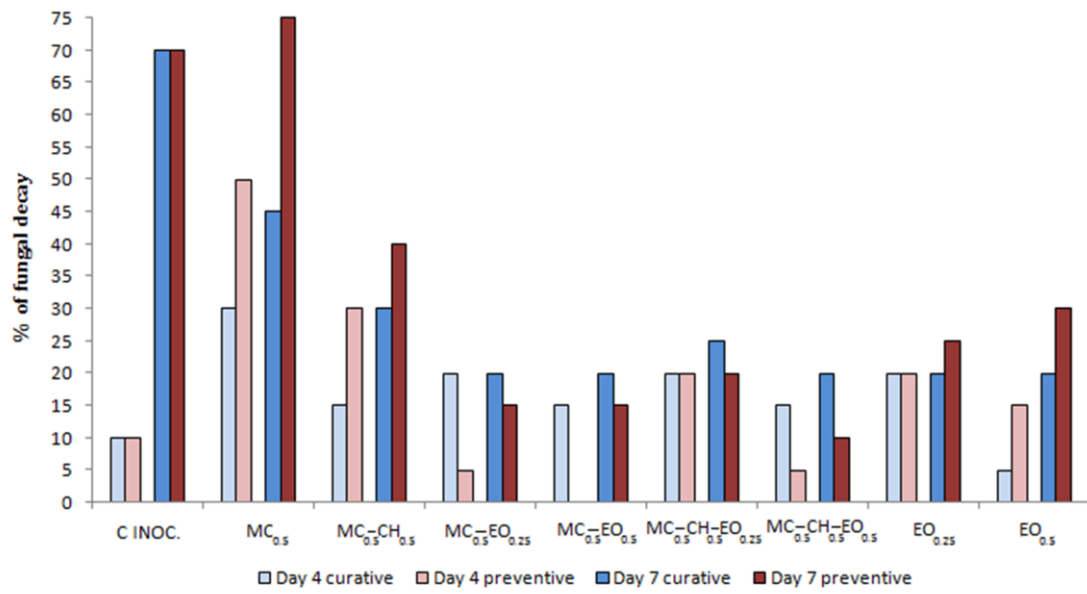


**Figure 4.** Total yield after pre-harvest applications: number of fruits and weight of yield.





**Figure 6.** Respiration rate in terms of CO<sub>2</sub> generation and O<sub>2</sub> consumption during storage at 25°C after (a) pre-harvest and (b) postharvest applications.



**Figure 7.** Fungal decay (% of fruits with visible signs of infection) for preventive and curative treatments 4 days and 7 days after inoculation with *R. stolonifer* spore suspension.