Scientia Horticulturae 245 (2019) 65–73

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Preharvest use of biodegradable polyester nets added with cinnamon essential oil and the effect on the storage life of tomatoes and the development of *Alternaria alternata*

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ARTICLE INFO

Keywords:
Solanum lycopersicum L.
Fibres
Chitosan
Polymers
Ripening
Biodegradability

ABSTRACT

Nets in agriculture are used during crop development to provide shade and protection against the pest attack, while fruit packaging during storage is used to extend and maintain the quality of horticultural products. This study has evaluated the *in vitro* antifungal activity of biodegradable nets and their effect on the shelf life and control of *A. alternata* in tomatoes. The biodegradability of the nets was also determined. The nets were made from extruded fibres of two biodegradable polymers, poly (lactic acid) (PLA) and poly (butylene adipate-*co*-terephthalate) (PBAT) and cinnamon bark essential oil (CEO). The fiber with 6.1% of CEO inhibited the *in vitro* mycelial growth of *A. alternata* in 72.7% and germination in 100%. The use of nets during the development of tomatoes in the plant had no effect on weight loss, firmness, TSS; titratable acidity and carotenoid content during storage, but values of the antioxidant capacity and ethylene were notably higher in those tomatoes grown in nets with CEO. The incidence of *A. alternata* in tomatoes was slightly higher in non-treated fruit compared with those grown only with nets and nets with CEO. The biodegradation of nets at 24 weeks was higher in those made with PLA followed by those with CEO. Our results lead us to continue to consider this technology for further pre- and postharvest issues.

1. Introduction

The tomato (*Solanum lycopersicum* L.) is one of the fresh vegetables which is in great demand worldwide. By 2015, Mexico exported about 53.3% of its production (1.43 million tons) to the United States as the main market with 99.3% of sales (Flores and Magña, 2017). However, the tomato is a fruit which continues its ripening process after being harvested. This entails an increase in its respiration and ethylene production and, therefore, a rapid deterioration and quality loss which reduces its shelf life. In addition, as it matures, the fruit becomes susceptible to attack by microorganisms (Bautista-Baños et al., 2008).

Among the main diseases of the tomato are those induced by fungal species of the genus *Alternaria*. They can attack during the development of the crop or in storage (Mamgain et al., 2013). The main species that occurs postharvest is *A. alternata* (black mould disease), which is mainly responsible for the reduction of the useful life of the tomato (Troncoso-Rojas and Tiznado-Hernández, 2014).

The use of natural products for the control of postharvest fungi has been studied, both in direct application, as well as in film components, coatings or packaging. Among these are essential oils (EOs) (Sivakumar and Bautista-Baños, 2014) which are volatile plant derivatives responsible for the plant's aroma. In the plant they also serve as messengers, attractors of pollinators and as a defence against herbivores and micro-organisms that produce diseases. This last characteristic is what is used in the control of plant pathogenic fungi (Başer and Buchbauer, 2010). According to the United States Food and Drug Administration (FDA, 2013), the EOs are considered as a product generally recognised as safe (GRAS). Several EOs are effective for *A. alternata* control including, among others, cinnamon (*Cinnamomum zeylanicum*) (CEO), cloves (*Syzygium aromaticum*), thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*) and epazote (*Dysphania ambrosioides*) include (Black-Solis et al., 2017).

Nets and bags in agriculture have been used mainly during the crop development for shade (Iglesias and Alegre, 2006), protection against

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https://doi.org/10.1016/j.scienta.2018.10.004
Received 21 June 2018; Received in revised form 1 October 2018; Accepted 3 October 2018

wind, hail, snow and rain (Bosco et al., 2018), protection against insects, birds and small animals (Briassoulis et al., 2007). Postharvest, they are used for fruit transport, protection and packaging (Castellano et al., 2008). Generally, these packing materials are made of polyethylene (PE) and polypropylene (PP) which are petroleum-based polymers (Castellano et al., 2008) which, when discarded, have a high environmental impact due to their slow degradation and accumulation in the environment (Dil et al., 2015). For this reason, the use of biomaterials derived from renewable natural resources has gained strength (Georgiopoulos et al., 2014).

Among these materials, the poly (lactic acid) (PLA), a biodegradable polyester derived from lactic acid, is widely used due to its biocompatibility, biodegradability and good mechanical properties (Liu et al., 2016). These are comparable with those of polyethylene terephthalate (PET) (Hongdilokkul et al., 2015); however, PLA has drawbacks due to its fragility (Wu and Zhang, 2017) and its limited gas barrier properties (Georgiopoulos et al., 2014).

To improve its characteristics, PLA has been blended, with other biodegradable polymers such as polycaprolactone (PCL), poly (butylene succinate-co-adipate) (PBSA), poly (butyl acrylate) (PBA) and poly (butylene adipate-co-terephthalate) (PBAT) (Arruda et al., 2015) which significantly improve its physical properties, including flexibility and elongation at break (Al-I Try et al., 2015). The PBAT is a biodegradable aromatic aliphatic copolyester (compostable) based on monomers of 1.4-butanediol, adipic acid and terephthalic acid. It is ideal for mixing with PLA for the compatibility between both, in addition to having excellent ductility and allowing a wide range of application temperatures (Dil et al., 2015).

Chitosan in solution has been incorporated into fibre formulations made by electrospinning and in the form of nanoparticles to PCL and PLA (Liu et al., 2017a,b; Tardajos et al., 2018) with enhanced antimicrobial activity. However, for PLA/PBAT blends incorporated with essential oils and chitosan as a coating, no information has yet been published in the literature. The incorporation into blends of biodegradable polymers of substances with antimicrobial properties, such as EOs and chitosan (the deacetylated form of chitin), might allow the use of these materials for the preparation of packaging with antimicrobial properties (Bautista-Baños et al., 2017; Stoica et al., 2015).

Based on this, the aims of the study were: 1. To evaluate the *in vitro* antifungal activity of biodegradable polymer fibres with CEO added and nets with chitosan and CEO added on *A. alternata*. 2. To determine the effect of the application of the nets in the preharvest stage on the quality and ripening of the tomatoes, 3. To assess the postharvest packing of tomatoes in nets on *A. alternata* development and, 4. To investigate the process of biodegradability of the nets.

2. Materials and methods

2.1. Fibre manufacture

Biodegradable polymer fibres were used to make the nets. The fibres were made by extrusion of the components, the base polymer (BP) and the CEO [dōTERRA, city of Mexico, Mexico].

The BP was made with a blend of two biodegradable polymers: PLA [IngeoTM Biopolymer 7001D, NatureWorks LLC, MN, USA] and PBAT [Ecoflex® F Blend C1200, BASF, city of Mexico, Mexico], in a 60/40 ratio (PLA/PBAT). The fibres were extruded using a Process 11, Thermo Scientific, MA, USA, with a temperature profile of 160/160/170/180/180/190/190/160 °C.

Five fibre formulations with CEO were tested. An additional three treatments consisted of BP only, BP with glycerol (J.T. Baker, Edo. de Mexico, Mexico) and a control with PDA only (Table 1).

2.2. Manufacture of nets coated with chitosan

Based on the results obtained *in vitro* from the above-mentioned formulations, the treatment selected was F4 (BP 93.9% and CEO 6.1%) for the manufacture of the nets.

Table 1
Formulations used of biodegradable polymer fibers.

Treatments	BP (%)	CEO (%)	Glycerol (%)
F1	97.1	2.9	0
F2	95.9	4.1	0
F3	94.6	5.4	0
F4	93.9	6.1	0
F5	87.1	12.9	0
F6	100	0	0
F7	95.7	0	4.3
F8 (control)	0	0	0

BP = base polymer, CEO = cinnamon essential oil, F1 - F5 = formulations with CEO, F6 = BP only, F7 = BP and glycerol, F8 = control treatment without fibre.

For knitting the nets, manual looms were used, which allowed us to obtain nets of two sizes: small nets of 5 cm^2 and large ones of 23 cm^2. In both cases, the crossing was diagonal with a separation of 5 mm between columns and 10 mm between rows. The small nets were used in *in vitro* evaluation assays and in biodegradation tests, while the large ones were used in *in vivo* assays.

2.2.1. Chitosan preparation

The chitosan solution (low molecular weight: 90 kDa, deacetylation degree: 90%, América Alimentos, Zapopan, Mexico) was prepared in three concentrations (1, 2 and 3%), dissolving it in 1% (v/v) aqueous acetic acid and the solution was maintained with constant agitation for 24 h at room temperature (28 ± 2 °C). After this period, the pH of the solutions was adjusted to 5.5 by the addition of sodium hydroxide (NaOH) 1 N (Hycel, Zapopan, Mexico) and stored under refrigeration.

Later, the nets were immersed in the chitosan solutions with the aforementioned concentrations. To achieve the adhesion of the chitosan, they were dried at room temperature (28 ± 2 °C) for 24 h. The nets were then treated as follows: nets with CEO 6.1% with chitosan coating 1% (NCEOQ1), nets with CEO 6.1% with chitosan coating 2% (NCEOQ2) and nets with CEO 6.1% with chitosan coating 3% (NCEOQ3). The control consisted of Potao Dextrose agar (PDA) only.

2.3. In vitro antifungal activity of biodegradable fibres and nets

2.3.1. Fungal isolate and variables measured in vitro

The *A. alternata* isolate was obtained from the fungi collection of the Laboratory of Tecnología Postcosecha de Productos Agrícolas (CEPROBI, Morelos, Mexico). The fungus, that had already been morphologically and molecularly identified, was grown in a potato-dextrose-agar culture (PDA, Bioxon, Mexico) for fourteen days at 28 °C.

For the *in vitro* evaluations, the fibres were cut into 4 cm long segments and squares of 1 cm^2 were formed. Six Petri dishes (50 mm diameter) with a PDA medium were divided into four equal parts. In each section, a fibre square was placed equidistant from the ends. A 5 mm diameter disc of *A. alternata* was placed in the centre of the Petri dish.

Petri dishes were sealed and incubated at 28 ± 2 °C for five days (time at which the mycelial growth of the control treatment reached the end of the Petri dish). At the end of the incubation period, the Petri dishes were photographed to establish, by means of image analysis with the ImageJ program (National Institute of Mental Health, MD, USA), the mycelial growth within each fibre square. For the evaluation of the variable percentage germination, 0.5 mL of a conidial solution of 21-day old colony (40 conidia mL^{-1}) was placed in six Petri dishes with a PDA medium. The Petri dishes were sealed and incubated at 30 °C for two days. At the end of the incubation period, the number of germinated conidia were counted.