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Microencapsulation of *Thymus vulgaris* and *Tagetes minuta* essential oils: Volatile release behavior, antibacterial activity and effect on potato yield

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ABSTRACT

Thymus vulgaris and *Tagetes minuta* essential oils (EOs) are recognized due to their antibacterial activity against *Streptomyces scabiei*, the main causal agent of potato common scab disease. However, EOs have phytotoxic characteristics and are susceptible to degradation by environmental factors, which make their storage and application difficult. Microencapsulation technology represents an alternative for EOs handle, allowing their protection and gradual spread of their compounds. In the present work, microcapsules of maltodextrin and hydroxypropyl methylcellulose containing *T. minuta* and *T. vulgaris* EOs were evaluated, analyzing their features, their stability at storage conditions and the control release of their volatile compounds when they are applied on humid substrate. Additionally, the *in vitro* antibacterial activity of microencapsulated EOs against *S. scabiei,* and their effects on potato crop yield were evaluated. The obtained microcapsules presented good yield (48.28–51.01 %), low moisture (3.87–3.98 %), spherical shape, and variable size. The release rate of volatiles was high and constant over the 29 days of storage, for almost every compound detected from microcapsules containing *T. vulgaris* EO. Additionally, the microcapsules containing *T. minuta* EO showed a variable but high release rate of its main compound dihydrotagetone. The application of microcapsules on humid substrate resulted in a greater release of volatiles for 14 days. The application of *T. vulgaris* and *T. minuta* EOs on potato plants (var. Spunta) showed no effect on plant growth and photosynthetic activity, but reduced tuber yield (9.07 and 9.40 g tubers fresh weight/ plant, respectively). The microencapsulation of these EOs reduced this effect (10.14 and 10.29 g tubers fresh weight/plant, respectively), and maintained the bacteriostatic activity on *S. scabiei*, making them a promising tool for potato common scab control.

1. Introduction

Potato common scab (PCS) is a bacterial disease that seriously affects potato crops. This worldwide disease alters tubers quality, damages potato surface and produces important economic losses (Loria et al., 2006). *Streptomyces scabiei,* a Gram positive actinobacteria, is the main causal agent of PCS. This pathogen has a worldwide distribution, and can survive on the soil as a saprophyte, as a pathogen in other vegetal species, or as a resistance spore in harsh environmental conditions (Loria et al., 2006). Its control is difficult, even though many control methods are applied (Dees and Wanner, 2012).

In search of new control strategies based on natural products, recently, a strong antibacterial activity of *Thymus vulgaris* and *Tagetes minuta* essential oils (EOs) against *S. scabiei* was reported

Abbreviations: BMC, microcapsules without EO; C, core; EOs, essential oils; HPMC, hydroxypropyl methylcellulose; I, irrigation; GLM, generalized linear models; MBC, minimum bactericidal concentration; MC, microcapsules; MD, maltodextrin; MIC, minimum inhibitory concentration; PCS, potato common scab; PEV, peak area electronic accounts value; SA, superficial application; SEO, *T. minuta* EO; SMC, *T. minuta* EO microcapsules; TEO, *T. vulgaris* EO; TMC, *T. vulgaris* EO microcapsules; WM, wall material.

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(Prieto et al., 2020). EOs are a complex mixture of bioactive volatile compounds (Burt, 2004) that are recognized due to their beneficial properties, including antimicrobial activity against a large diversity of phytopathogens (Aly et al., 2016; Sharmeen et al., 2020; Prieto et al., 2020; Hajian-Maleki et al., 2021). Additionally, EOs offer major advantages than synthetical agrochemicals making them an eco-friendly alternative. These compounds have low residuality in the environment and have shown low mammalian toxicity (Isman, 2000). In addition, it is rare that pathogens develop resistance towards them (Bedmutha et al., 2011).

However, EOs are susceptible to degradation by environmental factors such as heat, moisture, oxygen, UV radiation and light, and their quantities may be reduced by volatilization (López et al., 2014; Sharmeen et al., 2020). In addition, they are difficult to handle because of their low water solubility and hydrophobicity (Asensio et al., 2017). These features make the EOs storage and application difficult and compromise the effectiveness of the bioactive compounds, especially when they are applied on agronomic systems (Ayala-Zavala et al., 2008).

On the other hand, many EOs were described to have phytotoxic characteristics (Synowiec et al., 2017; Werrie et al., 2018; Alipour et al., 2019; Taban et al., 2021). These features are usually studied to evaluate their effectiveness on weed control, due to EOs being potential bioherbicides (Dayan et al., 2009; Alipour et al., 2019; Sharmeen et al., 2020). However, their phytotoxic activity is not limited to weeds and can affect crops development. In this context, phytotoxic reports of EOs on potato crops are limited. Faria et al. (2016) reported a phytotoxic effect of *Ruta graveolens* and *Satureja montana* EOs on potato hairy-roots cultures, while other works showed variable effects of *Origanum vulgare* EO on potato plants (Olanya and Larkin, 2006; Meng et al., 2013; Nikolova et al., 2022). The use of these natural products on potato tubers was mainly studied as postharvest inhibitors of potato sprouts (Vaughn and Spencer, 1991; Gómez-Castillo et al., 2013; Stupar et al., 2021) and as tuber quality preservative compounds (Hajian-Maleki et al., 2021). Even though it has been demonstrated that the use of some synthetic herbicides could alter potato yield (Pfleeger et al., 2008, 2011), the effects of EOs on this crop yield have not been explored yet.

To overcome all these problems, microencapsulation appears to be a good alternative for the use of EOs, providing protection (Dayan et al., 2009) and delivery control, and making its handling, storage, and application easier (Pereira et al., 2018). Microencapsulation is a process that allows the storage of active compounds into a protective coating, at a microscopic size, protect them and gradually spread them (Sobel et al., 2014). This technology is used in different industries like pharmaceutical, food, chemical and agronomic, due to its versatility (Sobel et al., 2014). There are many microencapsulation techniques. Among them, spray drying is one of the most economic methods (Pereira et al., 2018) commonly used in the industry. This technology allows easier microcapsules (MC) production, has a lower cost, and operates continuously compared with other techniques (Pereira et al., 2018). Different compounds are utilized as wall materials for microencapsulation of natural bioactive products (Lu et al., 2021). Among them, carbohydrates as maltodextrin and hydroxypropyl methylcellulose exist, being maltodextrin a starch-based material obtained by hydrolysis, that is characterized to be inexpensive, highly soluble in cold water and to provides protection against oxidation (Mohammed et al., 2020), and hydroxypropyl methyl cellulose a semi synthetic polymer based on cellulose, that is characterized to be a good emulsifier and to allows the control release of active ingredients (Zheng, et al., 2021). Both have been successfully utilized to encapsulate oregano EOs, and maintained its bioactivity after the microencapsulation process and allowed a gradual release of the encapsulated EO (Asensio et al., 2017). However, the effects of microencapsulation on the phytotoxic activity of EOs are not deeply studied.

The aims of this study were i) to develop and characterize microcapsules of *T. vulgaris* and *T. minuta* EOs and study their antibacterial activity against *S. scabiei* at *in vitro* conditions, ii) to evaluate the release of the volatile compounds of microencapsulated *T. vulgaris* and *T. minuta* EOs during their storage and application into the substrate, and iii) to study the EOs and their microcapsules effects on growth and photosynthetic parameters of potato plants, and potato crop yield.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Hydroxypropyl methylcellulose (HPMC, Methocel ™ K100 LVCR, Colorcon, Buenos Aires, Argentina), maltodextrin (MD) (Todo Droga Lab., Córdoba, Argentina), and neutralized peanut oil (Por-Nut SA, Ticino, Argentina) were used to perform the microcapsules. All the chemicals utilized in this work were pro-analysis grade.

2.1.2. Plant material and essential oils

Stems and leaves of *T. vulgaris* were obtained from the Experimental Station of the Agronomic Sciences College (National University of Córdoba, Argentina) and stems, leaves and flowers of *T. minuta* were collected near Pilar (Córdoba, Argentina). The EOs were extracted by hydrodistillation and chemically characterized by GC–MS. These data were published in Prieto et al. (2020), where the main compounds reported for *T. vulgaris* EO (TEO) were o-cymene (37.11 %), thymol (34.83 %), eucalyptol (4.89 %) and carvacrol (3.84 %), and for *T. minuta* EO (SEO) were dihydrotagetone (34.79 %), verbenone (31.39 %), *trans*-tagetone (11.80 %) and β-*cis*-ocimene (8.76 %).

2.1.3. Bacterial strain

For the antibacterial assay, a strain of *S. scabiei* (MK648101) previously isolated from infected potato tubers was used (Prieto et al., 2020).

2.1.4. Micropropagated potato plantlets

Micro-propagated potato plantlets provided by the Biotechnology Laboratory of the Agronomic Sciences College (National University of Córdoba, Argentina) were used for a subsequent micropropagation.

2.2. Preparation of microcapsules

The emulsions were performed following Asensio et al. (2017) with minor modifications. The aqueous phase was made by dissolving HPMC under magnetic stirring for 12 h and MD under magnetic stirring for 5 min. The core (C) was built using a mixture of neutralized peanut oil (90 % w/w) and EO (10 % w/w). Both phases were mixed, adding the oil phase (C) into the aqueous phase, and homogenized using a high-speed homogenizer (Ultra-Turrax T25 IKA Works, Wilmington, USA) during 6 min at 6000 rpm and at 10000 rpm for 6 more minutes. The ratio was 1:1:1 (HPMC: MD: C).

Microencapsulation was performed using a Büchi B-290 (Büchi Labortechnik, Flawil, Switzerland) spray dryer. The emulsions were atomized using hot air in the dry chamber. The inlet and outlet temperatures were 160 °C and 100 °C, respectively; Q-flow was 600L/h; the pump was used at 10 %; and the aspirator was set at 100 %.

2.3. Microcapsules characterization

2.3.1. Microcapsules yield

The microencapsulation yield was calculated as the ratio between the weight of the powder collected only from the collecting bottle after the spray drying process and the weight of all the ingredients used initially to prepare the microcapsules (including peanut oil, essential oil, HPMC and MD amounts expressed as weight, no water). It was expressed as percentage:

$$Yield\ (\%) = MC\ weight\ (g) \times 100/\ initial\ solids\ weight\ (g)$$

2.3.2. Moisture content

A gravimetric method was used. Briefly, 0.5 g MC were dried for 2 h at 105 °C. The results were obtained by calculate the ratio of final and initial powder weights and expressed it as percentage.

2.3.3. Microcapsules morphology and particle size distribution

The morphology and surface features of microcapsules were observed using a field emission gun scanning electron microscope (FEG-SEM Carl Zeiss, Oberkochen, Germany) coupled to an In-Lens detector for secondary electrons in Lamarx Laboratories (Astronomy, Mathematic and Physic College, National University of Córdoba, Argentina). For MC surface observation, the samples were laminated with gold. The particle size was measured using a laser scattering particle size distribution analyzer (Horiba LA-960, Japan).

2.4. Microcapsules antibacterial activity

The antibacterial activity was evaluated using the broth microdilution method, following Prieto et al. (2020). *S. scabiei* was inoculated on YME broth (Shirling and Gottlieb, 1966; without bacteriologic agar) and incubated at 28 °C for 72 h. To obtain appropriate cell density, 10 μL of resazurin sodium salt (Sigma-Aldrich, St. Louis, MO, USA) solution (0.01 % w/v) was added to 170 μL of different tenfold dilution of culture and incubated at 28 °C during 4 h. The first dilution unable to reduce resazurin was chosen. The MC were diluted in 10 serial twofold dilutions, using YME broth as solvent. The assays were performed using a sterile 96-well microtitre plate, adding 170 μL of *S. scabiei* culture into each well in the columns 1–10 plus 20 μL of the corresponding MC dilution. The columns 11 and 12 were used as the positive (*S. scabiei* culture) and negative (YME broth) controls, respectively. The plate was incubated for 20 h at 28 °C in darkness, and then 10 μL of the resazurin sodium solution were added to each well. The plate was then incubated for additional 4 h at 28 °C in darkness. The results were assessed visually to determinate the minimum inhibitory concentration (MIC), which is the first dilution unable to reduce resazurin, remaining blue color. From the wells where the resazurin was not reduced, 100 μL were taken and spread on YME medium (Shirling and Gottlieb, 1966), and incubated during 5 days at 28 °C, to assess the minimum bactericidal concentration (MBC), that is defined as the highest dilution at which 99.9 % final inoculums is killed.

2.5. Microcapsules stability

2.5.1. Volatile release analysis during storage

The release behavior of the major compounds for EO MC was monitored in a storage assay for 4 weeks. The assay was performed following Asensio et al. (2017) with some modifications. The MC were storage into open flasks covered with Parafilm®, in darkness at room temperature (~24 °C). To analyze the volatile profile, samples of MC (0.5 g) were added into 10 mL capacity vials, covered, and sealed. To capture the volatile compounds, a solid phase micro-extractor (SPME) fiber coated with divinylbenzene/ carboxen/ polydimethylsiloxane (Supelco, Sigma) was used. The vials were first heated at 70 °C during 20 min, and then the fiber was exposed to the headspace of the vial, during 10 min at the same temperature. The fiber was then injected into a PerkinElmer Clarus SQ8 GC–MS (Shelton, CT, U.S.A.) coupled with an ion trap mass detector (MS). The carrier gas was helium, which flow rate was 1 mL/min. The ionization was carried out by electron impact at 70 eV. The compounds of the samples were separated in a DB-5 capillary column (30 m × 0.25 mm, film thickness 0.25 mm) (PerkinElmer). The identification of volatile compounds was performed by comparison with published data and mass spectra library NIST (2.0). The analyzed compounds were selected due their prevalence in each EO and because of the antibacterial activity previously discussed in Prieto et al. (2020). The results were expressed as the peak area electronic accounts value (PEV)/ 1,000,000.

2.5.2. Total phenolic content (TPC)

TPC was measured on EO MC. A blank (BMC) was also obtained (as described in 2.2 subsection) without EO. MC (0.04 g) were dissolved in 0.5 mL of dimethyl sulfoxide. TPC of the MC was determined spectrophotometrically along the storage, utilizing the Folin-Ciocalteu method, based on Nepote et al. (2005). Calibration curve was performed using Gallic acid (GA, Sigma-Aldrich, USA) as standard and dimethyl sulfoxide as solvent. EO MC TPC values are the result of the subtraction of the BMC phenolic content values from each EO MC phenolic content original values. TPC was expressed as mg GA equivalents /g of MC.

2.5.3. Moisture content

Moisture content of MC was measured along the storage assay, using the method previously described in the 2.3.2 subsection.

2.5.4. Volatile release on humid substrate

To analyze the volatile release profile of the MC on humid substrate, 1 g of MC was applied on 120 mL pots with 25 g of a mixture of peat, perlite and vermiculite mixture (in a ratio of 1:1:1, respectively) previously autoclaved (121 °C, 20 min.). The pots were watered every-six days with sterilized water to maintain the substrate moisture along the assay. MC application was performed as follows (treatments): i) superficial application (SA): MC were mixed with substrate and applied superficially (earthing up), and ii) irrigation (I): MC were first added to 22 mL distilled sterile water and vortexed for 2–3 min to be dissolved, and then the solution was applied in the pots as first watering. Subsurface samples (1.5 g) were taken every-seven days (for 4 weeks) and added into 10 mL capacity vials to be analyzed by GC–MS, following the methodology previously explained in the 2.5 subsection. The results were expressed as the PEV/ 100,000.

2.6. Effect of EOs and their MC on potato crop

2.6.1. Potato plantlets micropropagation

Potato plants were multiplied by agamic reproduction using the micropropagation method following Li-li et al. (2020), with minor modifications. Nodal cuttings were obtained from *in vitro* serial propagated potato plantlets (Spunta variety) and were used as explants. Each explant was inoculated in a glass flask containing semisolid Murashige and Skoog medium (Murashige and Skoog, 1962) and α-naphthaleneacetic acid (0.01 mg/L) and was kept in a growth room at 17–24 °C with a 16 h light/8h dark photoperiod for 40 days until the plantlet showed 10–12 developed leaf.

2.6.2. Effect of EOs on potato plants

2.6.2.1. Effect of EOs on plant growth. *In vitro* potato seedlings were transplanted in 170 mL pots containing sterile substrate. After acclimation, the seedlings were grown in a growth chamber, with a temperature of 26 ± 2 °C, and a photoperiod of 16 h light/ 8 h dark, with a light intensity of 250 μmol s^{-1} m^{-2}. The seedlings were regularly watered and fertilized with ¼ Hoagland nutrient solution. After 10 days from transplant, TEO and SEO were mixed with 50 mL of substrate, and then earthing up (SA) was performed. EOs concentration was equal to twice the MIC. Substrate mixed with distilled water was added to control plants. Each treatment and control had eight replications. Stem length and number of nodes were recorded every-three days from the day of EOs application for 12 days. Increase of stem length and number of nodes were expressed as cumulative percentage. Root, stem and leaf biomass (dry weight) were analyzed at the end of the experiment as described by Vidoz et al. (2016).

2.6.2.2. Analysis of photosynthetic pigments and chlorophyll fluorescence parameters. Leaf disks of 7 mm of diameter were excised from the first expanded leaf and placed in tubes with 1 mL of 96 % ethanol in darkness and at 4 °C. Once the leaf disks were completely diaphanized, pigments (chlorophyll *a*, *b* and total carotenoids) in the ethanolic extract were quantified spectrophotometrically according to Lichtenthaler and Wellburn, (1983). Chlorophyll *a* and *b* content was expressed as micrograms per cm^2 of leaf.

Chlorophyll fluorescence was measured with a portable chlorophyll fluorimeter (PocketPEA, Hansatech, UK) on the first fully expanded leaf. After 20 min of dark acclimation, leaves were submitted to a light pulse of 657 nm for 1 s with an intensity of 3,500 μmol m^{-2} s^{-1}.The measurement was carried out on the same leaf for each replication along the experiment. Chlorophyll *a* fluorescence parameters (OJIP-test) were calculated using the software PEA plus (Hansatech, UK, Strasser et al.,2004).

2.6.3. Greenhouse assay

In vitro plantlets were washed and transplanted directly in a 3 L pot containing a mixture of peat, perlite and vermiculite (in a ratio of 1:1:1, respectively) previously autoclaved (121 °C, 120 min.), and placed in a greenhouse (Phytopathology Chair, Agronomic Science College, National University of Córdoba, Argentina). The crop was watered by spray irrigation and was regularly fertilized using Poly Feed™ GG (Haifa Iberia, S.A.), following the instructions of the manufacturer. The plants were cultivated with natural temperature and photoperiod, where the average minimum temperature was 12.9 °C and the average maximum temperature was 24.7 °C, and with a photoperiod of 13 h light/ 11 h dark (in February) at the beginning of the assay, and 11 h light/ 13 h dark (in May) at the end. No agrochemicals were used during the crop assay. Four treatments were performed, which consisted in the application by earthing up of a mixture of sterile substrate with EO or their MC in a concentration equal to twice the MIC. The treatments were i) TEO, ii) SEO, iii) MC with *T. vulgaris* EO (TMC), and iv) MC with *T. minuta* EO (SMC), and the control samples were plantlets without any application. The pots were distributed into 3 blocks that were arranged in a randomized design, with four replications per treatment into each block. The perimeter potato plants were not used in the assay to avoid the border effect. The treatments started 50 days after transplant, coinciding with the potato tuberization stage. This application time was selected because the susceptibility of tubers to *S. scabiei* infection starts at tuberization stage. The harvest was performed 80 days after transplanting. The tubers were air-dried for 24 h, and then counted and weighed. The yield was measured as the total fresh weight (g) of all tubers obtained per plant.

2.7. Statistics analysis

The volatile release of each compound was analyzed using generalized linear models (GLM), with time as fixed effect and PEV as the response variable. The relation between treatments and time for the volatile release of each compound on humid substrate was analyzed using GLM, with time, treatment and their interactions as fixed effects, and PEV as response variable. A multivariate analysis of variance (MANOVA) was performed to compare the release of volatiles of each EO between treatments.TCP and moisture content were analyzed using GLM, with time as fixed effect and phenol concentration and percentage of moisture as response variables, respectively.

Antibacterial activity (MBC and MIC) was analyzed with GLM, with treatments (MC/EO) as fixed effect and concentration as response variable. The effect of EOs on potato plantlets (growth parameters, biomass and photosynthetic parameters) were analyzed for each day and along days using GLM, with treatments as fixed effects, and each analyzed parameter as response variable. Time was added as fixed effect to analyze parameters along the assay. Potato yield was analyzed using GLM, with treatment as fixed effect, block as random effect and yield as response variable.

Predicted values were compared using LSD Fisher's multiple range tests, with a significance level of 5 % for all cases.

The data were analyzed using Infostat software (Di Rienzo et al., 2018).

3. Results

3.1. Microcapsules characterization

The microencapsulation yield was 48.28 % for TMC and 51.01 % for SMC, and the moisture content results were 3.87 % and 3.98 % for TMC and SMC, respectively. The observed morphologies for both MC were similar (Fig. 1), showing spherical particles, with smooth surfaces and, in some cases, concave depressions. Both types of MC showed a distribution of 3 main particle size populations, which represented 95.98 % and 98.52 % of the measured particles for TMC and SMC, respectively. The TMC presented smaller particles with mean sizes of 6.24, 117.29 and 667.15 μm for each population and for the entire sample (D_{10} = 5.5 μm; D_{50} = 6.5 μm; D_{90} = 374.47 μm) than SMC that showed 6.99, 110.78 and 3744.18 μm mean size for separate distributions (D_{10} = 7.06 μm; D_{50} = 3510.43 μm; D_{90} = 4228.35 μm).

3.2. Antibacterial activity

The results for the antibacterial activity of TMC showed a MIC of 0.1704 g/L, while MBC value was not found. For SMC, the MIC and MBC were 0.2439 g/L and 0.3661 g/L, respectively. These results presented no significant differences in comparison to those obtained by Prieto et al. (2020) where the pure EOs were analyzed and the MIC and MBC for TEO were 0.068 g/L and 0.137 g/L, and for SEO were 0.147 g/L and 0.147 g/L, respectively.

3.3. Storage assay

The TMC volatile release profile presented some variations between compounds (Table 1). o-cymene and γ-terpinene concentrations remained constant until the 21th day, and thereafter a significant increase on the volatile values ($p = 0.0010$ and $p < 0.0001$, respectively) were observed. Thymol presented similar values until the 9th day, while carvacrol and β-linalool maintained similar values until the 21th day. These three compounds thereafter showed a

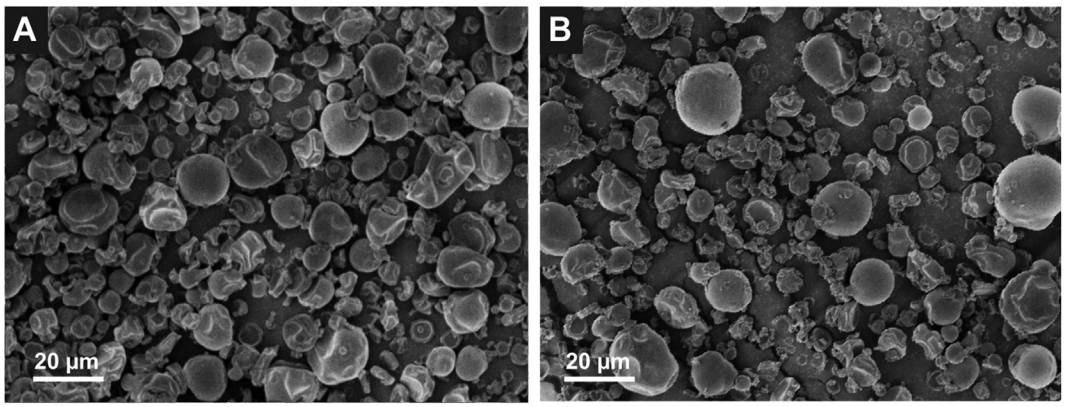

Fig. 1. Morphology of microcapsules of *T. vulgaris* (A) and *T. minuta* (B) essential oils, observed from scanning electron microscopy photographs.

Table 1
Release of volatile compounds for *T. vulgaris* and *T. minuta* microcapsules during storage assay.

MC***	Compound	Day 1**	Day 9**	Day 21**	Day 29**
	o-Cymene*	243.20 ± 3.09^{a}	243.65 ± 59.45^{a}	250.43 ± 4.43^{a}	323.78 ± 31.66^{b}
	Eucalyptol*	19.43 ± 1.33^{a}	6.60 ± 2.79^{b}	14.89 ± 0.52^{ac}	10.56 ± 3.43^{bc}
	ɣ-Terpinene*	11.16 ± 0.39^{a}	8.75 ± 2.47^{a}	10.03 ± 0.04^{a}	17.70 ± 4.69^{b}
TMC	Linalool*	14.40 ± 1.12^{a}	14.63 ± 5.34^{a}	23.12 ± 0.15^{a}	40.43 ± 8.05^{b}
	Thymol*	97.09 ± 4.34^{a}	102.37 ± 30.30^{a}	128.06 ± 7.48^{b}	228.19 ± 24.56^{c}
	Carvacrol*	12.18 ± 0.41^{ab}	11.41 ± 4.68^{b}	15.71 ± 1.22^{a}	36.19 ± 5.08^{c}
	β-Caryophyllene*	4.82 ± 0.29^{a}	1.39 ± 0.63^{b}	2.58 ± 0.10^{bc}	2.96 ± 0.84^{c}
	TPC****	11.98 ± 0.57	10.34 ± 2.44	11.50 ± 0.35	13.38 ± 1.28
	mc*****	3.87 ± 0.40^{a}	6.82 ± 0.48^{b}	4.24 ± 0.20^{a}	5.83 ± 0.25^{b}
	Limonene*	12.25 ± 1.47^{a}	19,96 ± 1.80^{b}	6.67 ± 0.26^{c}	10.15 ± 0.60^{a}
	β-(Z)-Ocimene*	75.32 ± 6.23^{a}	1,86 ± 0.94^{b}	0.31 ± 0.12^{b}	0.12 ± 0.0^{b}
	Dihydrotagetone*	169.58 ± 15.32^{a}	308,62 ± 8.46^{b}	158.68 ± 0.19^{a}	254.13 ± 2.51^{c}
SMC	*cis*-Tagetone*	7.28 ± 0.86^{a}	5,42 ± 1.66^{a}	2.09 ± 0.50^{b}	1.69 ± 0.32^{b}
	trans-Tagetone*	80.52 ± 9.89^{a}	9,44 ± 4.32^{b}	3.17 ± 0.62^{b}	2.39 ± 0.28^{b}
	Verbenone*	258.89 ± 29.44^{a}	60,38 ± 16.06^{b}	21.57 ± 2.70^{b}	14.28 ± 1.37^{b}
	TPC****	3.45 ± 2.09	3.45 ± 0.79	3.47 ± 0.79	3.41 ± 0.49
	mc*****	3.98 ± 0.85^{a}	6.91 ± 0.23^{b}	3.87 ± 0.50^{a}	5.75 ± 0.03^{b}

* Different letters in a row indicate significant differences in the volatile release of each compound along time, according to GLM and LSD Fisher test (α = 0.05, n = 2).
** Results expressed as peak area electronic accounts value/ 1,000,000 with standard deviation.
*** MC = Microcapsules; TMC = MC with EO of *T. vulgaris*; SMC = MC with EO of *T. minuta.*
**** TPC = Total phenolic content expressed as mg of gallic acid equivalents/g MC. (n = 3).
***** mc = moisture content expressed as percentage of moisture/MC weight. Different letters in a row indicate significant differences in the moisture content along time, according to GLM and LSD Fisheŕs test (α = 0.05, n = 2).

significant increase in the volatile values ($p < 0.0001$, $p < 0.0001$ and $p = 0.0166$, respectively). In SMC (Table 1), the compounds *cis*-tagetone, *trans*-tagetone, verbenone and β-*cis*-ocimene showed the maximum values in the first storage day, and decay towards the end of the assay. Conversely, dihydrotagetone and limonene showed an increase in the volatile release from days 1 to 9, then, the values decreased towards the day 21, and finally, the volatile profile changed again, increasing their values towards the end of the assay.

TPC (Table 1) remained constant along the assay for TMC and SMC, and had no significant differences between sampling days ($p > 0.05$). Moisture content (Table 1) showed some variations along the assay for MC, with maximum values of 6.82 % and 6.91 % of moisture content in TMC and SMC, respectively.

3.4. Volatile release on humid substrate

For both MC, the volatile release profile showed the highest values on day 1 (Fig. 2), with a subsequent decrease of volatiles captured by the SPME fiber. This trend resulted in minimal values of volatiles captured on day 21. In TMC, the compounds o-cymene, eucalyptol, ɣ-terpinene and β-linalool did not show significant differences between treatments SA and I. The same result was observed in SMC for the compounds limonene, β-*cis*-ocimene and *cis*-tagetone. On contrary, in TMC, the compounds thymol, carvacrol and β-caryophyllene showed differences between treatments SA and I ($p = 0.0067$, $p = 0.0035$, and $p = 0.0033$, respectively). For these compounds an interaction between the dependent variable treatments and time was observed, suggesting that both variables explained these differences. Moreover, the amount of these volatiles captured by the fiber in the first day was almost double in SA, in comparison with I. This difference continued towards day 14, when the obtained values for both treatments became similar. In SMC, similar results were observed for dihydrotagetone, verbenone and *trans*-tagetone, with significant differences between treatments ($p = 0.0124$, $p < 0.0001$ y $p < 0.0001$, respectively). An interaction between the variables treatment and time was also observed. The differences between treatments for the compounds dihydrotagetone, verbenone and *trans*-tagetone started with values close to double for SA in comparison with I, but in the day 7, the differences between the quantity of released volatiles increased for the compounds verbenone and *trans*-tagetone, at time that dihydrotagetone decreased. On the other hand, MANOVA results, that included the volatile profile for all compounds, did not show differences between treatments for TMC and SMC.

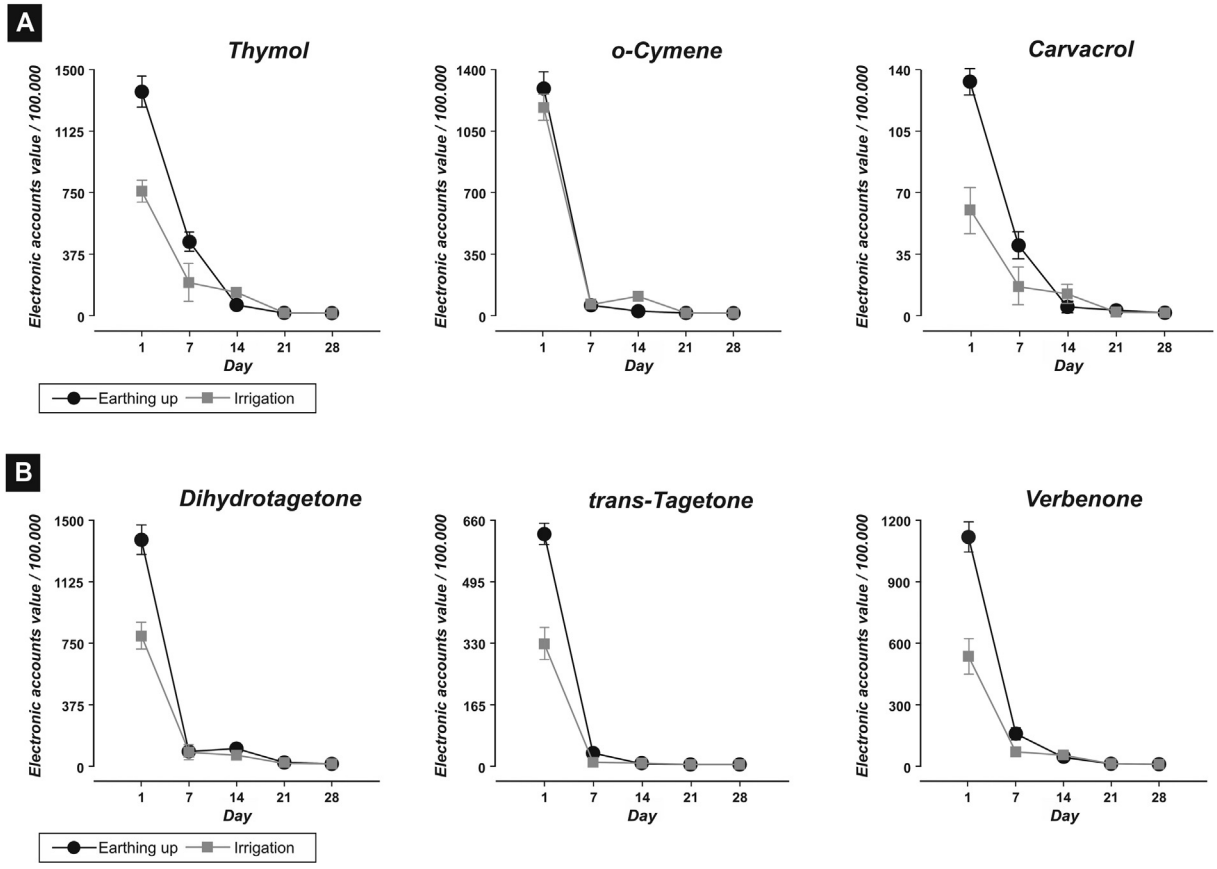

Fig. 2. Volatile release profile of (A) *T. vulgaris* EO microcapsules (Thymol, o-Cymene and Carvacrol) and (B) *T. minuta* EO microcapsules (Dihydrotagetone, *trans*-Tagetone and Verbenone), obtained in humid conditions.

3.5. Effect of EOs on plant growth and photosynthetic parameters

Potato plant growth and photosynthetic parameters are shown in Fig. 3. Stem length growth, expressed as percentage (Fig. 3-a), increased progressively over time both in control and treated plants. Control presented the highest values in all cases. At the end of the experiment, control remained the maximum value (69.47 %), followed by SEO (65.03 %) and TEO (64.55 %). However, GLM analysis for each day showed significant differences only at day 3 ($p = 0.0255$), where stem mean of SEO treatment was statistically shorter than control. No significant differences were observed between treatments along time ($p > 0.05$). Similarly, no statistical differences were observed in nodes number increase analyzed along time ($p = 0.2225$) (Fig. 3-b), and in leaf, stem and root biomass ($p = 0.6277$, $p = 0.4955$ and $p = 0.1230$, respectively; Fig. 3-c).

Photosynthetic parameters are shown in Fig. 3-d, 3-e and 3-f. Fv/Fm and PI_{TOT} are commonly used photosynthesis parameters. Fv/Fm is an index of PSII maximum quantum efficiency of primary photochemistry while PI_{TOT} is the performance index for energy conservation absorbed by PSII (Strasser et al., 2004). In this case, both parameters did not show significant differences between treatments throughout the experiment ($p = 0.9738$ and $p = 0.1769$, respectively). Fv/Fm showed constant values for all treatments (Fv/Fm $\sim$ 0.81) until day 9, and then slightly decreased (Fv/Fm $\sim$ 0.79) at day 12. PI_{TOT} remained at its highest level until day 3, being 2.59, 2.53 and 2.27 for SEO, control and TEO, respectively. A gradual decrease of the parameter was then registered showing values of 0.64, 0.55 and 0.44 for control, TEO and SEO, respectively at day 12.

Neither total chlorophyll ($a + b$) nor carotenoids showed significant differences between treatments ($p = 0.3098$ and $p = 0.8295$, respectively). Total chlorophyll values were 39.02, 31.11 and 36.15 μg/cm^{2}for control, TEO and SEO treatments, whereas carotenoids levels were 5.81, 5.54 and 5.38 μg/ cm^2 in leaves for control plants and SEO and TEO treated plants, respectively.

3.6. Effect of EO and their MC on potato yield

The results showed that the total fresh weight of tubers per plant show significant differences between treatments (Table 2). EO treatments resulted in a lower yield, expressed as fresh weight, in comparison to control. On the other hand, the MC treatments were statistically similar to control and to EOs treatments, showing intermediate values.

4. Discussion

4.1. Microcapsules yield and moisture

The microencapsulation yield presented similar values to those found by Asensio et al. (2017) for MC with wall material (WM): C ratio equal 2:1, and the moisture content resulted similar to previous studies (Karim et al., 2016; Asensio et al., 2017). The moisture content is a key attribute for MC, because high moisture content could result in core materials degradation probably by lipid oxidation (Karim et al., 2016), and therefore the further stability and bioactivity of the microcapsule affected.

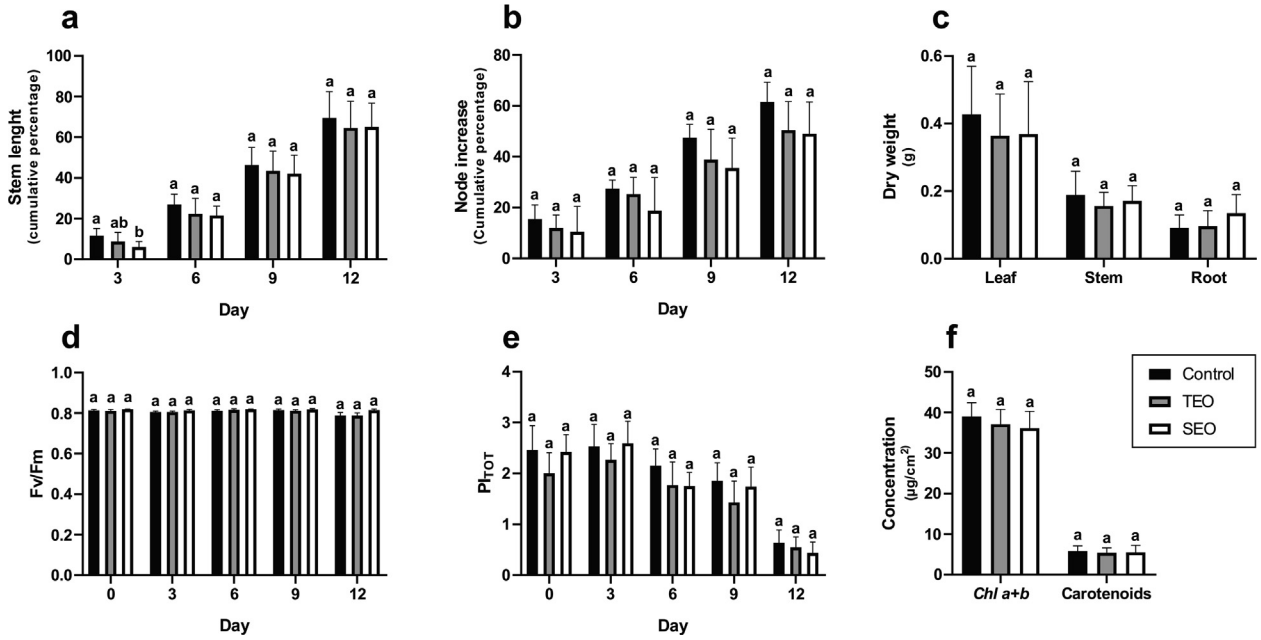

Fig. 3. Effect of *T. vulgaris* and *T. minuta* EOs on potato plant growth (Stem length, nodes increase and biomass dry weight) and photosynthetic parameters (Fv/Fm, PI_{TOT} and photosynthetic pigments).

Table 2
Mean and standard deviation of potato tubers yield expressed as total fresh weight of all tubers harvested per plant, obtained from potato plants treated with *T. vulgaris* EO and *T. minuta* EO and their microcapsules.

Treatment*	***Fresh total weigh(g)/plant*** ± SD**
Control	11.60 ± 3.36 [a]
TEO	9.07 ± 1.53[b]
SEO	9.40 ± 2.91[b]
TMC	10.14 ± 2.51[ab]
SMC	10.29 ± 1.00[ab]

* TEO = *T. vulgaris* EO; SEO = *T. minuta* EO; TMC = MC of TEO; SMC = MC of SEO.
** Different letters indicate significant differences between treatments, generalized linear models and LSD Fisher's test (α = 0.05, n = 9).

4.2. Microcapsules morphology and particle size distribution

Both MC presented morphologies expected for a spray drying process (López et al., 2014). The observed depressions on MC surfaces could be attributed to the MC collapses during the drying phase after achieving the largest size (Karim et al., 2016). The depressions may suggest that the obtained MC were empty and that the EO could be located on the internal surface of the capsule as small droplets (da Costa et al., 2013). Furthermore, MC with spherical morphology could be related to the used WM: C ratio. A high concentration of HPMC on the emulsion increases the viscosity and decreases MC elasticity, reducing the formation of depression on the MC surface (Karim et al., 2016). MC with spherical morphology and continuous smooth surfaces have a low permeability to volatiles, allowing an easier retention of the core material (López et al., 2014).

Particle size distribution was constituted by three main particle size populations, for both microencapsulated EOs. Similar results were observed by Tonon et al. (2011), who reported a variation in the particle size, with a distribution of three different peaks, where each one presented a predominant particle size. The differences obtained between both EOs particle size distributions could be related to the chemical composition of each EO, the interaction between their compounds and the interaction of these with the aqueous phase in the emulsion. Another parameter that can be involved is the agglomeration of the MC. Tonon et al (2011) indicated that the particles above 100 µm could be explained by an incipient agglomeration process, where the formation of link bridges lead to the production of bigger particles. Nevertheless, Reineccius (2004) reported that the retention of volatiles was not related to particle size, when a high infeed of solids was used, like in this study.

4.3. Antibacterial activity

The obtained results suggest that the antibacterial properties of EOs are generally not affected by the microencapsulation process. This agree with Moharreri et al. (2022), where microcapsules of thyme EO, with modified starch, whey protein and MD as wall materials, showed antibacterial activity against *Salmonella enteriditis*, with a MIC of 1.87 g/L and a MBC of 5 g/L. According to Matias et al. (2016) lower MIC concentrations than 1.024 g/L were considered relevant for antibacterial activity. The MIC and MBC values obtained in the present work for both MC allows considering them as good antibacterial agents.

4.4. Storage assay

The volatile profile of TMC showed an incremental release trend of the main compounds towards the end of the assay. However, some minor variations were observed along the 29 days. Thymol, o-cymene and ɣ-terpinene profiles resulted similar to the obtained by Asensio et al. (2017) for the same compounds from oregano EO microcapsules, where an increased release was observed close to the 30th day. This final trend suggested that the volatile delivery could continue before that time, assuming a longer shelf life than 28 days of these capsules.

Most of the compounds of SMC presented a volatile release profile with a decreasing trend at the end of the assay, except for dihydrotagetone and limonene, which finished it with a positive slope, suggesting a posterior release of compounds. The differences in the

behavior of the SMC main compounds could be related to the influence of each compound features on the adsorption to the SPME fiber (Asensio et al., 2017), on the interactions between compounds and WM (Del Toro-Sánchez et al., 2010), and on the interaction with other compounds inside de capsule. Locali-Pereira et al. (2020) reported a different limonene volatile release for pink pepper EO microencapsulated with MD, soy protein isolated and high methoxyl pectin as WM, where limonene mostly showed an incremental release trend along 20 days. In the same way, the differences in the composition of pink pepper EO and *T. minuta* EO, and the different composition of the WM, could be responsible for the differences observed in the limonene release behavior.

TPC values remained constant throughout the storage assay for both EO MC, showing higher values in TMC than in SMC. Bioactive compounds remained in high concentrations inside MC along the 29 days of storage, supporting the idea that volatile release probably continued before the storage time. Higher TPC values obtained in TMC could be related to the chemical composition of this EOs rich in phenols as thymol and carvacrol (Prieto et al., 2020).

Moisture contents varied along storage in both type of MC. These changes were similar between MC on each sampling day, suggesting that the moisture content was related to atmospheric humidity variations. However, the humidity percentage throughout the assay remained low (<7 %) for both MC, having no impact on MC quality.

4.5. Volatile release on humid substrate

The release of volatiles in wet conditions for both types of MC and for both treatments showed higher values at the first day, meaning a faster release of volatiles at the beginning of the assay and a later decreasing through the studied period. Interestingly, the volatile release was registered even before 21 days, with only one application. Similar results were obtained by Alipour et al. (2019), who worked with encapsulated rosemary EO, with starch as wall material, and analyzed its release in a solution of polyethylene glycol with a concentration that simulated the soil matric potential. They reported an initial phase of quick release of rosemary EO in less than a day, followed by a second phase, with a slow release of the EO until the 26th day.

In comparison with the volatile release profile of the storage assay, the obtained profiles for the release in wet substrate resulted faster. This difference could be related to a high solubility of HPMC in cold water (Al-Tabakha, 2010). Patterson et al. (2015) demonstrated that an increase in the relative humidity increased the degradation of MC made with HPMC acetate succinate as WM.

The volatile profile analysis suggested that the highest effectiveness for both MC could be along the first 14 days, with a maximum of bioactivity at application time. The multivariate analysis for MC indicated that both treatments were similar. However, when the individual compounds with highest antibacterial bioactivity (Prieto et al. 2020) were compared, significant differences between treatments were found, suggesting that earthing up could be a better application way for potato common scab control.

4.6. Effect of EOs and their MC on potato plants

In previous works, EOs have been recognized to have phytotoxic activity against different plant weeds and crops. This activity, defined as a negative impact on plant growth or fitness, is sometimes mediated by photosynthesis inhibition that could be produced by PSII alteration and decrease in photosynthetic pigments (Werrie et al., 2020). Despite this, the phytotoxic effect of EOs on potato plants growth and their photosynthetic activity have been poorly studied, while EOs effect has not been analyzed on potato yield. In previous works, TEO and SEO were reported as phytotoxic agents that could alter the germination and growth of plantlets of different species. Synowiec et al. (2017) observed alterations in the germination of some crop seeds (*Zea Mays*, *Brassica napus* and *Avena sativa*) and weeds (*Avena fatua*, *Bromus secalinus*, *Amaranthus retroflexus* and *Cetaurea cyanus*) when they were exposed to TEO. Additionally, Kordali et al. (2008) reported the effect of thymol, as a phytotoxic agent against different plant species, showing germination and growth alterations. The effect of TEO on potato plants was reported by Quintanilla et al. (2002), who observed that the foliar spray of a solution of TEO (1:500, v/v) produces chlorotic lesions on leaves, which later spread to other leaves and finally increase the ratio of foliar abscission. Furthermore, López et al. (2009) observed allelopathic effects of SEO that reduced the germination percentage on native cohabitant plants of *T. minuta* (*Bidens subalternans*, *Taraxacum officinale*, *Mikania cordifolia*, *Stipa eriostachya* and *Cynodon dactylon*) and, even on *T. minuta* seeds. Additionally, Scrivanti et al. (2003) reported allelopathic activity of SEO and its major components (o-cimenone, limonene and α-pinene) on the radical growth of *Z. mays* and suggested that lipid peroxidation was the mechanism of action. However, in those reports, a different chemotype of *T. minuta* (o-cimenone dominated) was analyzed, which is different to the one studied in the present research, for which, to our knowledge, there are not previous phytotoxic reports.

In the present work, EOs of *T. vulgaris* and *T. minuta* applied in a concentration equal twice de MIC (obtained for *S. scabiei*) did not show any negative effect on potato plants growth and photosynthetic parameters. This suggests that TEO and SEO applied in the concentration utilized could not affect the normal development of potato plants at early growth stage. However, these EOs, used in the same concentration, showed a reducing effect on potato yield expressed as tubers fresh weight.

The observed disparity on TEO and SEO effect on potato plants could be explained throughout the low concentration utilized in the present work. Werrie et al. (2020) indicate that some phytotoxic processes produced by EOs, as the decrease of photosynthetic pigments, could be affected in a dose-dependent way. Conversely, in the present work, the same low concentrations of TEO and SEO affected negatively potato yield. Similar results were found by Pfleeger et al., (2011), which analyzed the toxicity changes on potato tubers induced by the application of different herbicides (e.g. imazapyr, sulfometuron-methyl and glyphosate) utilized in low concentrations. In that work, the applications of herbicides were accomplished at 14 and 28 days after potato sprout and the harvest in the 4th month after that. However, to understand the mechanism of action utilized by these EOs on potato crops, additional studies are necessary.

Finally, MC treatments resulted not statistically different to control. This suggests that the microencapsulation reduced the observed EOs effects on potato crop yield, probably due to the volatile release control. This concentration reduction of the EOs compounds in the soil microenvironment could be altering their dose-dependent effects.

5. Conclusions

The obtained MC presents appropriate size and morphology to release bioactive compounds and keep the bacteriostatic activity of suico and thyme EOs against *S. scabiei*. The microencapsulation allows the control release of the EOs bioactive compounds, during storage and when applied to the soil. The release of volatiles in storage conditions result abundant and continuous for almost all compounds in TMC and for dihydrotagetone in SMC, suggesting a prolonged release before 29 days. On wet substrate, the highest effectiveness for both MC is around the 14th day, with a maximum

bioactivity at the first day. In those conditions, the application of MC results better by earthing up (SA). Regarding the effect of *Thymus vulgaris* and *Tagetes minuta* EOs on potato crops, they had no effect on plant growth and photosynthetic activity, but showed a negative effect on potato crop yield. However, the application of these EOs as MC minimizes that effect. Altogether, the microencapsulation of the studied EOs improves their utilization and maintains their antibacterial activity, whereby the application of TMC and SMC on potato crops can be a promising alternative to potato common scab control. Nevertheless, additional studies are necessary to analyze the effect of these MC on *S. scabiei* in a greenhouse assay.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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