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Chemical Composition and Antifungal Activity of Essential Oils of *Rosmarinus officinalis* L. and *Salvia officinalis* L. against *Botrytis cinerea* Pers

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ABSTRACT

Botrytis cinerea Pers. the causative agent of grey mould is commonly controlled by synthetic fungicides. Recently, studies have focused on the use of aromatic and medicinal plant extracts as safer alternatives. The aim of this study was to investigate the chemical composition and the antifungalactivity of essential oils of *Rosmarinus officinalis* L.(REO) and *Salvia officinalis* L.(SEO), against *B. cinerea* Pers.on tomato leaves and fruits. The EOs were extracted by hydrodistillation, and their chemical composition was analysed by gas chromatography-mass spectrometry (GC-MS). The antifungal activity of the EOs was evaluated on tomato leaves and fruitsinfested with *B. cinerea* Pers. using four treatment methods; simultaneous, preventive, curative, and combined preventive and curative treatments. GC-MS analysis showed that REO is composed mostly of α-pinene (29.01%), verbenone (21.59%), and camphor (7.32%), while SEO is predominantly composed of trans-thujone (29.01%), 1,8-cineole (22.78%), camphor (20.31%), and α-pinene (7.49%). REO and SEO demonstrated a concentration-dependent antifungal activity against *B. cinerea* Pers, especially in the combined preventive and curative treatment which exhibited 100% fungal growth inhibition for both EOs on tomatoleaves and fruits. On the tomato leaves, REO showed the least antifungal activity in the simultaneous treatment (IC_{50}= 7.62 ± 0.83µL/mL), while on tomato fruits, the curative treatment withSEOexhibited the least effective activity (IC_{50}= 9.15± 0.53µL/mL). Therefore, the essential oils of *R. officinalis* and *S. officinalis*. have potential for use in the biocontrol of tomato grey mould under green house and postharvest storage.

***Keywords*:**Antifungal effect, *Botrytis cinerea* Pers, Chemical composition, Essential oil, *Rosmarinus officinalis* L., *Salvia officinalis* L.

Introduction

Botrytis cinerea Pers. the causative agent of grey mould, is among the most pathogenic fungi in a wide spectrum of plants. It causes loss during culture, transportation and storage in more than 500 plant species across the globe.[1] It attacks many crops of economic importance, including many fruits, vegetables and ornamental crops during pre- and post-harvest period, especially crops under greenhouse conditions.[2]Among the crops infected by this fungus are tomatoes, (*Lycopersicon esculentum* L.),[3] the most important vegetable and main industrial crop around the world.[4] With the exception of the root, *Botrytis cinerea* infects all plant organs, causing significant losses in yield.[5] the difficulty in its control lies in its saprophytic nature (survival on plant debris) or in its sclerotia form.[6]

To fight against grey mould and to protect fruits postharvest and to keep them during storage, the synthetic fungicides are commonly used. However, the use of chemical fungicides is increasingly being discouraged due to their harmful effect on human health andenvironment.[7-11]

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In addition to causing harm to the environment, human health, and the economy, the use of chemical fungicides induces the development of resistant strains of pathogens to one or more active ingredient.[12-17]The selection and rapid development of fungicide-resistant *B. cinerea* Pers. genotypes have already been reported.[18-22] Thus, several studies have been carried out on eco-friendly and safer alternatives, namely aromatic and medicinal plants and have demonstrated their different biological activities, especially antifungal activity.[23] The interest in the study of the effectiveness of the aromatic and medicinal plants and their essences, particularly essential oils, has increased recently due to the ever-increasing demand in the international market.[24,25] Essential oils (EOs) have always played an important role in the daily lives of people, they have been widely used for perfuming, flavouring food or even for treatment, because of their potential as an antiseptic agent against infectious diseases, and also, for their properties as cytotoxic agents.[26]EOs contain a wide range of bioactive compounds, which are natural and biodegradable. EOs compounds can act alone or synergistically,[27-30]enhancing several activities including antimicrobial, herbicidal, insecticidal, antioxidants and fungicidal activities.[31-36]EOs have been the subject of several studies validating its effectiveness as alternatives to commercial pesticides for ecological conservation,[37] and also as a natural alternative to the control of antibiotic-resistant microorganisms.[38] Recently, several studies have demonstrated that essential oils have antifungal effect against plant pathogenic fungi namely *Botrytis cinerea*Pers.[39] The aim of this study is to demonstrate the effectness of the essential oils of *Rosmarinus officinalis* L. and *Salvia officinalis* L. against *Botrytis cinerea* Pers., for possible biological control against the deterioration of tomato crops.

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Materials and Methods

Fungal pathogen
Highly virulent and fungicide-resistant strain of *Botrytis cinerea* Pers. was used for the study.

Cultivation of Tomato Plant
The Campbell 33 variety of tomatoes (leaves and fruits) was used for the experiment. Healthy tomato plants were grown on sterile sand, as described by Mouria *et al*.[40]The plants were maintained in a culture chamber under controlled conditions (21°C, 16-hour photoperiod).

Extraction of Essential Oils
The essential oils were extracted by hydrodistillation as previously described.[41]Briefly, about 100 g each of the dried and crushed leaves of *Rosmarinus officinalis* L. and *Salvia officinalis* L. were placed in a 2 L flask to which 1 L of distilled water was added and placed on a heating mantle. After boiling for 3 h, the steam, after being cooled in the condenser, descends into the collector, where the essential oils and water separate by density difference. After removing the water, the oils were collected in a dark bottle and stored at 4°C in a refrigerator until use.

Gas Chromatographic-Mass Spectrometric (GC-MS) Analysis
The GC-MS analyses were carried out in Station d'Ionisation de Boukhalef (SIBO), of the Institut National de Recherche Agronomique (INRA) of Tangier, using gas chromatography coupled with electronically controlled mass spectrometry: GC-MS: Trace GC Ultra-ITQ900 thermoscientific, equipped with a TG-SQC column, (thermoscientific) with a length of more than 60 m. The starting temperature was set at 50°C, rising to 240°C at 5°C/min, with an automatic injection mode (TRIPLUS RSH- thermoscientific) by a 10µL micro syringe. The volume injected was 1µL (20 µL of HE diluted in 1000 of hexane). The carrier gas was helium with a fixed flow rate of 1 mL/min. The temperature of the injector was 250°C in split mode with ratio 1/20, with an Ion trap detector, at 230°C and hexane as eluent. The identification of the constituents was based on the comparison of their respective mass spectra (GC/MS) with the library spectra and on the basis of the calculation of the Kovats indices.

Determination of the antifungal activity of essential oils in vivo
The antifungal effects of different concentrations (0.25, 0.5, 0.75, 1, 10 and 20 µL/mL) of EOs of *S. officinalis* L. and *R. officinalis* L. were tested against *B. cinerea* Pers. on tomato leaves and fruits, using four types of treatments methods which include simultaneous treatment, preventive treatment, curative treatment, and a combination of preventive and curative treatment. The different concentrations of EOs were prepared in 1 mL of a previously sterilised mixture of tween 20 (0.1%) and ethanol (3%). For the simultaneous treatment, the treatment with EOs was carried out at the same time of inoculation with the pathogen. For the preventive treatment, the treatment with EOs was carried out 24 h before inoculation with the pathogen, for the curative treatment, treatment with EOs was done 24 h after inoculation with the pathogen, while for the combination of preventive and curative treatment, the EOs were applied 24 h before and 24 h after pathogen inoculation. Each test was carried out in seven replicates. The controls were treated with the mixture of tween 20 (0.1%) and ethanol (3%) only.

Leaf assays
Eight-week-old tomato seedlings were treated by spraying 1 mL of each concentration of the EOs on the leaves simultaneously, 24 h before, 24 h after, and both 24 h before and 24 h after inoculation with *B. cinerea* Pers. The treated leaves were inoculated with a mycelial explant (5mm) of seven-day-old pathogen. The seedlings were then placed in a growth chamber at 23±2°C and high humidity. After 5 days of incubation, the effects of SEO and REO was assessed by measuring the average diameter of the rot zone on the treated leaves.

Fruit assays
Uniform and healthy tomato fruits of the Campbell 33 variety were sterilised with 70% ethanol, rinsed and dried aseptically. Wounds of 3 mm depth were made with sterile needles (2 mm) at 3 different points on the equatorial zone. The fruits were subjected to the four types of treatments mentioned above for the leaf assay. A 20 µL of each concentration of EOs were placed in the wounds simultaneously, before, after inoculation. The wounds were inoculated with a mycelial explant (2mm) of seven-day-old *B. cinerea* Pers. The fruits were then placed in the dark at 21±2°C and high humidity conditions. After 7 days of incubation, the mean diameters of the rot zone and the spore zone were measured.

Evaluation of inhibitory activity
The antifungal index (AFI) of each essential oil was determined by the following formula:

$$AFI (\%) = ((d- di)/d) \times 100.$$

Where; d = Diameter of mycelial growth in the absence of essential oils.
di = Diameter of mycelial growth in the presence of essential oils.

Statistical analysis
All experiments were performed twice under the same conditions with seven replicates. The results obtained were subjected to one-way analysis of variance (ANOVA), and differences between means were determined using Tukey's Post-hoc test. Significant difference was set at $P \leq 0.05$.

Results and Discussion

Chemical composition of the essential oils
GC/MS analysis of the essential oils identified thirty-six constituents in REO, which represent a total of 98.84% of the essence and twenty-one constituents in SEO representing 99.69% of the essence (Table 1). REO is composed of α-pinene (29.01%), verbenone (21.59%), camphor (7.32%), camphene (4.98%), 1,8-cineole (3.54%), β-copaene (3.49%), borneol (3.43%) and limonene (3.02%) and other minor compounds. The chemical composition of REO obtained in this study is very similar to those reported in several works, with camphor, α-pinene, 1,8-cineole, camphene, β-pinene, Myrcene, borneol and piperitone as major compounds.[42-45] A studycarried out on the chemotaxonomy of Moroccan REO, identified three chemotypes: 1,8-Cineole (58-63%), camphor (41-53%) and α-pinene (37-40%).[46]Flamini *et al*. (2002)[47] found the highest alpha-pinene content (30.3%) in the essential oil extracted from the dried leaves of *Rosmarinus officinalis*. Ainane *et al*. (2018)[48] found that REO contains camphor (31.16%), β-caryophyllene (18.55%), 2,4-hexadiene, 3,4 -dimethyl-, (Z, Z) (9.08%), α-fenchene (4.67%), cis-verbenone (4.33%) and bornyl acetate (3.4%) as the major compounds, while the rest of the constituents represented less than 1%. Other researchers have demonstrated that *R. officinalis* extract are mainly composed of aromatic compounds such as borneol, camphene, camphor and cineol, flavonoids, tannins, rosmarinic acid, diterpenes, and rosmarin.[49-51]In fact many previous studies, have shown that the chemical composition of REO can vary qualitatively and quantitatively depending on several factors. The SEO were predominantly composed of trans-thujone (29.01%), 1,8-cineole (22.78%), camphor (20.31%), α-pinene (7.49%), camphene (4.99%) and myrcenol (2.96%), these compounds constituted more than three quarter of the total composition. In 2015, Rus *et al*.,[52] identified camphor (20.4%), eucalyptol (11.7%), camphene (11.5%), α-pinene (9.5%) as major compounds of the SEO. Similarly, several previous studies have confirmed that SEOcontain α-pinene, camphene, β-pinene, Myrcene, 1,8-cineole, camphor, borneol, bornyl acetate, (E)-caryophyllene, and viridiflorol as major compounds.[44,53-56]Also, the study ofAlexa, (2018)[57]identified caryophyllene (25.364%), camphene (14.139%), eucalyptol (13.902%), and β-pinene (11.230%), thymol (8.073%), camphor (4.028%), and valancene (5.525%) as themain compounds of SEO, Camphor has been reported as the main compound of SEO in several studies,[58-60] while other authors have reported α-thujone as the main compound of SEO,[58,61,62] which is in line with the results of this study which shows that REO and SEO are mainly composed of α-pinene, trans-thujone, 1,8-cinéole, and camphor.

Generally, despite the variations in the chemical composition, EOs are usually composed of two major chemical groups, the terpenoids, which are the most predominant (represented mainly by monoterpenes and sesquiterpenes),[63-66]and the phenylpropanoids.[63, 67-69] In fact, the

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variations in the results obtained in the different studies cited explains the role that environmental and climatic factors (geographical origin, time of harvest, light exposure)play in the chemical composition and quality of EOs.[70] In addition to the environmental and climatic factors, genetic factor also have a considerable influence on the quantitative and qualitative composition of EOs.[71]

In vivo antifungal activity of essential oils against B. cinerea Pers. *on tomato leaves*

The essential oils of *R. officinalis* L. (REO) and *S. officinalis* L. (SEO) showed aconcentration-dependent antifungal effect against *B. cinerea* Pers. (Figure 1). A recent study has proven the effictiveness of several EOs against several fungal pathogens including *B. cinerea* Pers.[39] The antifungal activity of EOs could be attributed to the major phytochemical group of compounds which are the monoterpenes with proven antifungal activity,[29,72-75]and the sesquiterpenes, which play a principal role in defence against bacterial and fungal attacks.[66] Generally, since terpenoids are non-polar with hydrophobic and lipophilic characters that facilitate their interaction with the membrane components of fungal cell, causing cell membrane damage and interferance in energy homeostasis. All substances rich in terpenoids are considered natural antifungals.[76,77]The compounds of EOs can act alone or in synergy with other compounds with a promise of greater effectiveness.[30,78,79]

With respect to the effects of the different types of treatments with REO against tomato leaf rot, the combined preventive and curative treatment was the most effective, resulting in 100% inhibition at concentration of 10 µL/mL (Figure 1A), followed by the curative treatment which gave 100% inhibition at concentration of 20 µL/mL, then the preventive treatment while the simultaneous treatment was the least effective producing slightly above 50% inhibition of the rot at 10 µL/mL (Figure 1A). In fact, the leaves treated with the simultaneous treatment at low concentrations showed no significant difference compared with the positive control leaves (inoculated with *B. cinerea* Pers. only), while the leaves treated with the other treatments were not significantly different from the negative control (healthy leaves) from a concentration of 10 µL/mL (Figure 2). On the other hand, SEO resulted in a significant inhibition of the rot (Figure 1B).The antifungal effect of SEO against *B. cinerea* Pers. and *Fusarium* sp. has been highlighted.[80,81] Also, Rguez *et al*. (2018)[82] found that SEO showed significant antifungal effect *in vitro* against *B. cinerea* Pers. With respect to the effectiveness of the type of treatment, the combined preventive and curative treatment consistently gave the most potent activity, resulting in 100% inhibition from a concentration of 1 µL/mL and no significant difference with the healthy control (Figure 2), this was followed by the preventive treatment and then the curative treatment, and lastly, the simultaneous treatment (Figure 1B).

Table 1: Chemical composition of *R. officinalis* L. and *S. officinalis* L. essential oils

Chemical group	Components	EO of *R. officinalis*	EO of *S. officinalis*
Terpenes	α-thujene	-	0.16%
	α-pinene	29.01%	7.49%
	Camphene	4.98%	4.99%
	β-pinene	0.88%	1.66%
	Myrcene	0.46%	0.94%
	α-terpinene	0.56%	-
	Limonene	3.02%	0.63%
	γ-terpinene	1.14%	0.28%
	Alloocimene	0.33%	-
	allo-Aromadendrene	-	1.01%
	β-bourbonene	0.33%	-
	α-cedrene	0.28%	-
	β-copaene	3.49%	1.81%
	β-acoradiene	2.22%	-
	Valencene	0.54%	-
	δ-cadinene	0.43%	-
	γ-bisabolene E	1.26%	-
Total *		48.93 % *	18.97*
Ketones	Camphor	7.32%	20.31%
	Menthone	-	0.33%
	Cryptone	1.32%	0.71%
	Dihydrocarvone	0.52%	-
	β-oplopenone	-	1.88%
	Verbenone	21.59%	-
	β-ionone,	0.58%	-
	Trans-thujone	-	29.01%
	Cis-thujone	1.8%	-
Total *		33.13 %*	52.24*
Alcohols	Linalol	0.84%	0.26%

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	Myrcenol	0.40%	2.96%
	Geraniol	0.23%	-
	α-terpiniole	1.64%	-
	Cubebol	2.46%	-
	Carotol	0.59%	0.62%
	β-bisabolol	0.37%	-
	E-sesqui- lavandulol	-	0.29%
	Borneol	3.43%	1.28%
	Menthol	0.29%	-
	Eudesm 7(11)-en-4-ol	0.43%	-
Total *		10.68 %*	5.41
Phenols	Thymol	1.61%	0.58%
Esters	Dillapiole	0.28%	-
	Citronellyl acetate	0.37%	-
Total *		0.65	-
Aldehydes	Benzenaldehyde	0.48%	-
Ethers	1,8-cineole	3.54%	22.78%
	NI	0.39%	-
	NI	0.27%	-
	NI	0.22%	-
	Total	99.9%	99.98%

NI: Unidentified
(-): Absent

On comparison of the IC_{50} values of the two EOs studied (Table 2), it was observed that the combined preventive and curative treatment with REO was the most effective (IC_{50}<0.25 µL/mL), followed by the curative treatment and the preventive treatment with IC_{50}values of0.53µL/mL and 0.66 µL/mL, respectively, while the simultaneous treatment was the least effective (IC_{50}=7.62 µL/mL). Like REO, the combined preventive and curative treatment of SEO was the most effective (IC_{50}< 0.25 µL/mL), followed by the preventive treatment and the simultaneous treatment (IC_{50}= 0.48 µL/mL and IC_{50}= 0.96 µL/mL, respectively), while the curative treatment was the least effective (IC_{50}= 4.21 µL/mL). On comparing the IC_{50} values of the different treatments of the two EOs, the simultaneous treatment of REO appeared to be the least effective, followed by the curative treatment of SEO. Moreover, the preventive treatment and the curative treatment of REO as well as the simultaneous treatment and the preventive treatment of SEO were not significantly different. The determination of IC_{90} allowed us to deduce that the combined preventive and curative treatment of SEO (IC_{90}= 0.61 µL/mL) was more effective than that of REO (IC_{90}= 7.64 µL/mL).The fact that the combined preventive and curative treatment, and the preventive treatment were more effective against the leaf rot, suggests that the EOs stimulate in the plant certain defense mechanisms against *B. cinerea* Pers. and consequently the accumulation of secondary metabolites in the plants. In this regard, differents studies revealed that EOs stimulate plant defense mechanisms against pathogenic infection on the basis of their antioxidant activity and ability to improve plant growth.[83-85]

The results obtained in the simultaneous treatments and curative treatments is justified by the fact that REO may contain substances that are capable of inhibiting the development of the disease at stages subsequent to infection, so if they are applied simultaneously with the infection by the pathogen, they evaporate before they act. Whereas, if they are applied after the infection (curative treatment), they act directly on the mechanisms of the disease development. On the other hand, SEO could be rich in substances that act at the earlier stages of the infection, so if they are applied simultaneously with the infection, they are effective, whereas when they are applied after the infection (curative treatment), the infection is not inhibited because the pathogen has already developed. It could be deduced that the EOs studied act differently, due to their diverse chemical constituents. In fact, EOs have several functional agents that enhance the antifungal activity and deactivate the fungus by the interaction between hydrophobic compounds and lipids of the fungal cell membrane,[63,86,87]interrupting its structure and function such as inhibiting nuclear material or protein synthesis,[69,87]and may also cause membrane expansion, affect the morphogenesis and growth of the hyphae.[88-90] Caccioni and Guzzardi (2011)[91] showed that monoterpenes such as α-pinene caused morphological changes in the fungal hyphae by disrupting cell membrane. They reveled that EOs with high concentrations of α-pinene had antifungal effects against differents pathogens. Similarly, Reguez *et al*. (2020)[92] showed that the pure α-pinene of *Tetraclinis articulata* EOs caused bulge of *B. cinerea* Pers. conidia which were probably attributed to a disturbance in membrane permeability. On the other hand, α-pinene reduces germination of *B. cinerea* Pers. conidia and alters their morphology. Also, Fernando *et al*. (2013)[65] proved that α-pinene targets the integrity of cell membrane, causing morphological alterations in the conidia such as rupture of membranes and vacuolization and disorganization of the cytoplasm. On the fungal mycelium, EOs act on the key proteins involved in the regulation of metabolism, growth and cell differentiation.[93,94] They lead to loss of cell membrane integrity and rigidity, causing disturbance of mycelium,[95] and alterations of fungal morphology (rupture, peeling, dehydration and interference with energy homeostasis).[29,76,77] According to Paula *et al*. (2020),[96] the antifungal effect of the EOs against *B. cinerea* Pers. could be attributed to the chemical composition of these EOs including monoterpenes, as α-pinene, α-thujene, limonene, α-terpinolene, 1,8-cineole, and α-terpinyl acetate.On the other hand, some authors suggest that the mechanism of EOs against fungal species, is probably attributed to the high content of lipid peroxides, such as the hydroxyl radicals, inhibition of cell membrane synthesis of ergosterol, mitochondrial fragmentation, and inhibition of respiratory enzymes.[97-99] Several other studies confirmed that EOs can cause cell death and/or inhibition of sporulation and germination of fungi by interfering with the mitochondrial respiratory chain complexes, leading to mitochondrial

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dysfunction, an imbalance in cellular metabolic activity, and consequently a drop in energy availability.[86,89,100-103]
The antifungal activity of Eos is attributed not only to a single compound,[104,105] but also to sevearal compounds which act synergistically.[90,106] Minor compounds of EOs such as alcohols, aldehydes and esters have been shown to have antimicrobial activity.[107] In fact, plants rich in phenolics seem also to be more effective against phytopathogens.[67-69]

In vivo antifungal activity of essential oils against B. cinerea Pers. *on tomato fruits*

On fruit rot, REO and SEO demonstrated a remarkable inhibitory activity against *B. cinerea* Pers. especially with the combined preventive and curative treatment which resulted in a 100% inhibition of the rot at concentrations of 0.25 µL/mL and 0.75 µL/mL for REO and SEO, respectively (Figures 3 and 4).The effect of the combined preventive and curative treatment was followed by that of the preventive treatment which produced slightly above 50% inhibition at 0.25 µL/mL and 0.5 µL/mL for REO and SEO, respectively. However, the simultaneous treatment and the curative treatment with SEO and REO were less effective, producing approximately 50% inhibition at 1 µL/mL.

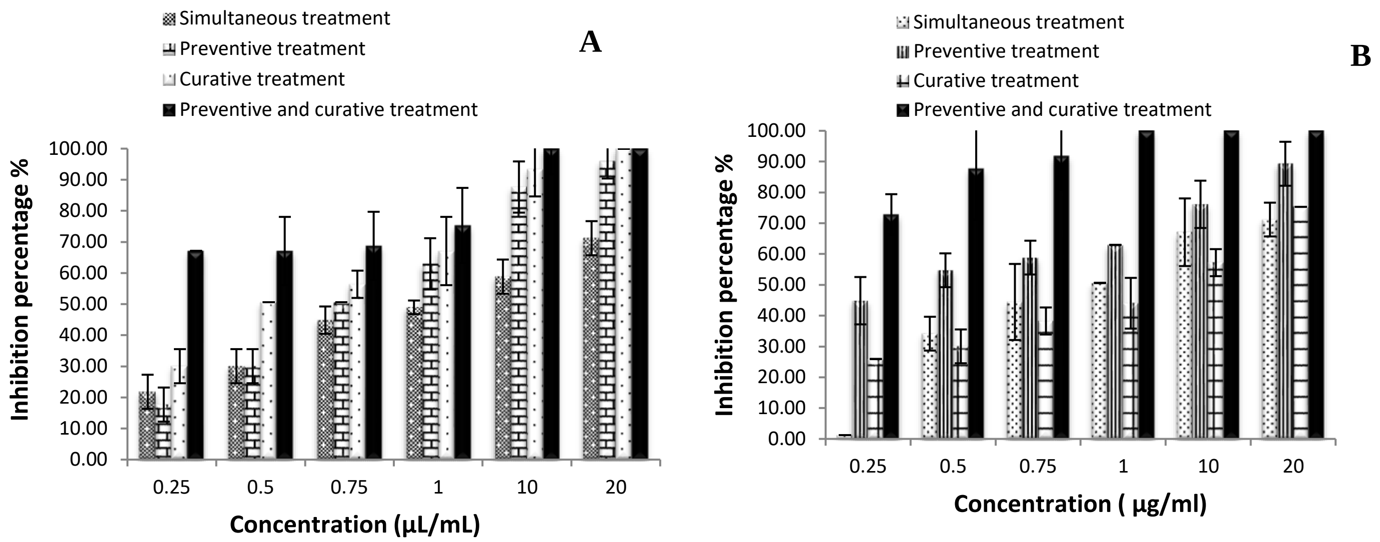

Figure 1: Antifungal effect of differents treatments against *B. cinerea* Pers. on tomato leaves. A: Treatments with *R. officinalis* L. essential oils, B: Treatments with *S. officinalis* L. essential oils

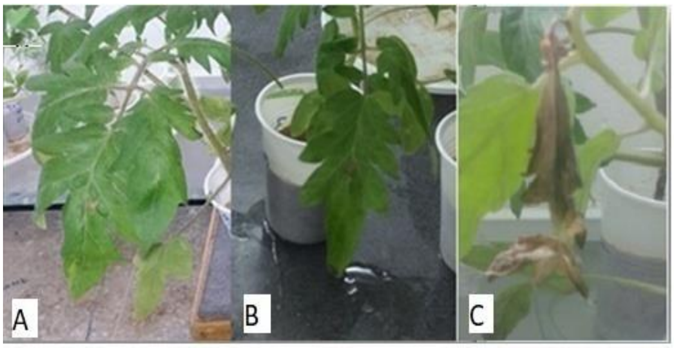

Figure 2: Effect of preventive and curative treatments with 10µL/mL of EOs against the rot of tomato leaves after 5 days of incubation. A: Leaf treated with *R. officinalis* L. essential oils, B: Leaf treated with *S. officinalis* L. essential oils, C: Positive control.

Determination of the IC_{50} of REO and SEO against *B. cinerea* Pers. growth on the fruits confirmed that the combined preventive and curative treatment was the most effective against fruit rot (IC_{50}<0.25 µL/mL), while the curative treatment with SEO was the least effective with IC_{50} of 9.15 µL/mL (Table 2). Although, the range of concentrations studied did not allow for the determination of IC_{90} for all the treatments methods, but the IC_{90} of only the combined preventive and curative treatments with the two essential oils were determined, and it was found out that the combined preventive and curative treatment of rosemary (IC_{90}< 0.25 µL/mL) was more effective than that of sage (IC_{90}= 0.39 µL/mL).

The results obtained are in agreement with the findings of other studies which have proven that 60% of the EOs, or of their derivatives, are effective against a wide spectrum of fungi. Thus these EOscan be used to control fungal diseases in fruits and vegetables, ensuring an extension of their storage life.[108,109] In fact EOs have been shown to be effective against *B. cinerea* Pers. Infection on fruit and against several other fungal infections. For example, on apple, *Satureja hortensis* and *Thymus vulgaris*, EOs have been shown to be effective against *B. cinerea* Pers. during postharvest.[85]
The fact that the combined preventive and curative treatment, and the preventive treatment were more effective against the fruit rot, suggests that the essential oils stimulated in the fruits certain defense mechanisms against *B. cinerea* Pers. According to Gholamnezhad (2019),[110] the treatment of apple fruits with plant extracts induced POX, PAL, β-1,3-glu and PPO and total phenolic contents in plants infected by *B. cinerea* Pers. The induction of phenolic compounds by plant extracts, suggests the roles of these compounds in resistance induction against fungal pathogens. Plant extracts, elicits production in apple fruits molecules that act against apple gray mould, especially phenol compounds which have a strong effect on the induction of POX, PPO, and PAL enzymes. Thus these plant extracts can be useful in the control of apple grey mould.
With regards to sporulation on fruits, the results obtained indicated that the combined preventive and curative treatment, as well as the preventive treatment with REO and SEO remain the most effective in reducing the growth of the sporulation zone, especially the combined preventive and curative treatment which caused 100% inhibition of sporulation even at the lowest concentration of the two essential oils (Table 3). On the other hand, for the curative treatment and the simultaneous treatment with low concentrations of the essential oils, it was found that the diameter of the sporulated zone on the treated fruits

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significantly exceeds that of the positive control, which means that these two treatments stimulated sporulation at low concentration, especially the curative treatment which only showed a slight inhibition at the 20 µL/mL concentration (Figure 5A). Figure 5B shows that during the simultaneous treatment, the antifungal effect was only observed from concentration of 10µL/mL (Table 3). This was confirmed by the fact that the REO and SEO at low concentrations are unable to inhibit fungal growth when applied simultaneously or curatively, but they create stressful conditions that stimulate sporulation of the fungus to ensure its dissemination and thus its survival. Comparing the inhibitory effect of the simultaneous treatment of the two EOs, it appeared that REO are more effective than SEO. On the basis of the results obtained for the tomato fruits, the REO and SEO hold promise for the biocontrol and the management of postharvest pathogen of tomatoes, especially *B. cinerea* Pers. which has been described as the most important postharvest pathogen.[111,112] However, the effectiveness of these biocontrol agents can be inflenced by the mode of their application which is a fundamental part of biocontrol. From the results of the present study, it appears that the application of EOs as biocontrol agents of *B. cinerea* Pers. should be both preventive and curative in order to stimulate the defence mechanisms of the leaves and/or fruit before infection and to act on the pathogen after infection.

Conclusion

The antifungal activity of the essential oils of *R. officinalis* L. and *S. officinalis* L. against *B. cinerea* Pers. on tomato leaves or fruits is concentration-dependent, the activity increases with increase in concentration. According to these results, the high antifungal activity observed in the essential oils of *S. officinalis* L. and *R. officinalis* L. could be attributed to the efficacy of its components which may act singly or synergistically.The preventive treatment as well as the combined preventive and curative treatment on tomato leaves and fruits with EOs of *R. officinalis* L. and *S. officinalis* L. reduced the severity of gray mould disease. There was no significant reduction in the severity of *B. cinerea* Pers. with the simultaneous treatment. Based on the findings from this study, postharvest losses of stored crops may be reduced if crops are protected with EOsin a preventive manner. Thus the essential oils of *R. officinalis*L. and *S. officinalis* L. have a great potential as antifungal compounds that could be used in the biocontrol of grey mould disease. Additionally EOs of *R. officinalis* L. and *S. officinalis* L. may be safer alternatives to synthetic fungicides in the control of both fungal diseases in the field and during storage. Further studies is therefore encouraged to explore the mechanism of action of isolated and/or combined essential oil components and the formulation of biofungicides based on these components.

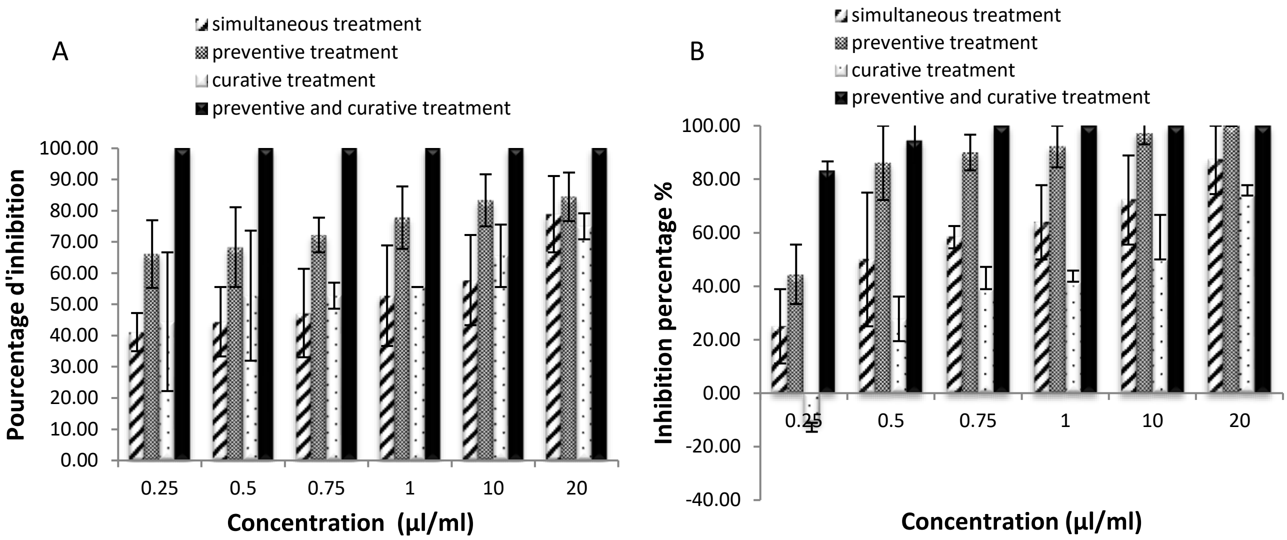

Figure 3: Antifungal effect of differents treatments against *B. cinerea* Pers. on tomato fruits.
A: Treatments with *R. officinalis* L. essential oils, **B:** Treatments with *S. officinalis* L. essential oils

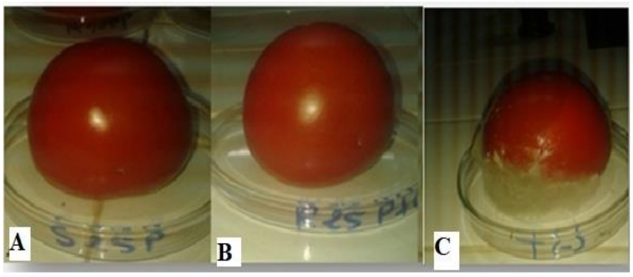

Figure 4: Effect of preventive and curative treatment with 0.25 µL/mL of EOs, against the rot of tomato fruits after 7 days of incubation. **A:** Tomato fruit treated with *S. officinalis* L. essential oils, **B:** Tomato fruit treated with *R. officinalis* L. essential oils, **C:** Positive control.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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Table 2: Antifungal activity of *Rosmarinus officinalis* L. and *Salvia officinalis* L. essential oils against *Botrytis cinerea* Pers.

	Leaves		**Fruits**	
	IC_{50} (µL/mL)	**IC_{90}**(µL/mL)	**IC_{50}**(µL/mL)	**IC_{90}**(µL/mL)
***R. officinalis* L.**				
Simultaneous treatment	7.62 ± 0.83^{a}	>20	0.91 ± 0.6^{a}	>20
Preventive treatment	0.66 ± 0.21^{b}	14.25 ± 0.39^{a}	<0.25	>20
Curative treatment	0.53 ± 0.06^{b}	8.96 ± 0.01^{b}	0.52 ± 0.03^{a}	>20
Preventive + curative treatment	<0.25	7.64 ± 0.48^{c}	<0.25	<0.25
***S. officinalis* L.**				
Simultaneous treatment	0.96 ± 0.01^{b}	>20	0.6 ± 0.06^{a}	>20
Preventive treatment	0.48 ± 0.08^{b}	>20	0.37 ± 0.02^{a}	6.48 ± 0.86^{a}
Curative treatment	4.21 ± 0.11^{c}	>20	9.15 ± 0.53^{b}	9.15 ± 0.4^{b}
Preventive + curative treatment	<0.25	0.61 ± 0.21 ^{d}	<0.25	0.39 ± 0.11^{c}

Values are means ±SD. Values in the columns followed by the same superscript letter are not significantly different.

Table 3: Diameter (cm) of the spore zone on tomato fruits after treatment with *Rosmarinus officinalis* L. and *Salvia officinalis* L. essential oils

Treatment	**Concentrations of EO (µL/mL) + *B. cinerea***						**Positive control *B.C***	**Negative control**
	0.25	**0.5**	**0.75**	**1**	**10**	**20**		
***R. officinalis* L.**								
Simultaneous	1.88 ± 0.19^{b}	0.75 ± 0.15^{c}	0.60 ± 0.10^{cd}	0.30 ± 0.15^{de}	0.25 ± 0.15^{e}	0.13 ± 0.19^{e}	1.03 ± 0.49^{a}	0.00 ± 0.00^{e}
Preventive	0.68 ± 0.18^{b}	0.50 ± 0.15^{bc}	0.43 ± 0.08^{bc}	0.35 ± 0.15^{bc}	0.30 ± 0.10^{c}	0.08 ± 0.11^{c}	1.03 ± 0.49^{a}	0.00 ± 0.00^{c}
Curative	1.88 ± 0.19^{b}	1.63 ± 0.19^{bc}	1.55 ± 0.13^{bc}	1.30 ± 0.20^{c}	0.88 ± 0.19^{d}	0.60 ± 0.25^{d}	1.03 ± 0.49^{a}	0.00 ± 0.00^{e}
Preventive and curative	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	1.03 ± 0.49^{a}	0.00 ± 0.00^{b}
***S. officinalis* L.**								
Simultaneous	1.88 ± 0.19^{b}	1.38 ± 0.19^{c}	1.25 ± 0.15^{c}	1.13 ± 0.04^{cd}	0.88 ± 0.08^{d}	0.45 ± 0.08^{e}	1.03 ± 0.49^{a}	0.00 ± 0.00^{f}
Preventive	0.45 ± 0.08^{b}	0.33 ± 0.18^{bc}	0.10 ± 0.15^{cd}	0.08 ± 0.11^{cd}	0.05 ± 0.08^{d}	0.00 ± 0.00^{d}	1.03 ± 0.49^{a}	0.00 ± 0.00^{e}
Curative	2.00 ± 0.00^{b}	1.63 ± 0.19^{c}	1.50 ± 0.20^{c}	1.18 ± 0.09^{d}	1.00 ± 0.00^{d}	0.83 ± 0.18^{d}	1.03 ± 0.49^{a}	0.00 ± 0.00^{e}
Preventive and curative	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	1.03 ± 0.49^{a}	0.00 ± 0.00^{b}

Values are means ±SD. Values in the columns followed by the same superscript letter are not significantly different.

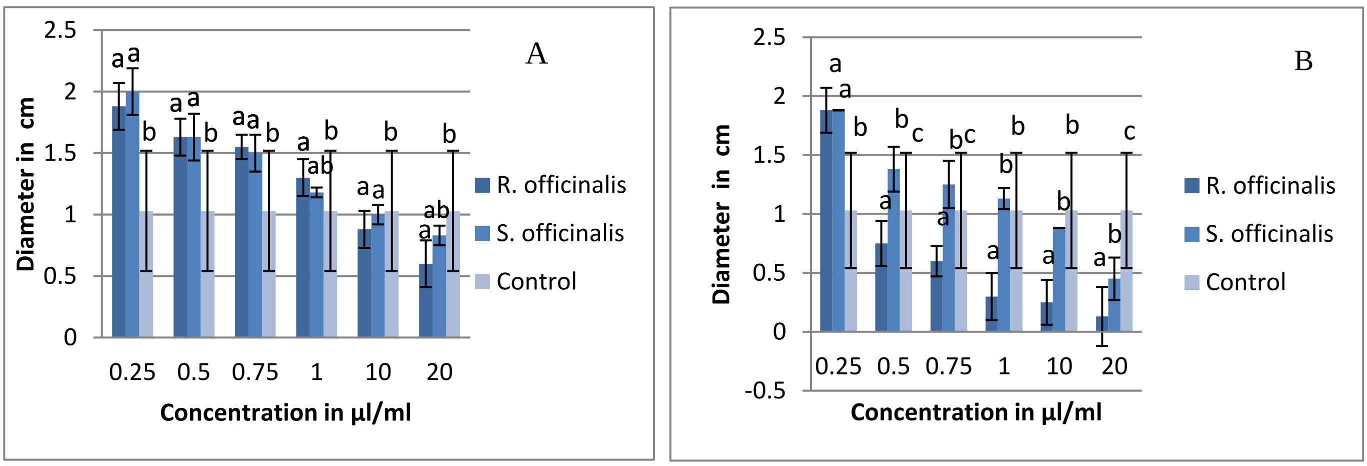

Figure 5: Effect of *R. officinalis* L. and *S. officinalis* L. essential oils on sporulation. **A:** Curative treatmnt, **B:** Simultanous treatmnt
Bars for each concentration, with the same letters represent values that are not significantly different.

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