Essential oils and Gras salts for preventing postharvest fruit rot and reducing the need for chemical fungicides

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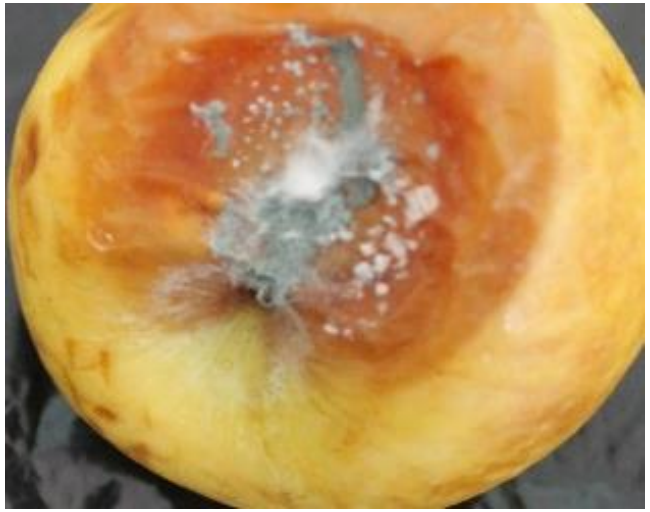
INRAT- Tunisia

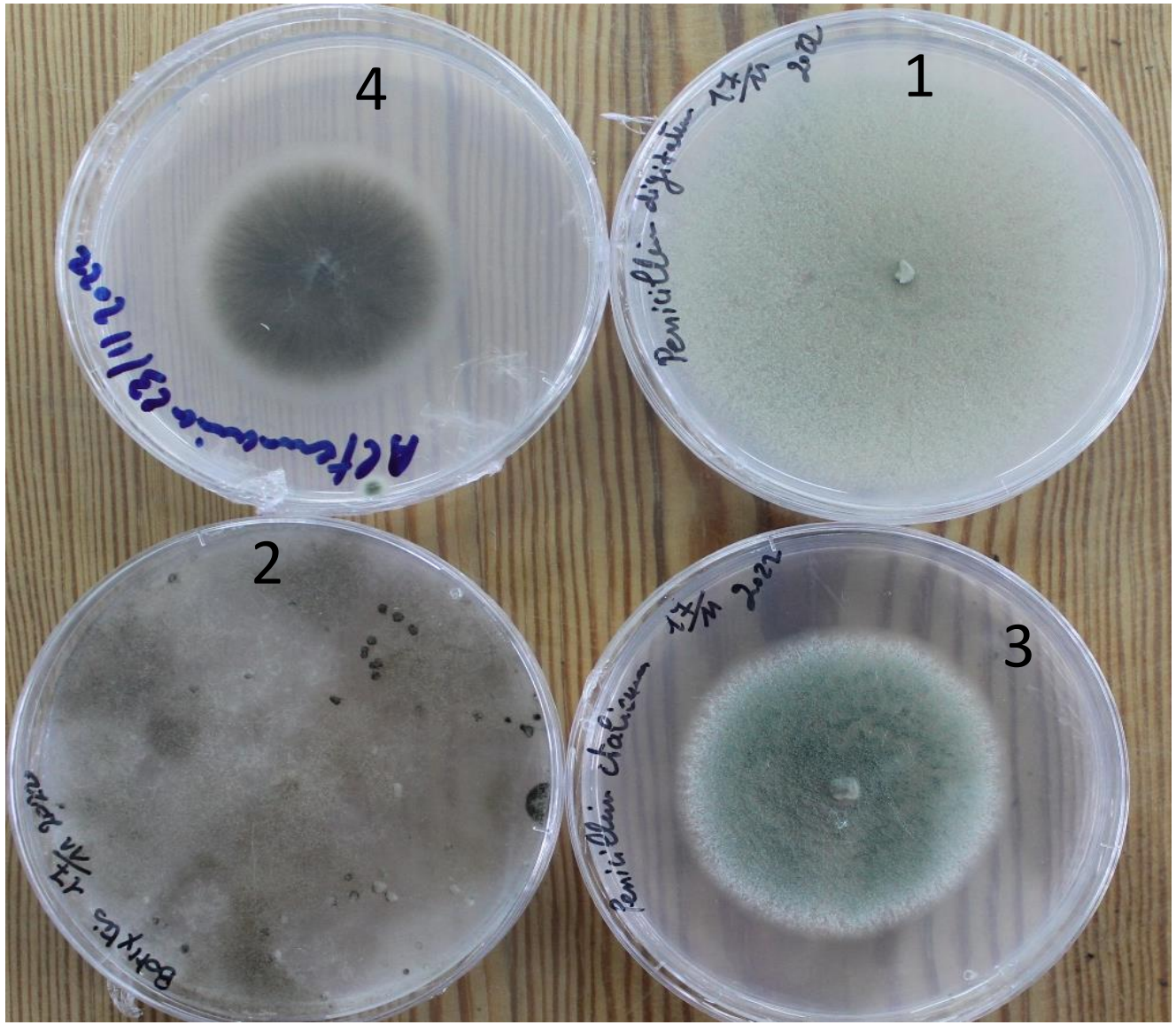
January 23, 2024 Ancona

High amount of post harvested fruit are lost due to rots



- fungal species : *Penicillium digitatum, Botrytis cinerea, Penicillium italicum and Alternaria alternata*
- These fungi were isolated from fruits collected in storage locations of several companies, then morphologically identified and conserved until use,
- These are considered the most important fungi causing fruit decay during storage in our conditions.

Devastating fungal species on fruit postharvest in Tunisia



1. *Penicillium digitatum* (green mold)

2. *Botrytis cinerea* (gray mold)

3. *Penicillium italicum* (blue mold)

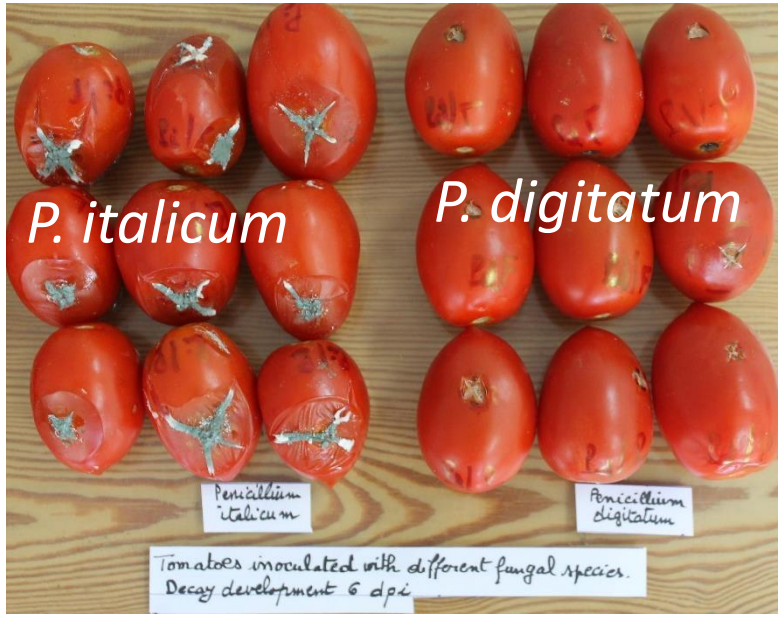
4. *Alternaria alternata* (black rot)

Pathogeneicity of different fungal species

P. digitatum

P. italicum

B. cinerea

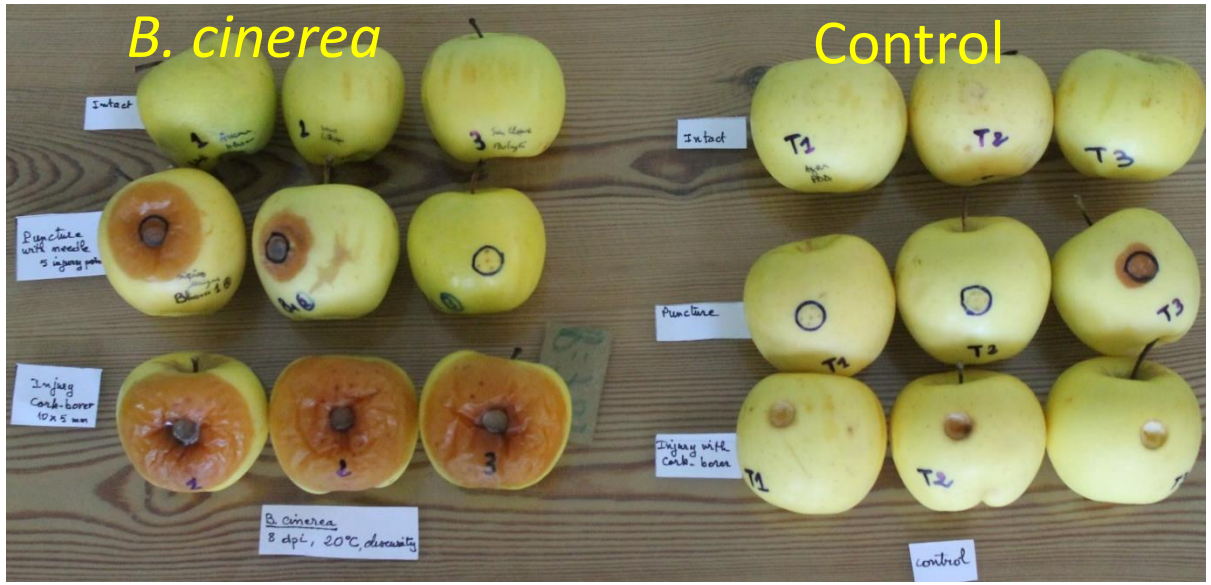


Importance of wounds for fungal infection

Wounds performed on fruit (10 mm diameter with cork-borer, 5 punctures with needle, intact fruit)

Diameter of the decay (mm) 8 dpi (20°C)

	Wide wound (10 mm diam with cork-borer)	5 small wounds with needle	Intact fruit
Botrytis cinerea	68.5	23	0
Fruit wounded not infected	0	5.6	0



The larger the wound, the greater the diameter of decay

Biological control of fruit decay

- Essential oils
- GRAS salts
- Compound mixture

Essential oils

- Essential oils (EOs) extracted from aromatic or medicinal plants are biodegradable, safe and considered as an environmentally solution to reduce fungal species attacking different crops.
- Several EOs have been tested to evaluate their ability to extend the shelf life of postharvest fruit and vegetables during storage.
- 30 Eos were tested *in vitro*

antibiotics

MDPI

Article

Antifungal Activity of Thirty Essential Oils to Control Pathogenic Fungi of Postharvest Decay

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Abstract: Essential oils (EOs) extracted from aromatic or medicinal plants are biodegradable, safe, and regarded as alternatives to chemical pesticides to reduce fungal species attacking different crops. In this study, thirty EOs at 0.5 mg/mL were evaluated for in vitro growth inhibition of the main postharvest fungi, which are *Alternaria alternata*, *Botrytis cinerea*, and *Penicillium italicum*. *Cinnamomum verrum* EO completely inhibited the mycelial growth of *A. alternata* and *B. cinerea*, and *Syzygium aromaticum* EO completely inhibited the mycelia of *A. alternata*. *B. cinerea* mycelial growth was completely inhibited by *Gaultheria fragrantissima*, *Cymbopogon nardus*, *Pelargonium asperum*, and *Cupressus sempervirens* EOs. *G. fragrantissima* EO inhibited the mycelia growth of *P. italicum* by 98%. Overall, *B. cinerea* displayed the highest sensitivity to EOs than *P. italicum* and *A. alternata*. *G. fragrantissima*, *C. sempervirens*, *C. nardus*, *P. asperum*, *Mentha piperita*, *Foeniculum vulgare*, *C. verrum*, and *S. aromaticum* EOs showed the highest inhibition for these three pathogens. Minimum inhibitory concentrations were lower for *C. verrum* and *S. aromaticum* EOs, ranging between 0.31 and 0.45 mg/mL and 0.37 to 0.57 mg/mL, respectively, against the three pathogens. The tested EOs inhibited the in vitro growth of three of the main postharvest fungal pathogens. Further studies are needed to confirm these activities in vivo.

Keywords: blue mold; essential oils; fruits; gray mold; shelf life

Citation: Allagui, M.B.; Moumni, M.; Romanazzi, G. Antifungal Activity of Thirty Essential Oils to Control Pathogenic Fungi of Postharvest Decay. *Antibiotics* **2024**, *13*, 28. https://doi.org/10.3390/antibiotics13010028

Academic Editors: Luis Alberto Ortega-Ramirez and Jesus Fernando Ayala-Zavala

Received: 24 October 2023
Revised: 14 November 2023
Accepted: 17 November 2023
Published: 28 December 2023

1. Introduction

Food loss and waste are issues of importance to global food security, and according to the Food and Agriculture Organization of the United Nations, 45% of all fruits and vegetables are lost or wasted every year [1]. This waste occurs along the entire food chain (from field to consumer) and needs to be analyzed and monitored due to its impact on the development of the food sector. Contamination of fruit and vegetables by pathogenic microorganisms is a major factor in reducing yields and market quality. The use of fungicides is a common practice as a postharvest treatment to control fruit decay. In recent years, it has been necessary to achieve the United Nations' Sustainable Development Goals (SDGs) and the Farm to Fork Strategy of the European Green. In addition, fresh fruit loss,

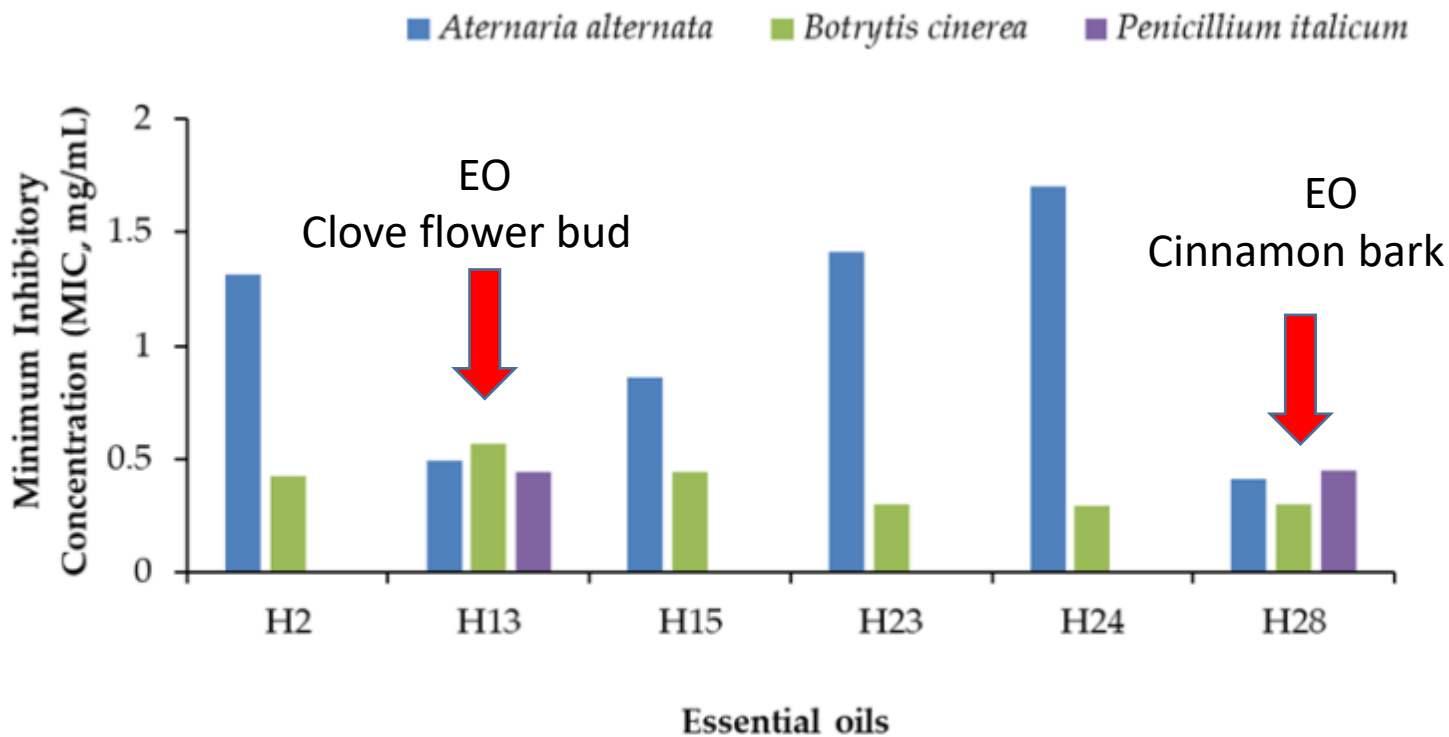
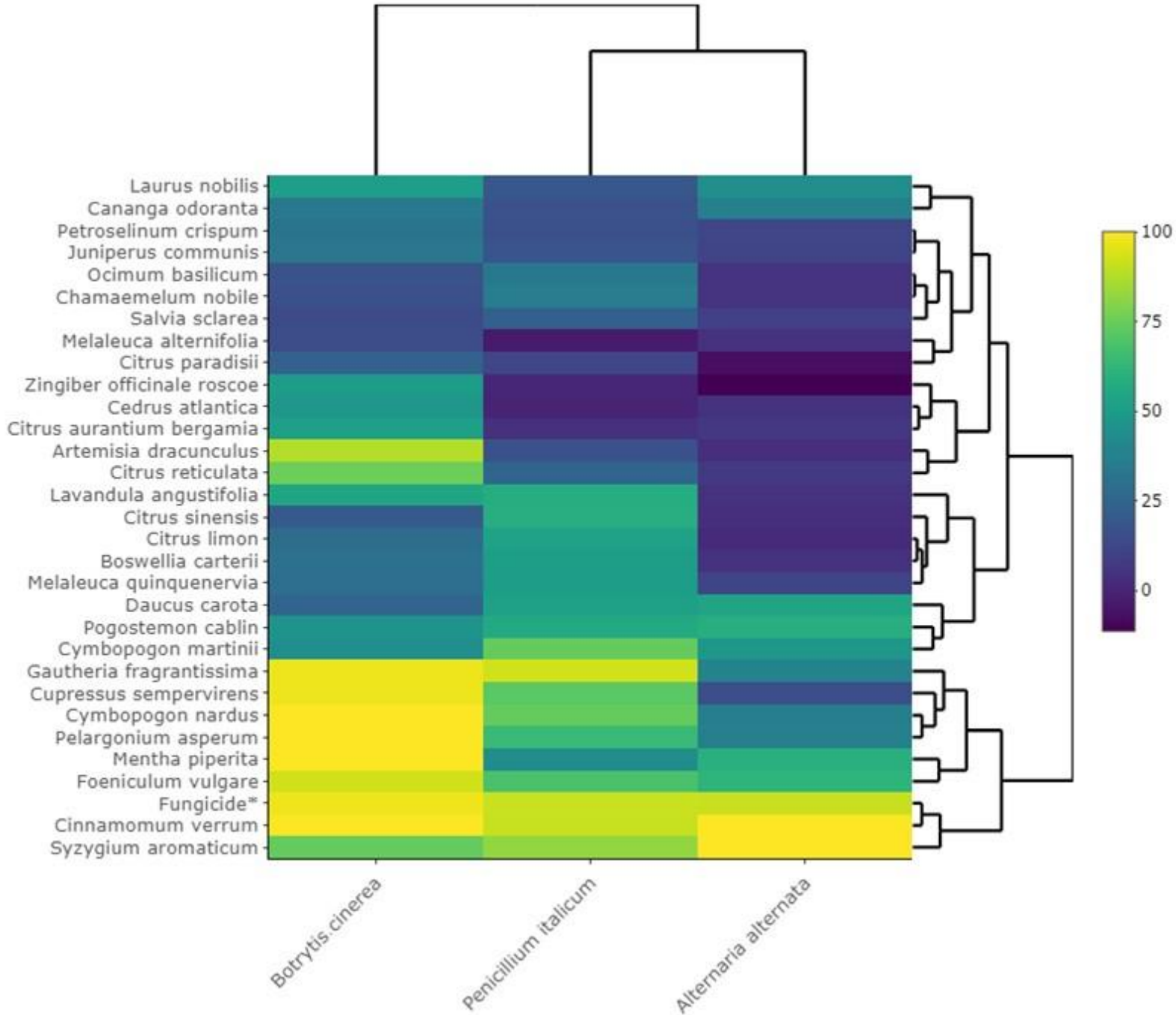


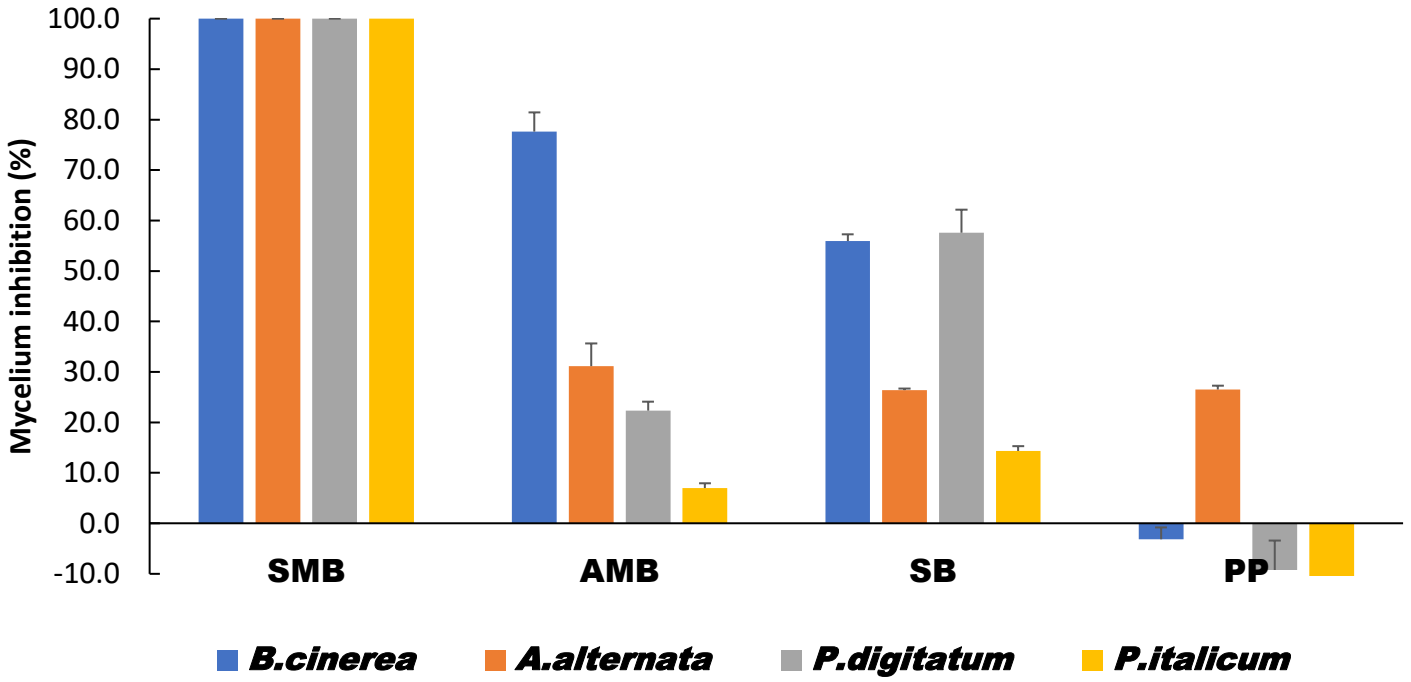
Figure 8. Minimum Inhibitory Concentration (MIC) of six essential oils against *A. alternata*, *B. cinerea*, and P. italicum. H2, *F. vulgare*; H13, *S. aromaticum*; H15, *G. fragrantissima*; H23, *C. nardus*; H24, *P. asperum*; H28, *C. verrum*.


Laurus nobilis
Cananga odoranta
Petroselinum crispum
Juniperus communis
Ocimum basilicum
Chamaemelum nobile
Salvia sclarea
Melaleuca alternifolia
Citrus paradisii
Zingiber officinale roscoe
Cedrus atlantica
Citrus aurantium bergamia
Artemisia dracunculus
Citrus reticulata
Lavandula angustifolia
Citrus sinensis
Citrus limon
Boswellia carterii
Melaleuca quinquenervia
Daucus carota
Pogostemon cablin
Cymbopogon martinii
Gautheria fragrantissima
Cupressus sempervirens
Cymbopogon nardus
Pelargonium asperum
Mentha piperita
Foeniculum vulgare
Fungicide*
Cinnamomum verrum
Syzygium aromaticum
Botrytis cinerea
Penicillium italicum
Alternaria alternata
100
75
50
25
0

Biological control of fruit decay

- Essential oils
- GRAS salts
- Compound mixture

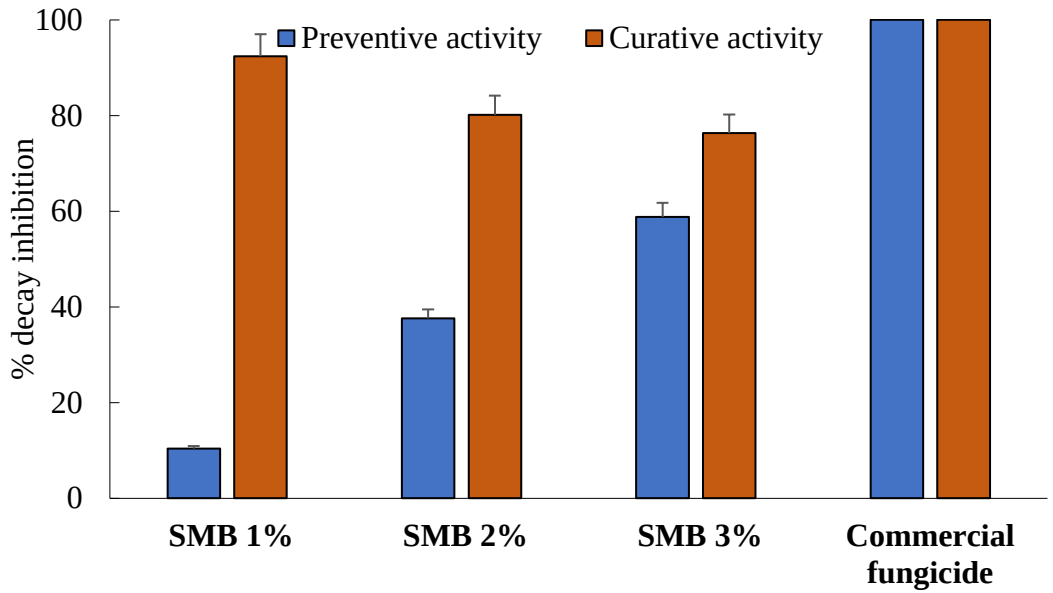
GRAS salts activity in vitro :
Sodium Metabisulfite (SMB), Ammonium Bicarbonate (AMB), Sodium Bicarbonate (SB), Potassium Phosphate (PP)



Inhibition of mycelial growth (%) of studied pathogens on PDA amended with GRAS salts at **0.2 %** and incubated at 20 °C.

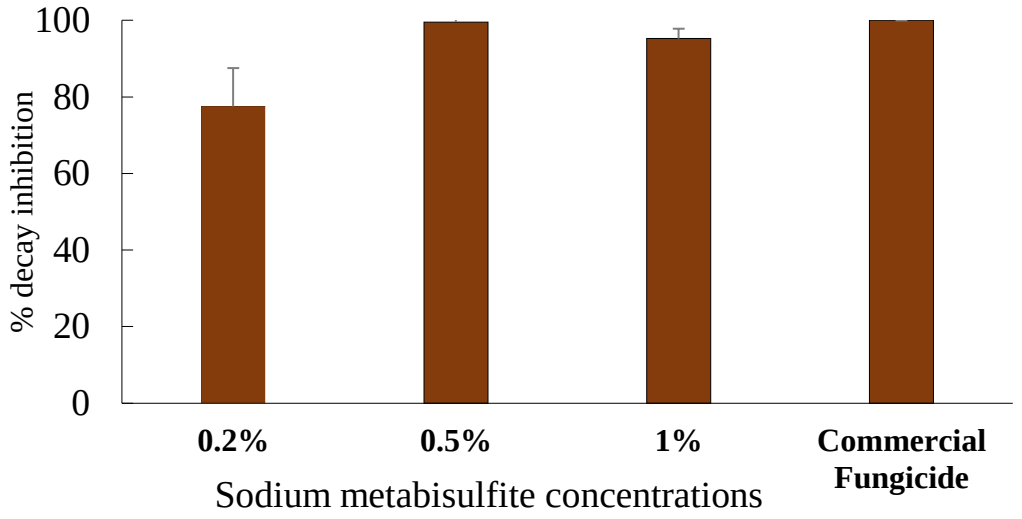
Significance P≤0.05: GRAS salt (*) Pathogen (*) Interaction (*)

PREVENTIVE AND CURATIVE ACTIVITY OF SODIUM METABISULFITE GRAS SALT *IN VIVO*



Decay inhibition in apple fruit var 'Golden' inoculated with Botrytis cinerea and dipped in different concentrations with sodium metabisulfite preventively and curatively and incubated at room temperature for 7 d.

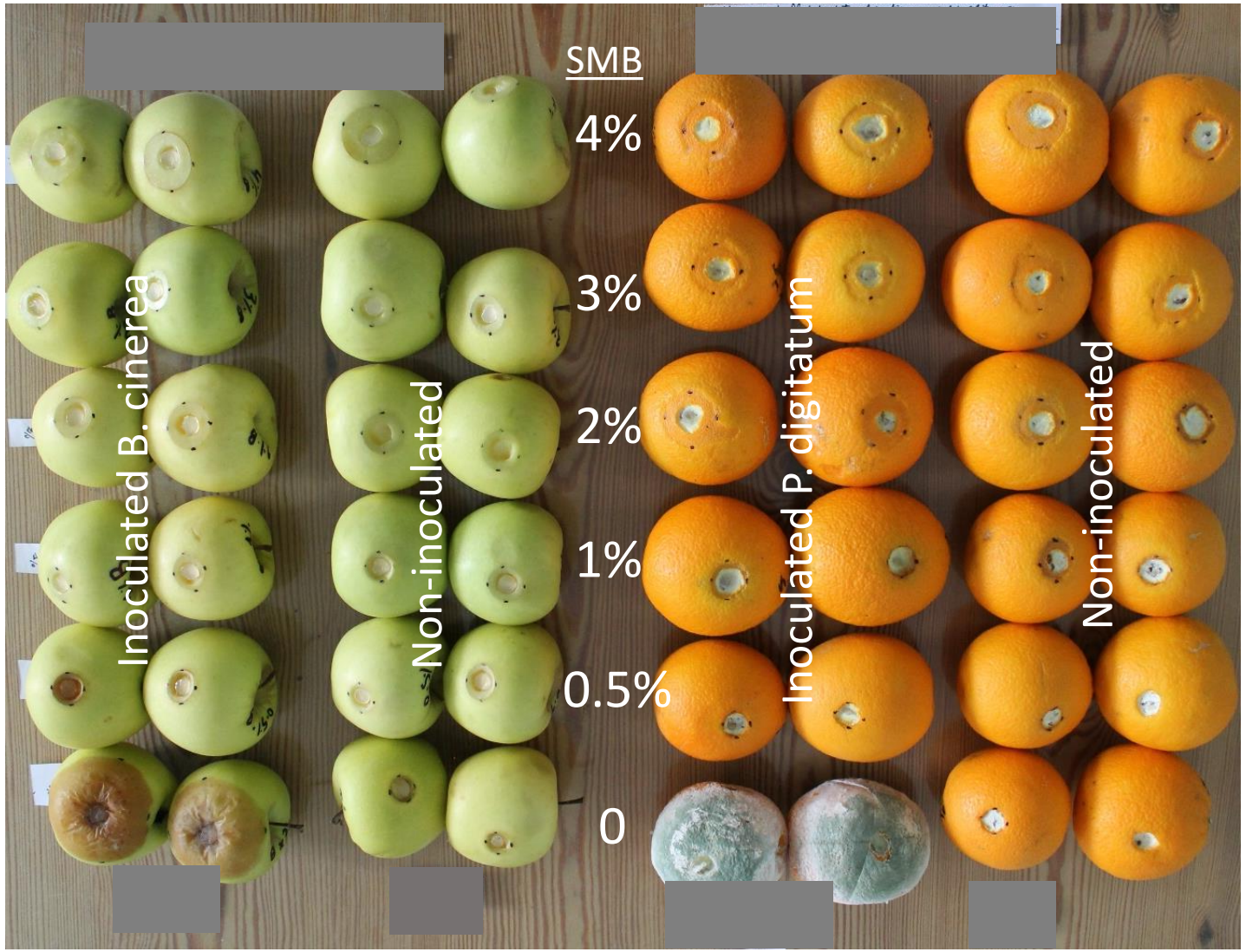
Significance P ≤ 0.05: Treatment (*) Concentration (*) Interaction (*)

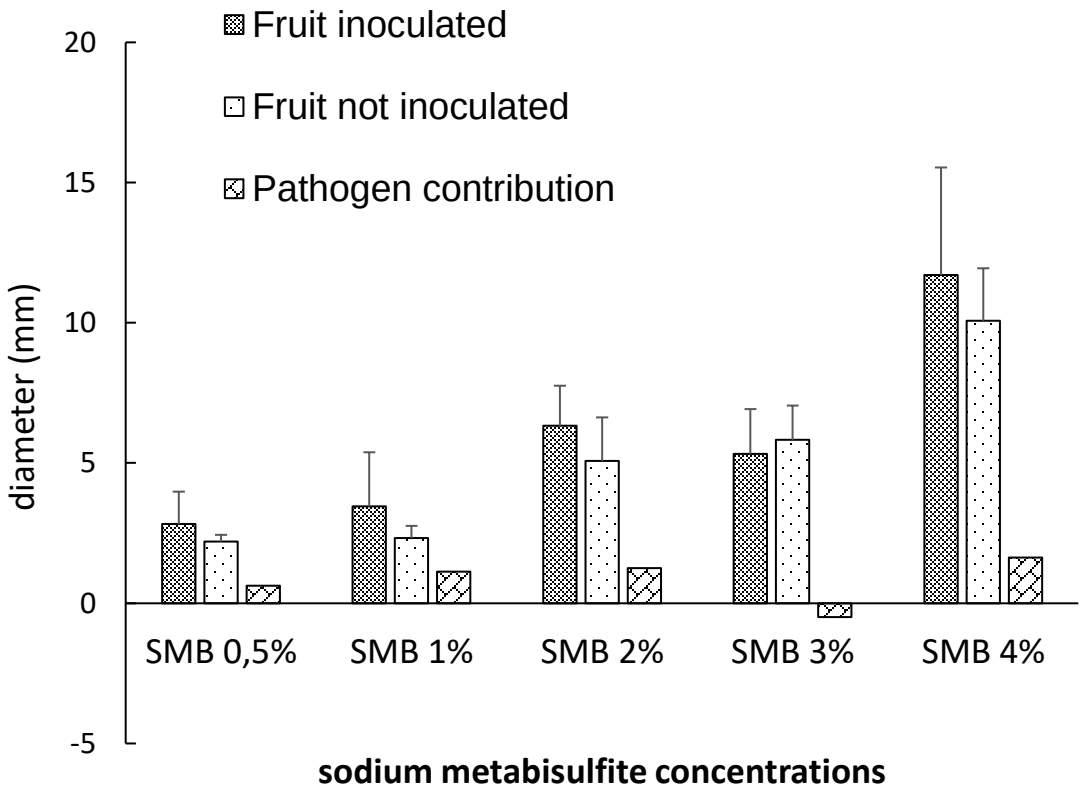


Decay inhibition in apple fruit var 'Golden' inoculated with B. cinerea and dipped with sodium metabisulfite curatively and incubated at room temperature for 7 d.

0.5% SMB the best concentration *in vivo*

PHYTOTOXICITY OF SODIUM METABISULFITE GRAS SALT (0, 0.5, 1, 2, 3 and 4%)
Orange /P. digitatum and Apple/ B. cinerea





Biological control of fruit decay

- **Essential oils**
- **GRAS salts**
- **Compound mixture**

Orange (maltaise)/*P. digitatum*
5 dpi

Apple/*B. cinerea*
8 dpi

Conclusion

- ***Penicillium digitatum*** **is the most damaging post-harvest pathogen, mainly on citrus fruits,** ***Botrytis cinerea*** **is a serious pathogen on a wide range of fruit types, followed by** ***Penicillium italicum*** **and** ***Alternaria alternata*****.**
- **After prolonged cold storage and during shelf life, all these fungal species are capable of causing considerable damage.**
- **Wounds on fruit are the main factor in the spread of fungal rot. Fruit with wounds should be sorted before cold storage to prevent further fungal attack.**
- **Certain essential oils such as cinnamon bark and clove flower bud were effective in reducing the mycelial growth of these fungi by 98.5% and 92.2% respectively at 500 ppm, showing a high effectiveness in vitro.**
- **The GRAS salts, in particular sodium metabisulphite, was effective in vitro and on fruit treated curatively at low dose (0.5%). Higher concentrations of this salt were phytotoxic.**
- **We suggest the use of innovative methods such as nanoencapsulation for the practical formulation of these environmentally-friendly compounds, helping to reduce the need for chemical fungicides.**

PRIMA

RÉPUBLIQUE TUNISIENNE
MESRS
Ministère de l'Enseignement Supérieur et de la Recherche Scientifique

STOP MED WASTE

Meeting

INNOVATIVE SUSTAINABLE STRATEGIES TO CONTROL POSTHARVEST DECAY OF FRESH FRUIT AND VEGETABLES AND REDUCE FRUIT LOSS AND WASTE

Thursday, 15 June 2023

Espace de l'innovation (amphithéâtre), INRAT, TUNIS

08.30 REGISTRATION

09.00 WELCOME ADDRESS

Mondher Ben Salem, Director of INRAT
Mourad Bellassoued, Director of DGRS
Chedly Abdelli, Director of ANPR
Hichem Ben Salem, Director of IRESA

TALKS

09:20	Presentation of the PRIMA StopMedWaste project - Gianfranco Romanazzi, *UNIVPM, Italy*
09:40	Progress of StopMedWaste Project at INRAT - Mohamed Bechir Allagui, *INRAT, Tunisia*
10:00	Use of chitosan, essential oils, other natural compounds and ozone for the management of postharvest decay of fresh peaches - Gianfranco Romanazzi, Marwa Moumni, *UNIVPM, Italy*
10:20	Novel antifungal edible coatings combined with modified atmosphere packaging to reduce pomegranate postharvest losses - Lluis Palou, *IVIA, Spain*
10:40	COFFEE BREAK
11:00	Innovative strategies for controlling postharvest diseases of pomegranates - Annamaria Mincuzzi, Antonio Ippolito, *UNIBA, Italy*
11:20	Efficacy of biological compounds to preserve fruit freshness during cold storage and shelf life - Mouna Ben Amara, Mohamed Bechir Allagui, *INRAT, Tunisia*
11:40	Effect of postharvest UV-C applications on postharvest decays on strawberry fruits - Pervin Kinay, *UE, Turkey*
12:00	Decco innovative solutions for postharvest industry - Julio Marin, Citrus Commercial Manager for North Africa, *DECCO IBERICA, Spain*
12:20	Contribution of Tunisian company managers about fruit packaging and the use of pesticide: Hichem Aoun Allah (Bioprotection, pesticide company); Hassen Ghidhaoui (Fertiplant, pesticide Company); Tarek Tira (GIFruit); Nabil Ben Meftah (SODEA, packed fruit company); Aymen Arfaoui (Select fruits, packed fruit company); Hichem Kalech (Mabrouka, packed fruit company)
13:20	GENERAL DISCUSSION
14:00	LUNCH
15:00	On-site demonstration by commercial companies of packed fruit and recommended antifungal products for the management of postharvest diseases of fresh fruit and vegetables

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Cyprus University of Technology
UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO
Institut National de la Recherche Agronomique de Tunisie
DECCO Naturally Postharvest
ivia Institut Valencià d'Investigacions Agràries
UNIVERSITY OF EGE, IZMIR (UE), TURKEY


ESPACE INNOVATION
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STOP
MED WASTE

Effect of CMC-Beeswax composite edible coating amended with antifungal agents on physicochemical proprieties of nectarine fruits during cold storage

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[1]Marche Polytechnic University, Agricultural, Food and Env Sciences Dept., Via Brecce Bianche, 60131 Ancona, Italy
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Abstract

Edible coatings maintain fruit and vegetables integrity as well as shelf life against bruising, tissue damage and, in general, physical injury caused by pressure, vibrations, and other mechanical factors. Researches on postharvest coating are steadily increasing towards new coating formulations based on biopolymers such as polysaccharides and proteins. These edible coatings should be safe to meet consumer's interest in health and nutrition. Carboxymethylcellulose (CMC) (0.5%), beeswax (0.2%), sodium bicarbonate (0.5%) and potassium sorbate (1%) were formulated as edible coating for postharvest fruits of Snow Queen' nectarine (*Prunus persica* var. nucipersica). These coated fruits inoculated with *Alternaria alternata* were stored at 10 °C during one month before assessing physiochemical and sensory criteria. Results showed that the edible coating reduced significantly lesions produced by the fungus delaying changes in color, firmness and minimized weight loss of nectarine fruits. The results demonstrated the potential of selected edible coatings containing salt GRAS (Generally Recognized As Safe) to extend postharvest life of fresh nectarine fruits, although further studies should focus on improving some properties of the coatings to enhance gas barrier and storability.

Matrials and method

Biological matrial

➢ **Fruit**: Snow Queen nectarine (*Prunus persica* var. *nucipersica*).

➢ **Fungal specie : *Alternaria alternata***

Methods of edible coating formulations

- **Gras salts used**

Sodium bicarbonate (0.5%) and potassium sorbate (1%)

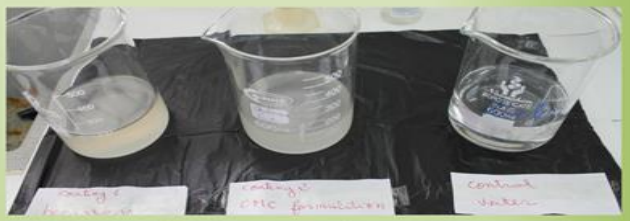
- **Coating application**

Carboxymethylcellulose (CMC) (0.5%), beeswax (0.2%),

✓Coated fruits were inoculated with ***Alternaria alternata*** and stored at 10 °C during one month before evaluating physiochemical and sensory fruit quality

Resultats

❑the potential of selected edible coatings containing GRAS salt could extended the shelf life of post harvested fresh nectarine fruits,



Conclusion

- ❑ The use of GRAS Salts in edible coatings and CMC films together with BeesWAx have shown positive effect in stopping waste decay of post-harvested Fruit,
- ❑ Further studies should focus on improving some properties of the coatings to enhance gas barrier and storability

Fundings:

This work was conducted within the framework of the PRIMA StopMedWaste project, which is funded by PRIMA, a programme supported by the European Union

Acknowlegments

Special Thanks to **Dr.Khadija Ouerghi** for her help and guidance in statistical analysis of data of project and development presentation

Limassol (Cyprus)
June 2022

Category: 04.09 - POST-HARVEST - Part 2: Sustainable managements of postharvest diseases: new technologies and approaches

EVALUATION OF CHITOSAN ALONE OR MIXED WITH SODIUM METABISULFITE IN CONTROLLING POSTHARVEST FRUIT DECAY

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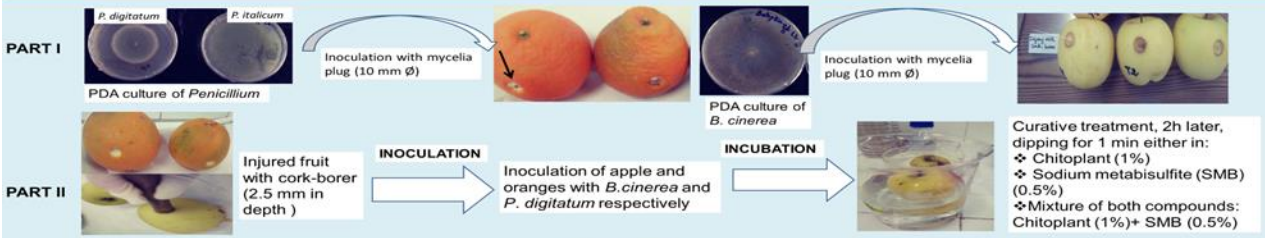
INTRODUCTION

Fruit decay during postharvest storage is a critical point that have required efficient biological treatments to prevent deterioration of fresh fruit quality and to reduce waste. Chitosan, a deacetylated derivate of chitin, and GRAS salt are recognized as antifungal compound in preventing decay.. In this study, fruit of apple *var. 'Golden'* and of citrus *vars. 'Maltaise', 'Thompson' and 'Clementine' were* inoculated separately with *Botrytis cinerea*, *Penicillium italicum* and *Penicillium digitatum* to test their pathogenicity, Then, the efficacy of chitosan and GRAS salts sodium metabisulfite (SMB) alone or in mix were evaluated for their ability to reduce decay incidence on apple and orange fruit wounded and inoculated with *Botrytis cinerea* and *Penicillium digitatum* respectively.

MATERIAL AND METHODS



RESULTS

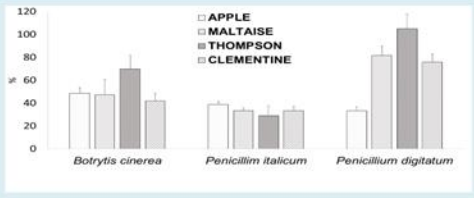


Fig 1. Decay incidence (%) of main fungal species on apple and citrus fruit

Fig 2. Inoculation of citrus species by different fungal species (6dpi)

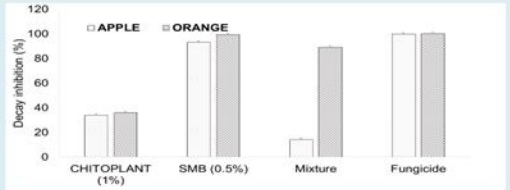


Fig 3. Decay inhibition (%) in apple var 'Golden' (8dpi) and orange var 'Maltaise' (5dpi) inoculated with *B.cinerea* and *P.digitatum* respectively, dipped in different solutions and incubated at 17°C

CONCLUSION

- Orange var 'Thompson' and, to a lesser extent, var 'Maltaise' were the most sensitive to rots of *Penicillium digitatum* and *Botrytis cinerea*.
- Apple fruit were the least susceptible mainly *to Penicillium digitatum*.
- The least non significant pathogen was *Penicillium italicum* whatever the fruit used.
- Chitosan was slightly effective in comparison to sodium metabisulfite in decreasing fruit rot percentage.
- Mixture of both compounds showed a depressive effect compared to each single component in the case of apple.
- On orange, this mixture was more effective compared to apple since the inhibtion increased from 14.3% (for apple fruit) to 89.2% (for orange fruit).

ACKNOWLEDGEMENT

This work was conducted within the framework of the PRIMA StopMedWaste project, which is funded by PRIMA, a programme supported by the European Union.

Efficacy of the GRAS salt sodium metabisulfite to control curatively postharvest fruit decay

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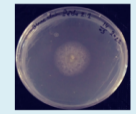
INTRODUCTION

Injuries on fruit during harvest, postharvest handling, and commercialization are the primary cause of fungal infections. Control of postharvest fruit decay is based on synthetic fungicides. However, Organic and inorganic salts, generally recognized as safe (GRAS) ingredients, are reported powerful antifungal compounds and postharvest fruit preservatives. This study evaluate the effectiveness of the GRAS salts, sodium metabisulfite (SMB), ammonium bicarbonate (AMB), sodium bicarbonate (SB) and potassium dihydrogen orthophosphate (PDO) firstly *in vitro* against the main fungal species of postharvest fruit decay, *Alternaria alternata*, *Botrytis cinerea*, *Penicillium italicum* and *Penicillium digitatum*. *In vivo* experiments were runned against *B. cinerea* on apple fruit treated with SMB.

MATERIAL AND METHODS

In vitro



PDA media amended with each GRAS salt (SMB, AMB, SB and PDO) at 0.2 %,

24h later, isolates were placed in the media in Petri dishes and incubated at 20 ± 2 °C in obscurity during several days (until control fills the medium) before measuring le diameter of the resulted colony.

In vivo

Injured apple fruit with cork-borer (Ø10mm x 2.5 mm deep)

Inoculation with mycelia plug (Ø=10 mm) of *B.cinerea* placed on the produced wound

2h later, inoculated fruit were dipped in SMB solutions (0.5 %, 1%, 2%, 3% and 4%) for 1 min, air dried in aseptic conditions, then incubated at 20 ± 2 °C in obscurity.

RESULTS

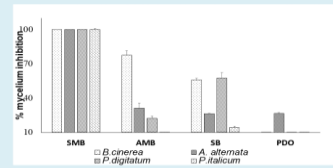


Fig 1. Inhibition of mycelial growth (%) of *B. cinerea*, *A. alternata P. digitatum* and *P. italicum* on PDA amended with one of the four GRAS salts at 0.2 % and incubated at 20 °C

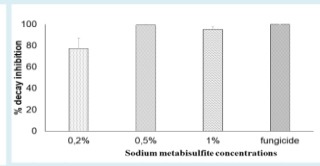


Fig 2. Decay inhibition (%) in apple fruit var 'Golden' inoculated with *Botrytis cinerea* and dipped in different concentrations with sodium metabisulfite curatively and incubated at room temperature for 7 d.

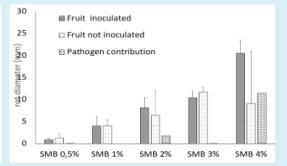


Fig 3a. Phytotoxicity of sodium metabisulfite with increased concentrations on apple var 'Golden' inoculated or not with *Botrytis cinerea*

Fig 3b. Phytotoxicity of sodium metabisulfite on apple var 'Golden' inoculated or not with *Botrytis cinerea*

CONCLUSION

In vitro
- SMB at 0.2% inhibited completely mycelium growth of the tested fungal species.
- Ammonium bicarbonate and sodium bicarbonate were less efficient.

In vivo (inoculated fruit)
- SMB was highly efficient at 0.5 % and 1 % in curative treatment since the decay was entirely blocked at these concentrations.
- SMB starts to induce visible phytotoxicity on fruit from a concentration of 1% onward by softening the compactness of fruit skin tissues (the phytotoxicity is like a necrosis area around the produced wound also in control fruit (treated with SMB but not inoculated).
- The appropriate concentration of SMB retained for postharvest treatment is 0.5% in curative treatment.
- Further experiments in semi-commercial trials should be conducted during cold storage.
- To highlight its effectiveness, commercial formulations including SMB may improve its efficacy and reduce phytotoxicity reaction.

ACKNOWLEDGEMENT

This work was conducted within the framework of the PRIMA StopMedWaste project, which is funded by PRIMA, a programme supported by the European Union.

EFFICACY OF PYTHIUM OLIGANDRUM AND SODIUM METABISULPHITE IN CONTROLLING POSTHARVEST FUNGAL DISEASES OF ORANGES, TOMATOES AND NECTARINES

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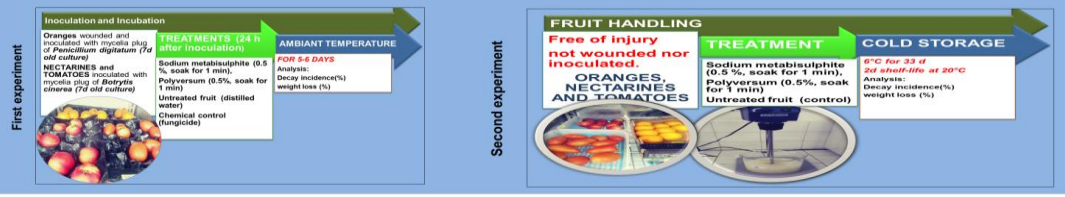
INTRODUCTION

Fresh fruits are vulnerable to pathogens such as *Penicillium digitatum* and *Botrytis cinerea*, which can cause significant losses during cold storage. Less toxic natural compounds are currently being investigated to reduce the severity of these infections on fresh fruit after harvesting.
We conducted two experiments on two compounds, the first compound is a commercial fungicide (Polyversum®) formulated from a biological agent, *Pythium oligandrum*, and the second compound is a GRAS salt, sodium metabisulphite (SMB). The aim of this study is to assess the effectiveness of these compounds in reducing rot on fruit, whether inoculated and treated then stored at room temperature (20 °C) for 5-6 days for the first experiment. For the second experiment, fruit were treated and stored at 6 °C for 33 days and further 2 days of shelf life at room temperature.

METHODOLOGY



RESULTS

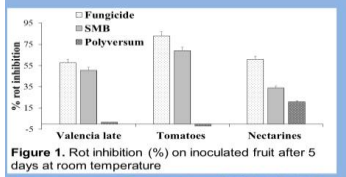


Figure 1. Rot inhibition (%) on inoculated fruit after 5 days at room temperature

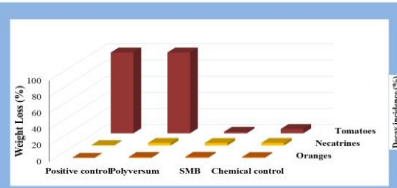


Figure 2. Weight loss (%) of stored fruit after 30 days at 6°C and 2 days shelflife at room Temperature

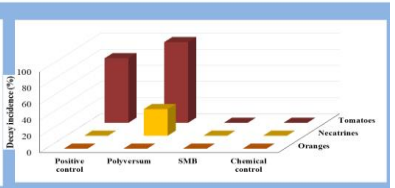


Figure 3. Decay incidence (%) of stored fruit after 30 days at 6 °C and 2 days shelflife at room Temperature

CONCLUSION

- SMB proved its efficacy on rot inhibition by 50% on oranges, 69 % on tomatoes and 34% on nectarines.
- Polyversum was less effective, 20% rot inhibition only on nectarines.
- No rot was detected on un-inoculated fruit and treated with SMB assessed after shelf life. However, Polyversum recorded the highest rate, mainly with tomatoes (100 %) and nectarines (33%).
- Tomatoes treated with SMB showed the lowest weight loss of 1.9 %.
- Results confirmed the effectiveness of SMB against fruit rot. Polyversum, needs further tests to optimize its concentration and method of treating the fruit.

ACKNOWLEDGEMENT

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ANALYSIS OF THE QUALITY OF APPLES AND ORANGES TREATED WITH SODIUM METABISUPHITE DURING COLD STORAGE

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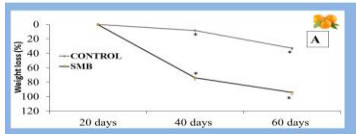
INTRODUCTION

The quality of fresh fruit after cold storage is of paramount importance to consumers and retailers. The quality may be altered by postharvest pathogens that deteriorate the fruit and reduce shelflife.
During cold storage, natural compounds, including GRAS salts, are gaining of interest as alternative methods for treating fruit and maintaining their freshness and quality after harvesting. The aim of the present study is to assess the physicochemical quality including decay incidence, weight loss, pH, TSS and bioactive compounds (Total phenols and flavonoids) of apple and orange fruit treated with sodium metabisulphite and stored for up 60 days at 6 °C. Fruit were assessed after 20, 40 and 60 d.

METHODOLOGY

RESULTS



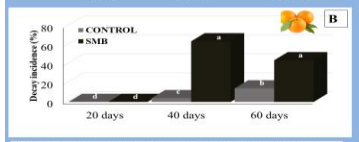


Figure 1. Weight loss (A) and decay incidence (B) of oranges during storage at 6 °C

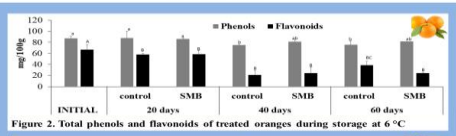


Figure 2. Total phenols and flavonoids of treated oranges during storage at 6 °C

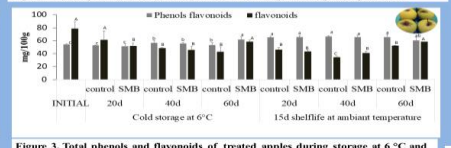


Figure 3. Total phenols and flavonoids of treated apples during storage at 6 °C and after shelflife

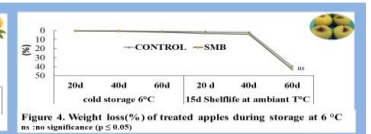


Figure 4. Weight loss(%) of treated apples during storage at 6 °C
ns :no significance ($p \leq 0.05$)

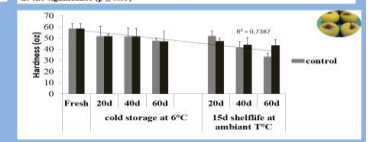


Figure 5. Hardness of treated apples during storage at 6 °C

CONCLUSION

Oranges stored at 6 °C for up to 20 days without significant fungal spoilage or impairment of physico-chemical quality. Storage could be extended to 40 days at 6 °C with low risk of *Penicillium digitatum* growth and decrease of flavonoids.
Apples could be stored for up to 60 days without no incidence of rot, while decrease of flavonoids and increase of phenols were registered after shelflife, this result could be linked to progress of ripening during long storage.
After shelflife, weight loss and loss of hardness of apples were registered from 40 days onwards.

ACKNOWLEDGEMENT

This work was conducted within the framework of the PRIMA StopMedWaste project, which is funded by PRIMA, a programme supported by the European Union.

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Thank you very much