Experience of the Tunisian partner in the PRIMA 'STOP MED WASTE' project

Mohamed Bechir Allagui, Mouna Ben Amara

INRAT- Tunisia

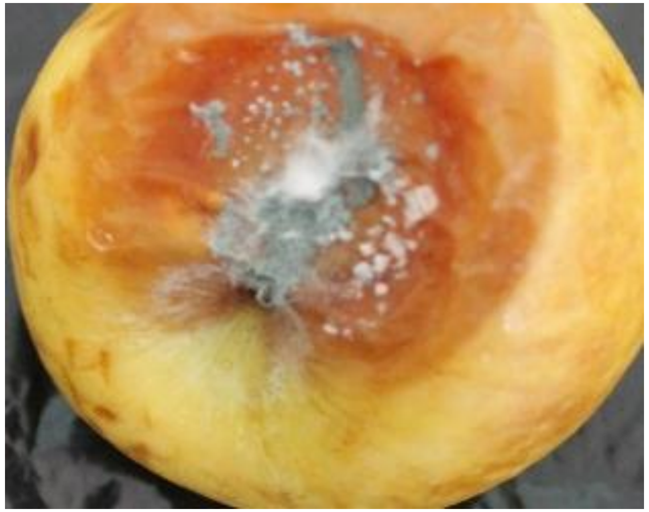
Cordoba, March 4-5, 2025

STOP MED WASTE PROJECT

- 8 Research Units : Extend the shelf life of fresh fruit, vegetables and aromatic plants
- 3 Specific objectives: Reduce
  - Food waste from 30% to 15%
  - Discarded fruit by 20%
  - Application of postharvest pesticides by 20%

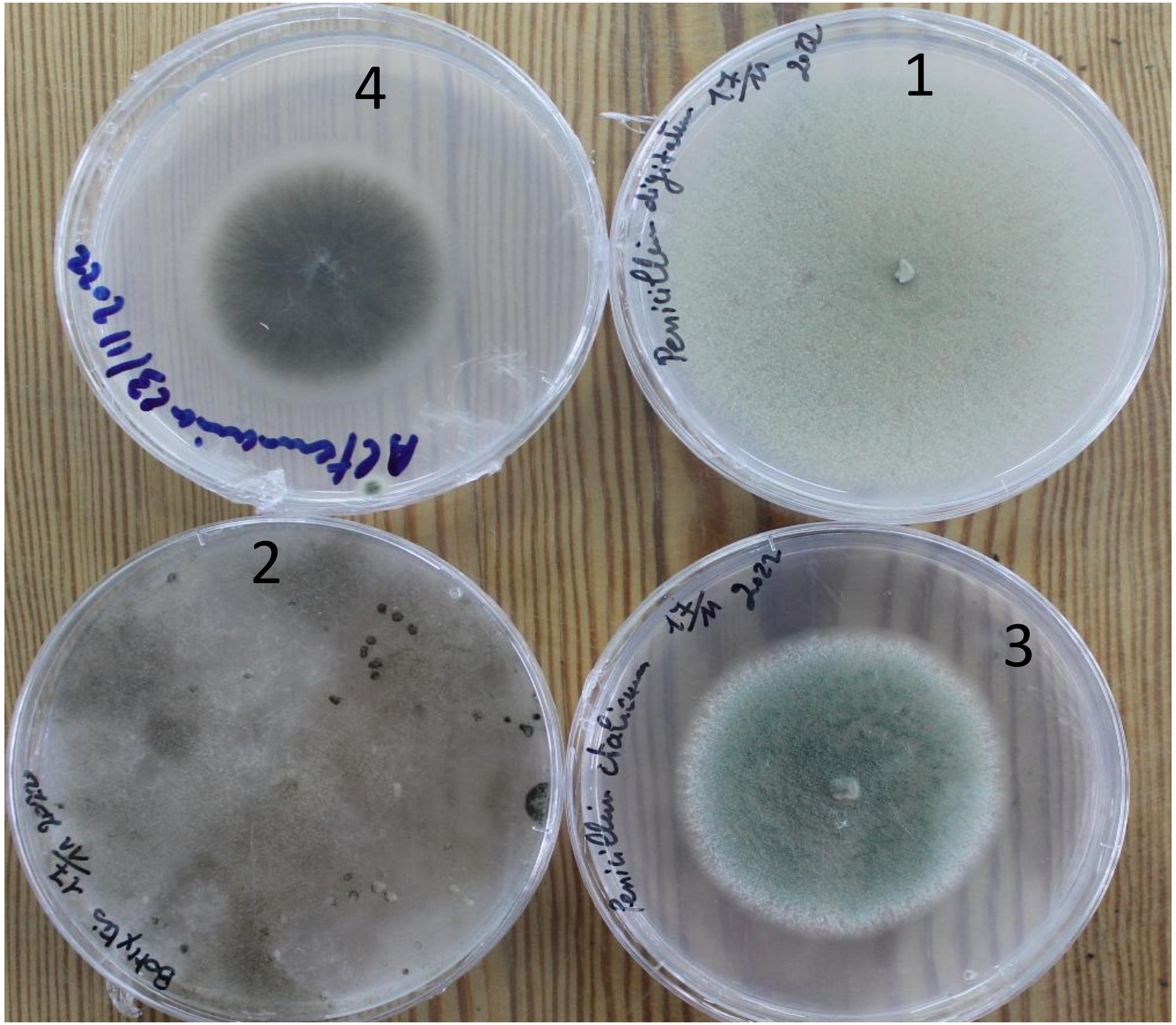
The question is: are we in line with these goals?

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)

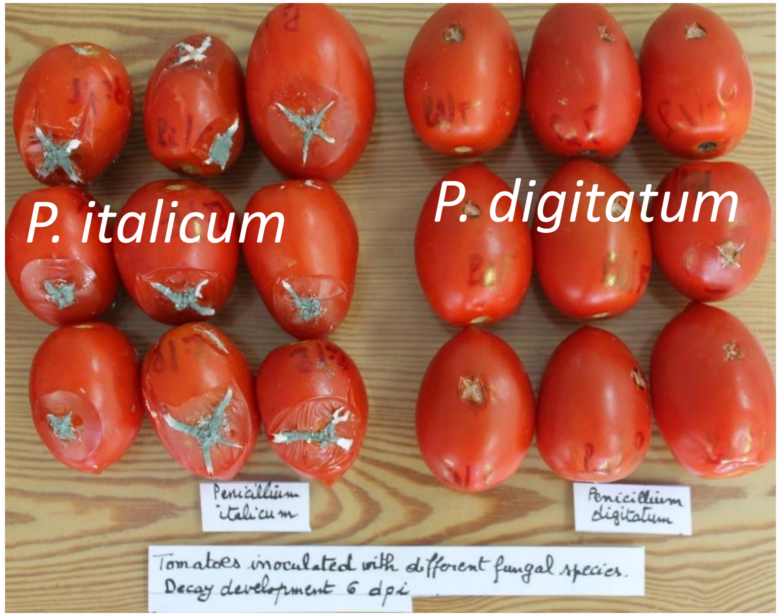
4. *Alternaria alternata* (black rot)

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)

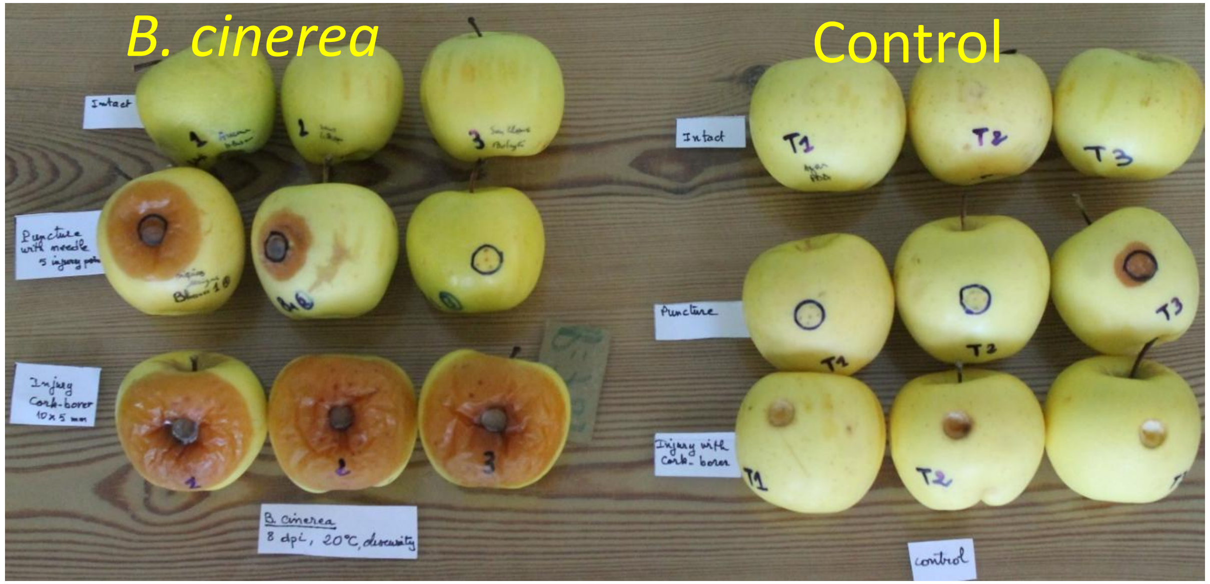
Diameter of the decay (mm) 8 dpi (20°C)

	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

intact

Small puntures

Wide wound



intact

Small puntures

Wide wound

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

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 *Gautheria 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

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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,

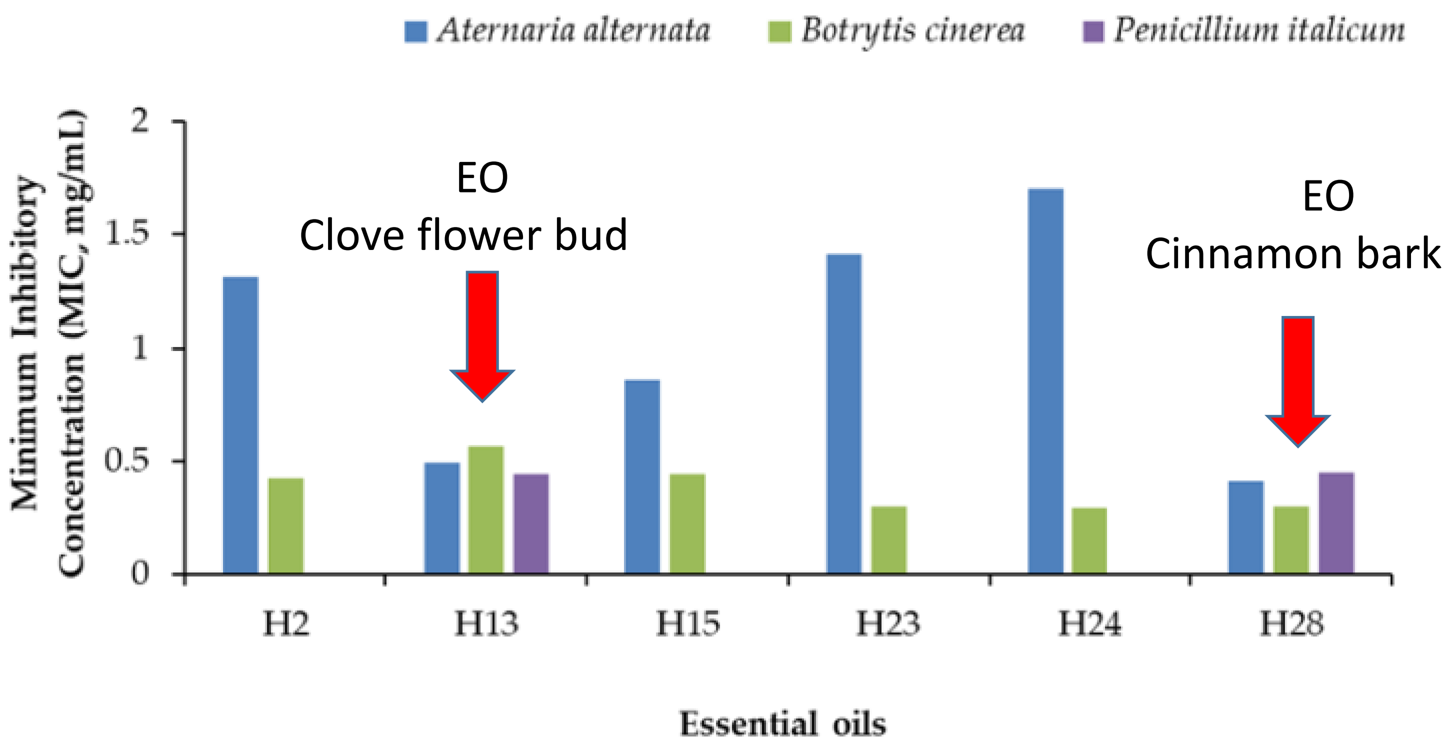

Figure 8. Minimum Inhibitory Concentration (MIC) of six essential oils against *A. alternata*, *B. cinerea*, and P. italicum. H2, *F. vulgare*; H13, *S. aromaticum*; H15, *G. fragrantissima*; H23, *C. nardus*; H24, *P. asperum*; H28, *C. verrum*.

Biological control of fruit decay

- Essential oils
- GRAS salts
- Compound mixture

Journal of *Fungi*

MDPI

Article

Effectiveness of Several GRAS Salts against Fungal Rot of Fruit after Harvest and Assessment of the Phytotoxicity of Sodium Metabisufite in Treated Fruit

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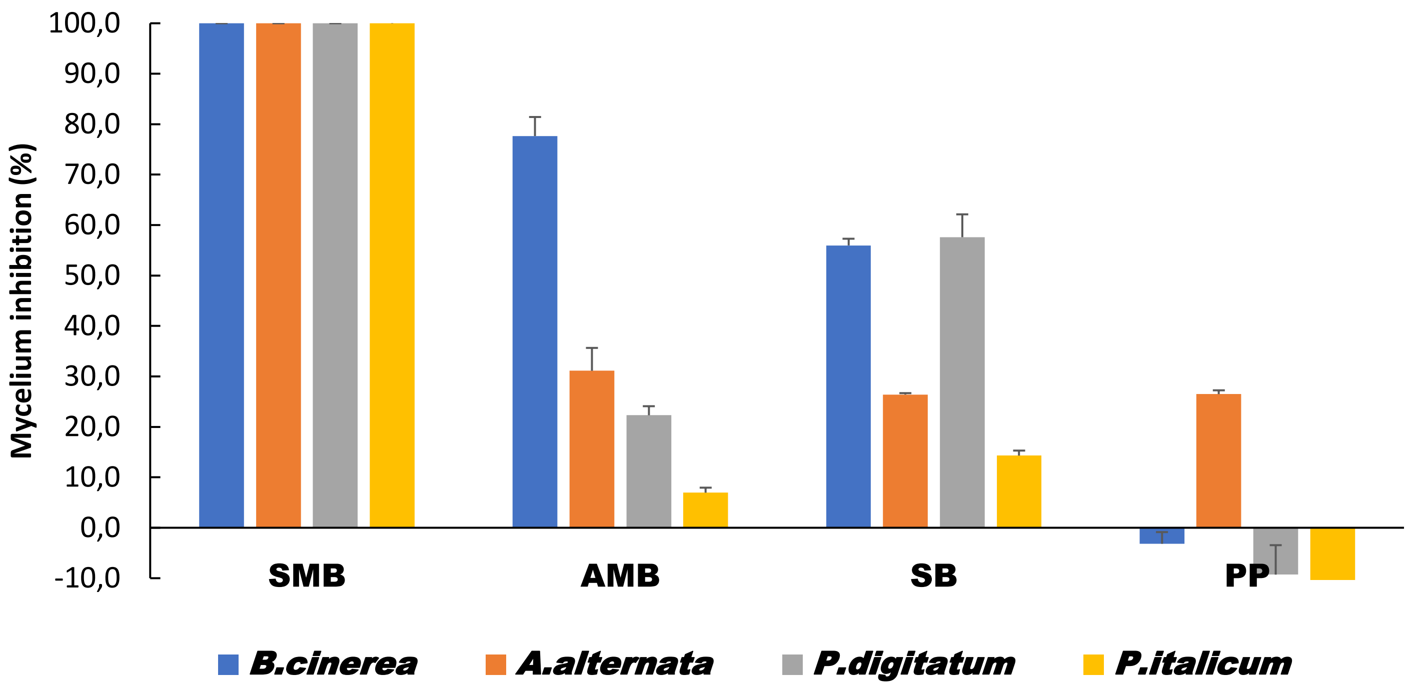
Abstract: This study evaluates the efficacy of the salts sodium metabisulfite (SMB), ammonium bicarbonate, sodium bicarbonate, and potassium dihydrogen orthophosphate first in vitro against the main postharvest fruit rot fungi, *Alternaria alternata*, *Botrytis cinerea*, *Penicillium italicum*, and *Penicillium digitatum*. Results showed that 0.2% SMB completely inhibited the mycelium growth of the fungal species. Ammonium bicarbonate and sodium bicarbonate were less effective at 0.2% in inhibiting mycelial growth, ranging from 57.6% to 77.6%. The least effective was potassium dihydrogen orthophosphate. Experiments were also performed in vivo on wounded apples inoculated with the most pathogenic fungus, *B. cinerea*, and treated with SMB at concentrations of 0.2, 0.5, 1, 2, and 3%, both preventively and curatively. Results based on the decay size showed that SMB, when used as a preventive treatment, had a reduced efficacy, even with the highest concentration. However, this salt proved to be very effective at 0.5% in curative treatment since the decay was completely blocked. Our results suggest that the appropriate concentration of SMB for post-harvest treatment is 0.5% as a curative treatment. On the other hand, the 1% dose induced the onset of phytotoxicity around the wound. To assess the extent of the phytotoxicity reaction, higher concentrations of 1–4% SMB were applied to wounded fruit. Apples and oranges were inoculated or not with *B. cinerea* and *P. digitatum*, respectively. Doses of 1–4% induced phytotoxicity in the form of a discolored ring surrounding the wound on the epidermis of the fruit; this phytotoxicity enlarged as the concentration of SMB increased. The phytotoxic features were similar on apples and oranges. The methodological procedure made it possible to carry out a quantitative assessment of SMB phytotoxicity. This method is proposed as an easy-to-use technique for quantitatively estimating the phytotoxicity of antifungal compounds on post-harvest fruit.

Keywords: control; decay; GRAS salts; postharvest; sodium metabisulfite

Citation: Allagui, M.B.; Ben Amara, M. Effectiveness of Several GRAS Salts against Fungal Rot of Fruit after Harvest and Assessment of the Phytotoxicity of Sodium Metabisufite in Treated Fruit. *J. Fungi* **2024**, *10*, 359. https://doi.org/10.3390/jof10050359

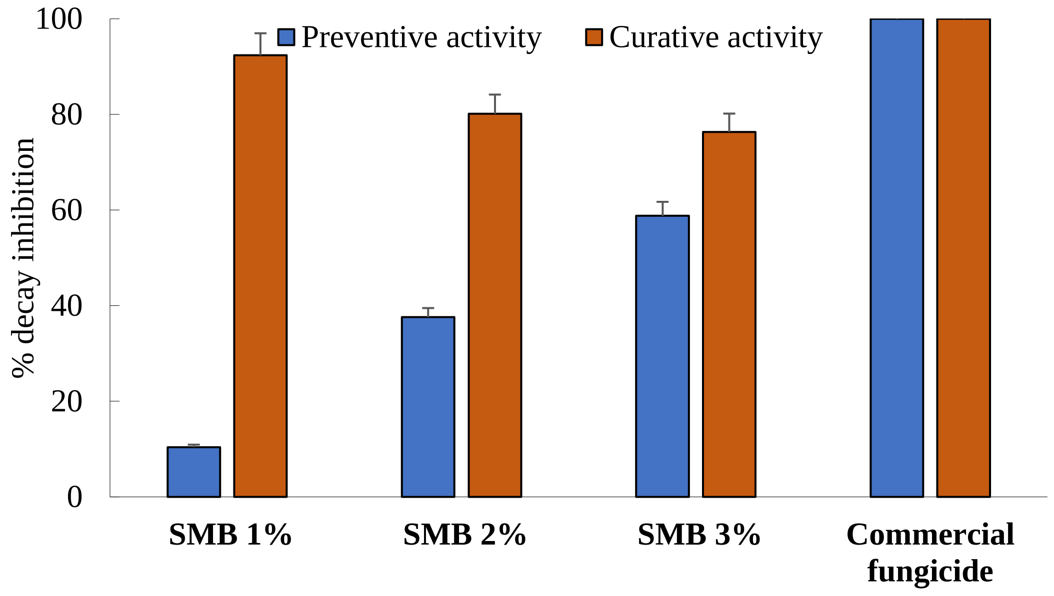
GRAS salts activity in vitro : Sodium Metabisulfite (SMB), Ammonium Bicarbonate (AMB), Sodium Bicarbonate (SB), Potassium Phosphate (PP)



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

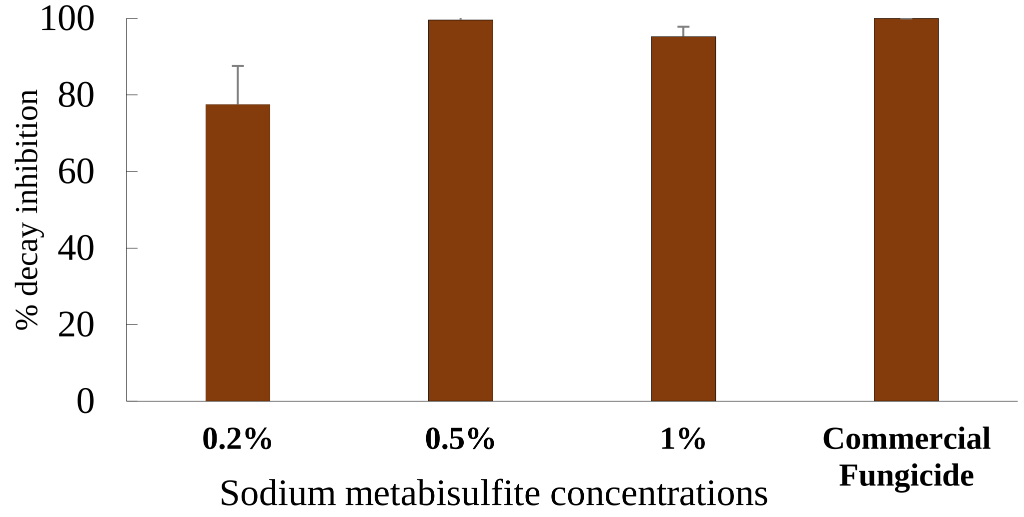
Significance P≤0.05: GRAS salt (*) Pathogen (*) Interaction (*)

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.

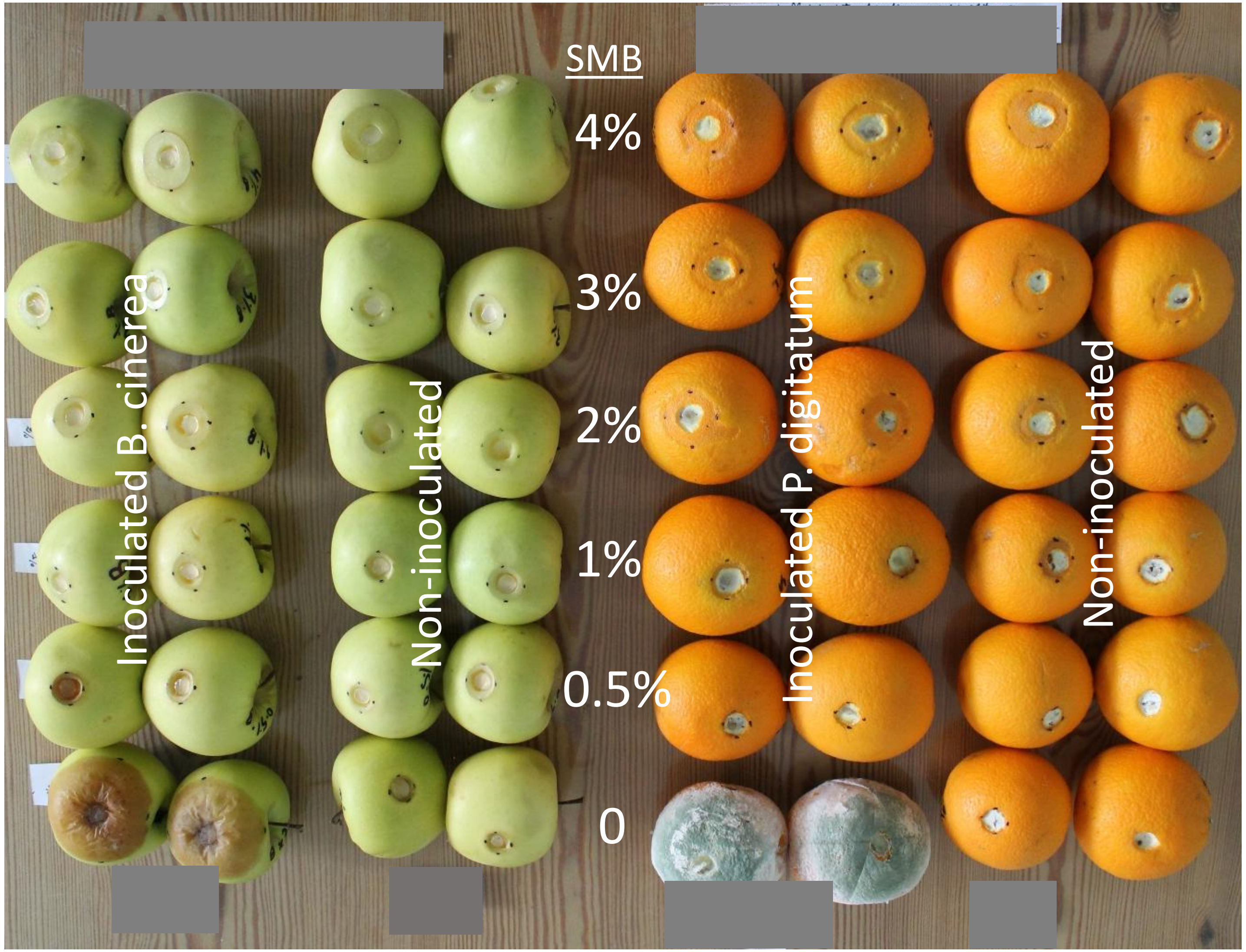
Significance P ≤ 0.05: Treatment (*) Concentration (*) Interaction (*)

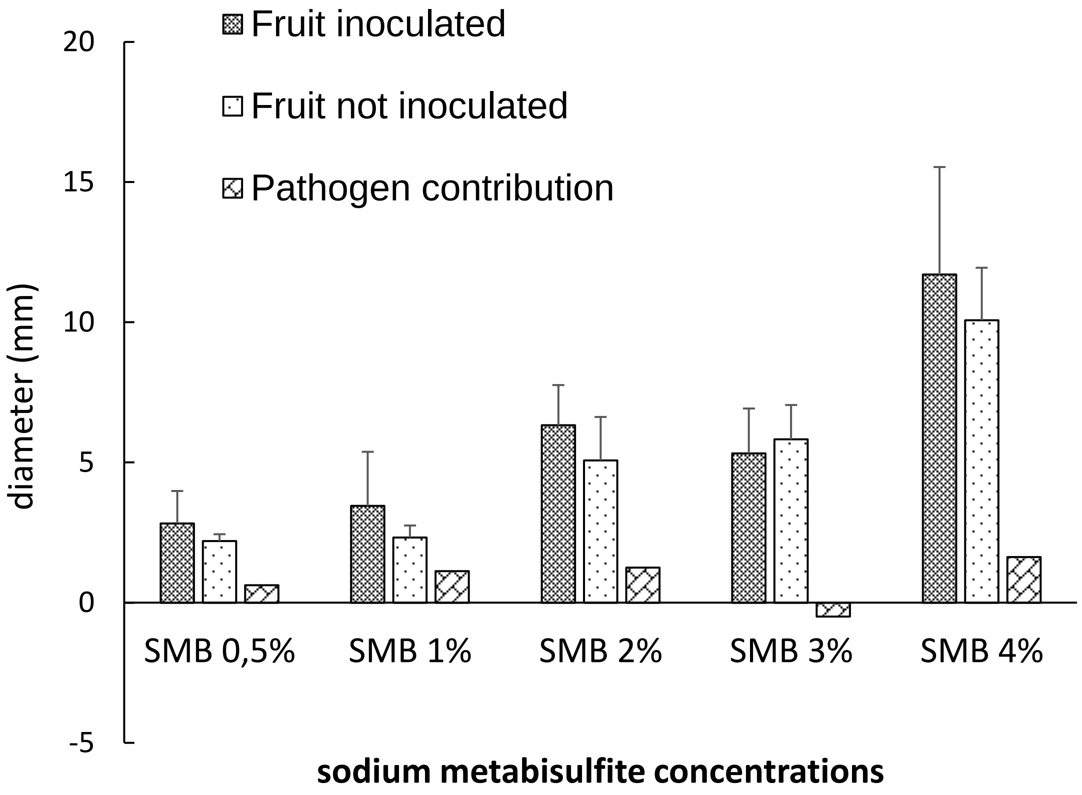


Decay inhibition in apple fruit var 'Golden' inoculated with B. cinerea and dipped with sodium metabisulfite curatively and incubated at room temperature for 7 d.

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





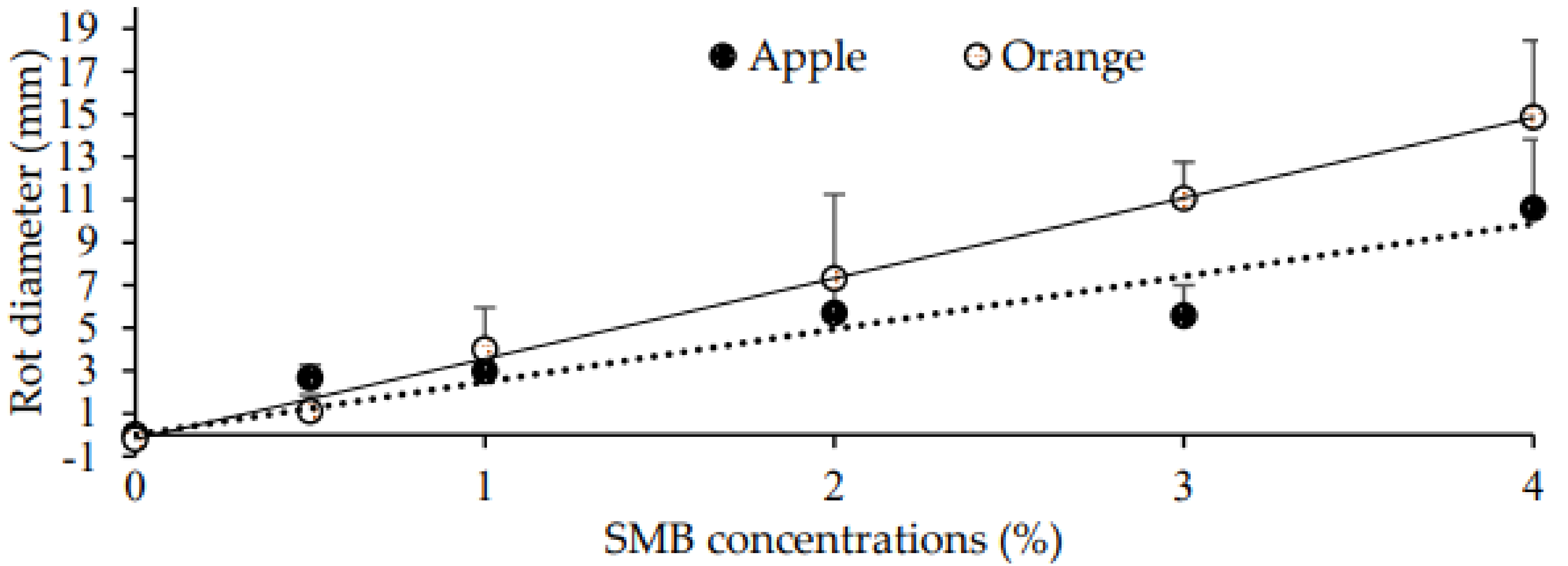


Figure 7. Linear regression between sodium metabisulfite concentration (x-axis) and rot diameter size (y-axis) was measured on oranges (6 dpi) and apples (8 dpi). Each point is the mean diameter of eight replicates (fruit). Vertical bars represent standard deviations.

Biological control of fruit decay

- Essential oils
- GRAS salts
- Compound mixture

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

Apple/*B. cinerea*
8 dpi

frontiers

Unravelling during cold storage and shelf life the physicochemical and pathological characteristics of postharvest apples and oranges treated with sodium metabisulfite

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Keywords: Postharvest, pathogen, severity, physicochemical, bioactive compounds

Abstract

Fruits are susceptible to a diverse range of postharvest rots that can reduce quality if preventive measures are not taken in time. In this study, samples of orange cv. 'Maltaise' and apple cvs. 'Golden Delicious' and 'Richared' were sorted without infection or injury, treated or not with sodium metabisulfite (SMB), then placed in cold storage for 20, 42 or 59-61 days, followed by a shelf life of 6 or 15 days. The physicochemical characteristics, degree of fruit infection and weight loss were analyzed at each storage period. Our results indicate that adequate postharvest storage depends on the type of fruit, the duration of cold storage and shelf life. The heat map grouped 'Richared' apples close to its fresh state, without developing rot or perceptible weight loss for 60 days at low temperature (6°C) and 15 days of shelf life. These red apples performed better during storage than the "Golden", especially in terms of storability and total flavonoids. Apples of 'Golden' showed better storage stability than 'Maltaise', which could be stored properly for up to 20 days at 6°C, followed by a 15-day shelf life, regardless of treatment with sodium metabisulfite. The longer the oranges were stored, the greater the risk of infection and the physicochemical properties, in this case flavonoids, decreased. The chemical criteria (TSS, pH) of apples and oranges were not affected by soaking in SMB, being similar to that of untreated fruit. However, treating such fruit with SMB is regarded as unlikely due to its low effectiveness in preventing fruit decay during long-term storage. Cluster analysis showed that total polyphenols were linked to poor storability, while flavonoids, hardness and TSS were clustered with better storability. This suggests that flavonoids may be a more reliable indicator of storage suitability than total polyphenols.

1 Introduction

Hierarchical cluster analysis for predicting the link between different physicochemical properties and fungal infection criteria in oranges of cv. 'Maltaise' and apples of both cvs. 'Richared' and 'Golden' during different storage periods

Red apples (Richared):

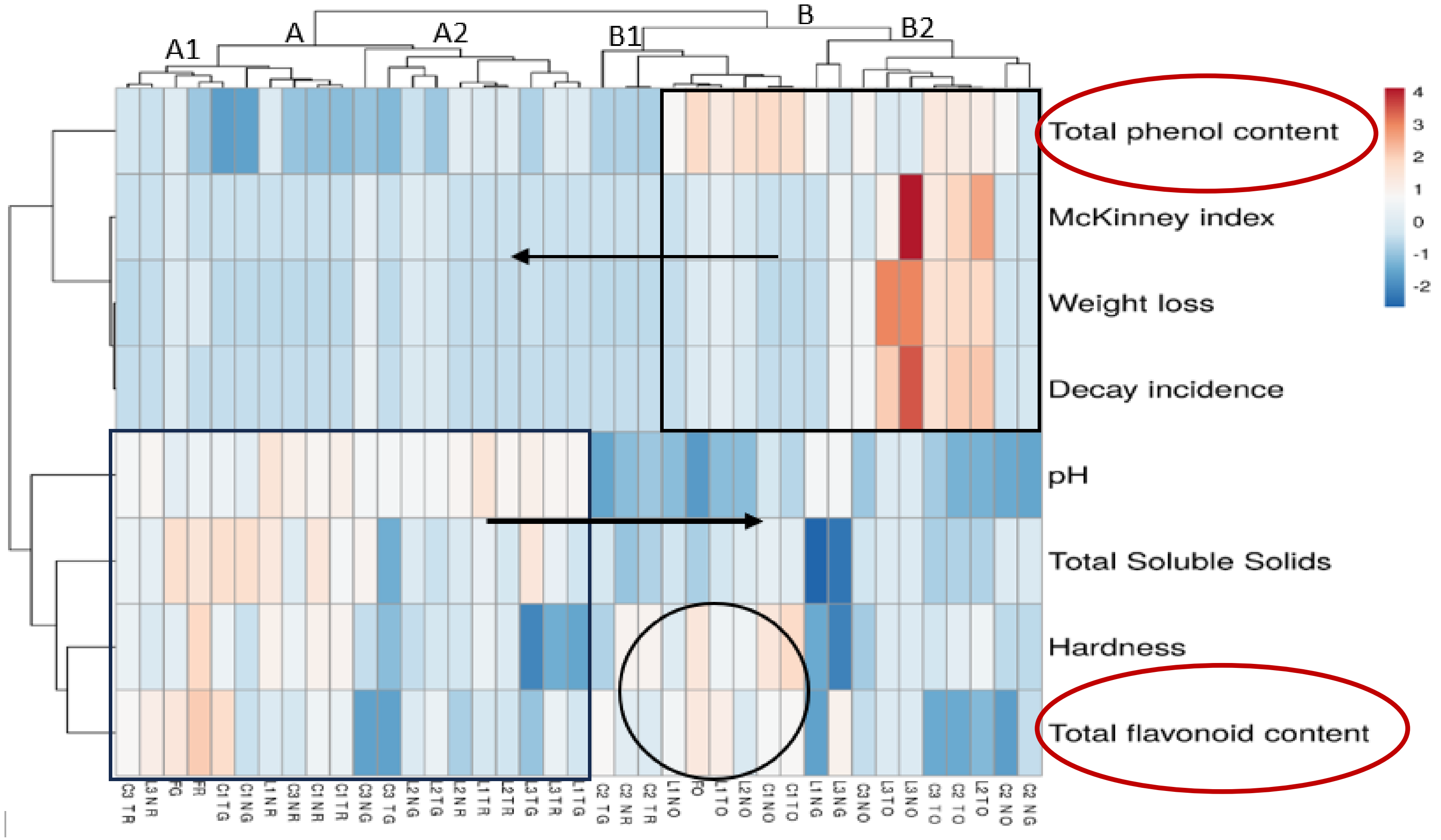
60d (6°C) + 15d (≈ 20°C)

Yellow apples (Golden):

42d (6°C)+less than 15d(≈ 20°C)

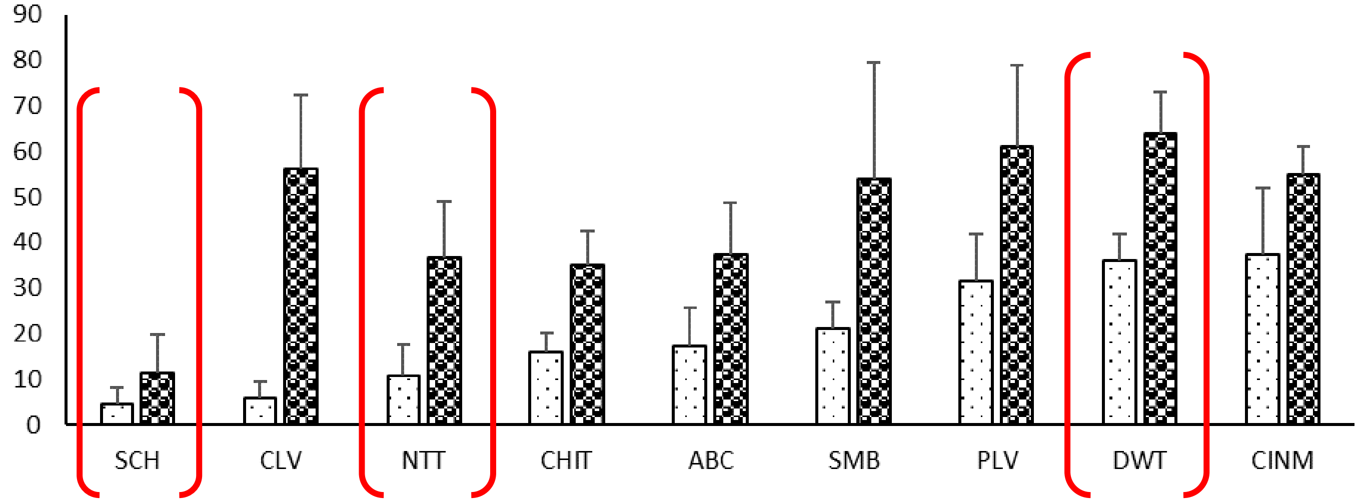
Oranges (Maltaise):

20d (6°C)+15d (≈ 20°C)

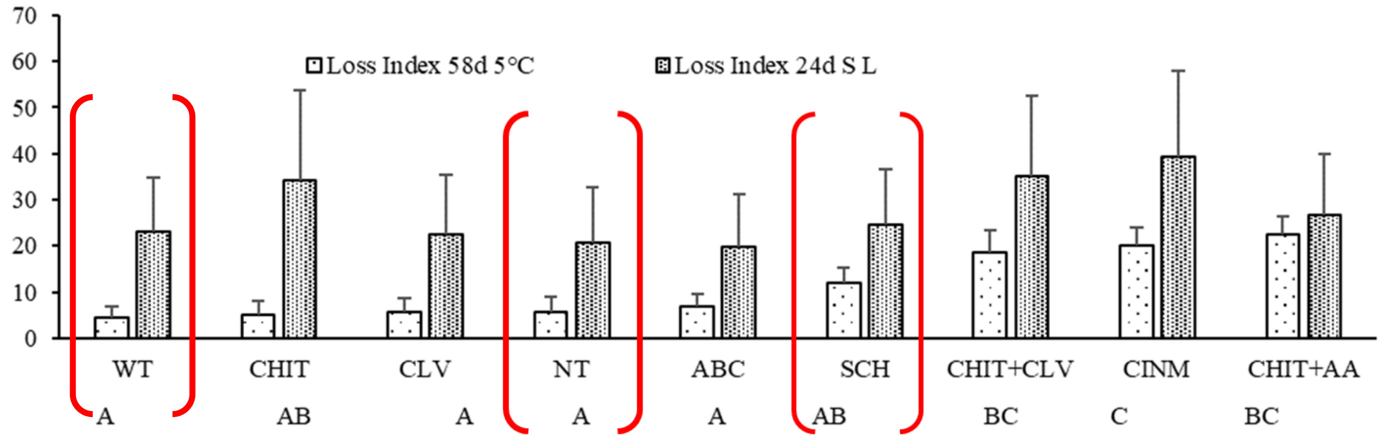


Loss index formulated from weight loss, disease severity and disease incidence, in relation to each treatment method for oranges of the cultivar 'Valencia Late' after 58 days at 5 °C and 24 days of shelf-life and cv 'Maltaise' after 35 days at 5 °C and after 7 days shelf life at ambient temperature

Maltaise:
35d (5°C)+7d SL



Valencia late:
58d (5°C)+24d SL



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*.

- Fruit wounds are the main factor in the spread of fungal decay. Fruit with wounds should be sorted before cold storage to prevent further fungal attack.

- Well-sorted but untreated red apples showed the longest storage period, followed by yellow apples and the 'Maltaise' oranges.
- Our results suggest that it is possible to dispense with treatments for healthy fruit (free of wounds) intended for cold storage.

- We suggest the use of innovative methods such as nanoencapsulation for the practical formulation environmentally-friendly compounds (effective essential oils), helping to reduce the need for chemical fungicides.

- These results could have a positive impact on the reduction of synthetic pesticides and food losses (main objectives of the project)

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

www.stopmed...
StopMedWast...

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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ESPACE INNOVATION
INRAT
فضاء التجديد
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


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Thank you very much