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MARIA BRUNA MEDEIROS ARAÚJO

SPECIES OF *Fusarium* CAUSING PEDUNCULAR ROT IN MELON IN BRAZIL AND ALTERNATIVE MANAGEMENT METHODS

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Thesis submitted to the Universidade Federal Rural do Semi-Árido, in partial fulfillment of the requirements for the degree of Doctor of Science in Plant Science.

Research area: Phytopathology

Advisor: Profª. Dra. Márcia Michelle de Queiroz Ambrósio

Co-advisor: Profª. Dra. Selma Rogéria de Carvalho Nascimento

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To God.
To my mother, Raimunda Maria, and aunt Raimundinha, for all the love and affection.
To my companion, Ikaro Cezar, for all the encouragement and inspiration.

I dedicate.

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Chico Xavier

RESUMO

Araújo, Maria Bruna Medeiros. **Espécies de *Fusarium* causando podridão peduncular em melão no Brasil e métodos alternativos no manejo da doença**. 2020. 96f. Thesis (D. Sc. in Plant Science) – Universidade Federal Rural do Semi-Árido (UFERSA), Mossoró-RN, 2020.

O presente estudo teve como objetivo conhecer a diversidade de espécies de *Fusarium* que causam podridão peduncular (PP) em melão na maior região de produção do Brasil, e verificar o efeito de métodos alternativos no manejo da doença. Um total de 28 isolados foi obtido de diferentes cultivares e áreas de produção de melão do Nordeste do Brasil. Por meio das análises filogenéticas e de caracteres morfológicos, foi possível identificar cinco espécies de *Fusarium* pertencentes a quatro complexos distintos: *Fusarium falciforme* do complexo de espécies *F. solani* (FSSC), *F. sulawesiense* e *F. pernambucanum* do complexo de espécies *F. incarnatum-equiseti* (FIESC), *F. kalimantanense* do complexo de espécies *F. oxysporum* (FOSC) e *Fusarium* sp. do complexo de espécies *F. fujikuroi* (FFSC), como uma provável nova espécie. A patogenicidade dos isolados foi testada em melão Amarelo e todos os isolados induziram sintomas de podridão, com os isolados de *F. falciforme* e *F. sulawesiense* mostrando maior agressividade. Para o manejo da PP, foram realizados três experimentos. O primeiro consistiu no teste *in vitro*, testando diferentes produtos sobre o crescimento micelial de *F. falciforme*, utilizando os tratamentos: Controle; Magnate® (2 mL/L); Compost Aid® (2, 4, 8 e 10 g/L), Nem Out® (2, 4, 8 e 10 g/L), Serenade® (2, 4, 8 e 10 mL/L), Enzimatic (2, 4, 8 e 10 mL/L), Copper Crop® (2, 4, 8 e 10 mL/L); Óleo essencial (OE) de citronela (1, 2 e 2,5% v/v); OE de melaleuca (1, 2 e 2,5% v/v); Tween 20 (1% v/v) e cloreto de cálcio ($CaCl_2$) (2% v/v). Os outros dois experimentos consistiram na avaliação *in vivo* da PP, utilizando frutos inoculados e não inoculados com *F. falciforme*. Os tratamentos consistiram na associação, ou não, da termoterapia (58 °C por 30 segundos) com os melhores tratamentos observados no experimento *in vitro* (Magnate®, 2 mL/L; Compost Aid®, 2 g/L; Nem Out®, 8 g/L; Serenade®, 10 mL/L; Enzimatic, 10 mL/L; Copper Crop®, 8 mL/L; OE de citronela, 2% v/v; OE de melaleuca, 2,5% v/v; $CaCl_2$, 2% v/v), avaliando-se a incidência (INC) e a severidade (SEV) da PP, aos 30 e 40 dias de armazenamento em câmara fria a 10 ± 2 °C. A qualidade dos frutos também foi avaliada quanto às aparências externa e interna, firmeza e teor de sólidos solúveis. Em ambos os experimentos *in vivo*, os frutos tratados apenas com água quente apresentaram menor INC e SEV da PP, na comparação com os que não receberam o tratamento térmico. Em condições de alta umidade do ambiente (precipitação média de 265 mm durante o período de produção do melão), a combinação da termoterapia com Copper Crop® (8 mL/L) ou com

OE de citronela (2% v/v), foi eficiente no controle da podridão peduncular em melão por até 30 dias, e a termoterapia associada ao Serenade® (10 mL/L) ou ao $CaCl_2$ (2% v/v) controlou a doença por até 40 dias de armazenamento. Na ausência da termoterapia, Compost Aid® e o OE de citronela controlaram a PR até 30 e 40 dias, respectivamente, nessa mesma condição. Sob baixa umidade do ambiente (precipitação média de 4 mm durante período de produção do melão), a combinação da termoterapia com o Compost Aid® (2 g/L) controlou a doença por 30 dias e, quando em associação com Nem Out® (8 g/L), Enzimatic (10 mL/L), Copper Crop®, OE de citronela, OE de melaleuca (2.5% v/v) ou ao $CaCl_2$, promoveu proteção até 40 dias de armazenamento. Nessa mesma condição, o Enzimatic e o Copper Crop® foram eficientes no controle da doença em até 30 e 40 dias, respectivamente. Para o nosso conhecimento, este é o primeiro relato de espécies dos complexos FSSC, FOSC e FFSC ocorrendo em melão. Nenhum tratamento influenciou negativamente a qualidade dos frutos, mantendo-os dentro dos padrões de mercado. A termoterapia a 58 °C por 30 segundos é uma alternativa eficiente para o manejo de PP no melão causada por *F. falciforme*. A combinação da termoterapia com o Compost Aid®, Nem Out®, Enzimatic, Serenade®, Copper Crop®, OE de citronela, OE de melaleuca ou com o $CaCl_2$ é eficiente no controle da PP do melão até 40 dias, em ambiente refrigerado. Os produtos Enzimatic, Compost Aid® e o OE de citronela, sozinhos, são capazes de inibir a PP em melão até 30 dias de armazenamento, e o Copper Crop® fornece proteção até 40 dias, em ambiente refrigerado, não afetando a qualidade dos frutos.

Palavras-chave: Análise filogenética. Controle. *Cucumis melo*. Patogenicidade. Pós-colheita.

ABSTRACT

Araújo, Maria Bruna Medeiros. **Species of *Fusarium* causing peduncular rot in melon in Brazil and alternative management methods**. 2020. 96p. Thesis (D. Sc. in Plant Science) – Universidade Federal Rural do Semi-Árido (UFERSA), Mossoró-RN, 2020.

The present study aimed to know the diversity of *Fusarium* species that cause peduncular rot (PP) in melons in the largest production region in Brazil, and to verify the effect of alternative methods in the management of the disease. A total of 28 isolates were obtained from different cultivars and melon production areas in Northeastern Brazil. Through phylogenetic analyzes and of morphological characters, it was possible to identify five *Fusarium* species belonging to four distinct complexes: *Fusarium falciforme* from the *F. solani* species complex (FSSC), *F. sulawesiense* and *F. pernambucanum* from the *Fusarium incarnatum-equiseti* species complex. (FIESC), *F. kalimantanense* from the *F. oxysporum* species complex (FOSC), and *Fusarium* sp. from the *F. fujikuroi* species complex (FFSC), as a probable new species. The pathogenicity of the isolates was tested on Canary melon and all isolates induced symptoms of rot, with the isolates of *F. falciforme* and *F. sulawesiense* showed to be more aggressive. For the management of PR, three experiments were carried out. The first consisted of an *in vitro* assay, testing different products on the mycelial growth of *F. falciforme*, with treatments: Control; Magnate® (2 mL/L); Compost Aid® (2, 4, 8 and 10 g/L), Nem Out® (2, 4, 8 and 10 g/L), Serenade® (2, 4, 8 and 10 mL/L), Enzimatic (2 , 4, 8 and 10 ml/L), Copper Crop® (2, 4, 8 and 10 ml/L); Citronella essential oil (EO) (1, 2 and 2.5% v/v); Melaleuca (EO) (1, 2 and 2.5% v/v); Tween 20 (1% v/v) and calcium chloride ($CaCl_2$) (2% v/v). The other two experiments consisted of an *in vivo* evaluation of the PR, using fruits inoculated and not inoculated with *F. falciforme*. The treatments consisted of the combination, or not, of thermotherapy (58° C for 30 seconds) with the best treatments observed in the *in vitro* assay (Magnate®, 2 mL / L; Compost Aid®, 2 g / L; Nem Out®, 8 g / L; Serenade®, 10 mL / L; Enzimatic, 10 mL / L; Copper Crop®, 8 mL / L; Citronella EO, 2% v / v; Melaleuca EO, 2.5% v / v; $CaCl_2$, 2% v / v), evaluating the incidence (INC) and severity (SEV) of PR, at 30 and 40 days of storage in cold chamber at 10 ± 2° C. Internal and external quality of the treated fruits, firmness, and soluble solids content were also assessed. In both *in vivo* experiments, fruits treated only with hot water, presented lower INC and SEV of PR, compared to those that did not receive heat treatment. Under conditions of high humidity (average precipataion of 265 mm during the period of production), the combination of thermotherapy with Copper Crop® (8 mL/L) or with Citronella EO (25 v/v) was efficient to control peduncular rot in melon for

upt to 30 days, and thermotherapy with Serenade® (10 mL/L) or $CaCl_2$ controlled the disease for 40 days in storage. In the absence of thermotherapy, Compost Aid® and Citronella EO controlled the PR for 30 and 40 days, respectively, under the same conditions. Under low humidity (average precipitation of 4 mm during the period of production), combination of thermotherapy with Compost Aid® (2 g/L) controlled the disease for 30 days, and the combination with Nem Out® (8 g/L), Enzimatic (10 mL/L), Copper Crop®, Citronella EO and Melaleuca EO (2.5% v/v) or $CaCl_2$, provided protection for up to 40 days in storage. At this same condition, the Enzimatic and Copper Crop® were efficient at controlling the disease for up to 30 and 40 days, respectively. To our knowledge, this is the first report of species from the FSSC, FOSC and FFSC complexes occurring in melon. No treatment negatively influenced the quality of the fruits, keeping them within the market standards. The thermotherapy at 58° C for 30 seconds is an efficient alternative for the management of PR in melon caused by *F. falciforme*. The combination of thermotherapy with Compost Aid®, Nem Out®, Enzimatic, Serenade®, Copper Crop®, Citronella EO, Melaleuca EO or $CaCl_2$ are effective in controlling the melon PR for up to 40 days, in a refrigerated environment. The products Enzimatic, Compost Aid® and Citronella EO, by themselves, are able to inhibit PR in melon for up to 30 days of storage, and Copper Crop® provides protection for up to 40 days in a refrigerated environment, without affecting the quality of the fruits.

Keywords: Phylogenetic analysis. Control. *Cucumis melo*. Pathogenicity. Postharsvest.

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CHAPTER I

1 GENERAL INTRODUCTION

Brazil is one of the largest producers of fruits and vegetables in the world, occupying the 9th place in vegetable production, with average annual production of 3.2 million tons, of which melon (*Cucumis melo* L.) accounts for 18% of the total (FAO, 2018). Brazil's melon production is concentrated in the Northeast region, with favorable climatic conditions for the crop (low humidity and high temperatures), enabling the production through almost the whole year (CAMPELO et al., 2014). Among the main melon producing states are Ceará (CE) and Rio Grande do Norte (RN), responsible for 95% of national production. These states together exported 194.5 thousand tons of the fruit to Europe in 2018, with Spain, the Netherlands, and the United Kingdom as main markets, generating a revenue of US$ 136 million (Kist et al., 2019). In addition to its great importance in the economic scenario, melon production provides employment and income for many families in the semi-arid region, where small producers associate with cooperatives or larger producers to sell their products, generating about 25,000 jobs for the local population every crop season (ABRAFRUTAS, 2018).

Even though the Northeast region provides excellent conditions for melon cultivation, the sector faces several challenges, such as the reduction in fruit productivity and quality due to phytosanitary difficulties. Lack of crop rotation and the expansion of cultivated area contribute to the increase of diseases in the field, also reflecting in the postharvest, with, according to Brazilian exporters, about 15% of the exported melon being lost due to postharvest diseases, generating irreversible commercial losses (OSTER et al., 2018). One of the main diseases observed in melon after harvest is the peduncular rot, often caused by fungi of the genus *Fusarium*. Symptoms were first reported in 2000, when fruits produced in the RN presented water-soaked rot accompanied by fissures in the peduncular zone, with abundant mycelial growth of the pathogen, which was later identified as *F. incarnatum* (Desm.) Sacc. (syn. *Fusarium pallidoroseum* (Cooke) Sacc) (GADELHA, 2002). Since then the disease has been attributed to *F. incarnatum* on the basis of morphological characters, however, with the use of molecular markers, another species is being reported to be related with the disease in different parts of the world. For instance, *Fusarium equiseti* (Corda) Sacc., belonging to the *Fusarium incarnatum-equiseti* species complex (FIESC), was reported in plantations in China (LI et al., 2019) and Thailand (NUANGMEK et al., 2019).

The phylogenetic analysis of the species, in general, is a reliable and efficient identification method in taxonomic studies, especially in cases of similarity among fungi of the same complex. Although there is a higher incidence of FIESC fungi causing postharvest rots in melon, it is necessary to verify whether there are other species of this genus associated with the disease in fields in Brazil, as the knowledge of the diversity of species related to peduncular rot of melon will allow to a more accurate diagnosis of the causal agent, contributing to the planning of more efficient disease management strategies, since each species may behave differently in the face of different control methods. The process of *Fusarium* infection in melons has not yet been well elucidated, however, some researchers believe that it may still occur in the field (pre-harvest), during the harvest, in postharvest procedures (in the packing-house) (OSTER et al., 2018) or even colonizing as an endophyte, later becoming pathogenic. Because the symptoms appear late (between 5 to 10 days after harvest, at room temperature, and 15 to 20 days, under refrigeration), the adoption of preventive methods in the management of the disease is difficult, especially when dealing with fruits for exportation, being the symptoms observed only in the importing country, making them unacceptable to the consumer (TERAO et al., 2006).

The methods of disease control in the melon postharvest are insufficient. Currently, products containing imazalil as active ingredient are used as the fungicide recommended by the Ministério da Agricultura, Pecuária e Abastecimento (MAPA), although it does not present any indication of efficiency against *Fusarium* rots. According to the growers, such fungicide is no longer efficient, and increasing fruit loss is observed every season; also, the discussing of the suspension of imazalil by the European Food Safety Authority (EFSA), due to residue problems (EUROPEAN FOOD SAFETY AUTHORITY et al., 2018) motivates the search for safer management practices in the food-producing field with efficient results against the peduncular rot of the melon. The use of pesticides in postharvest is very limited due to the reduction of the residual limits allowed. The search for safe and environmentally friendly approaches to control postharvest diseases is becoming a global trend and, among the different methods, thermal treatments have been shown to be efficient in the control of diseases in melons (SIVAKUMAR; FALLIK, 2013), as well as antagonistic microorganisms, which is becoming an emerging and advantageous alternative, because it is a safe application method, free of toxic residues and more profitable (BONATERRA et al., 2012; DUKARE et al., 2019). Alternative management does not rule out traditional methods, but tends to manage more harmoniously with the environment to maintain high agricultural productivity

(FISCHER et al., 2018). With this, the combination of methods for the management of diseases in the postharvest of melon may be more effective than the use of an isolated method.

Physical treatments, such as the use of heat, UV radiation and radio frequency, in the treatment of postharvest fruit diseases, have gained a lot of interest due to the absence of residues in treated products, and for its minimum environmental impact. However, there are also limitations, such as adverse effects on the final quality of the product, technological difficulties for commercial scale application and low persistence (USALL et al., 2016). Thermotherapy is the best physical method known in the control of postharvest diseases, and can be applied by immersion of fruits in hot water, washing and brushing in hot water, or by steam, for a short period of time after harvest, to prevent infection by pathogens present in the superficial layer of the fruits (SIVAKUMAR & FALLIK, 2013).

The treatment of fruits with hot water has been used by Israeli companies in the postharvest of melon to reduce *Fusarium* and *Alternaria* rots, besides providing higher fruit quality (USALL et al., 2016), however, in Brazil, this practice is not yet used on a commercial scale. Sui et al. (2014) confirmed the efficiency of thermotherapy in the control of *F. oxysporum* in melons, by immersing fruits in hot water at 45 °C for 10 to 25 minutes, maintaining the quality of the fruits within the marketable standards. Similar result was reported by Sivakumar and Fallik (2013), when treating Galia melons in hot water for 15 seconds at 58 °C, reporting as an efficient alternative in the management of postharvest rots, also discussing that temperature and duration of treatment will depend on the size, weight and maturation stage of the fruit.

The demand for organic products is increasing, also due to concerns with the resistance of pathogens to the traditionally used phytosanitary products, as well as the interest in organic foods free of residues, which consequently increases the demand of products composed by biocontrolling agents in the market. Biological control refers to the use of living organisms or their metabolites, extracts of plants or microorganisms, which present inhibitory or antimicrobial effect (BALOUIRI; SADIKI; IBNSOUDA, 2016). In the main antagonistic relationships used in biological control, it is prevalent the use of bacterial genera *Bacillus*, *Lactobacillus*, *Pseudomonas* and *Streptomyces*, with higher prevalence of the former, because it presents different mechanisms of action, such as antibiotic production, substrate competition, production of hydrolytic enzymes and induction of resistance (BRAGA JUNIOR et al., 2017). The biocontrol system in the postharvest involves the interaction among the antagonist, the pathogen and the host, which are influenced by the environmental conditions, and different modes of biostatic action may occur, such as competition for nutrients and

space, parasitism, induction of host resistance, production of antibiotics and volatile metabolites (DUKARE et al., 2019).

Essential oils (EOs) are extracted from different parts of plants, rich in volatile secondary metabolites, with insecticide and antimicrobial properties (SEOW et al., 2014). Citronella essential oil (Cymbopogon nardus (L.) Rendle) is commonly used for its repellent properties but has also performed well in the control of fungi and bacteria (ALI et al., 2017). Endowed with antifungal activity of great potential for use in the treatment of postharvest diseases, Citronella EO is constituted of citral, citronellal, citronellol and geraniol compounds, being an alternative to the use of synthetic chemicals (CHEN et al., 2014; DE TOLEDO et al., 2016). Melaleuca EO is also an antimicrobial suppressor, rich in terpinen-4-ol, which can act on the integrity of membranes, and on the pathogen's cellular respiration (YADAV et al., 2017). Thus, these EOs can show satisfactory results on the peduncular rot in melon caused by *Fusarium* and may be an alternative to synthetic products. It is also important to highlight the use of chemical compounds that can improve resistance of fruits to pathogen infections, such as calcium chloride ($CaCl_2$), commonly applied in fresh fruits, increasing pulp firmness, delaying the ripening process and, consequently, hindering early development of diseases (FERRAZ et al., 2016).

In view of the above, the combination of different management techniques may be more efficient in controlling postharvest diseases than those ones used separately. Thus, the objective of this study was to identify and characterize the species of *Fusarium* that occur in melon produced in the main production areas of Northeastern Brazil, through phylogenetic, morphological and pathogenic analyses, as well as to evaluate the effect of different commercial products currently used in agriculture, essential oils and calcium chloride ($CaCl_2$), in combination with thermotherapy, in the management of peduncular rot of melon.

REFERENCES

ABRAFRUTAS, Associação Brasileira dos Produtores Exportadores de Frutas e Derivados. Cenário Hortifruti Brasil, 2018. Disponível em: <https://abrafrutas.org/wp-content/uploads/2019/09/Relatorio-Hortifruti.pdf>. Acesso: 10 mar. 2020.

ALI, E. O. M. et al. Antifungal activity of nano emulsions of neem and citronella oils against phytopathogenic fungi, *Rhizoctonia solani* and *Sclerotium rolfsii*. **Industrial crops and products**, v. 108, p. 379-387, 2017.

BALOUIRI, M.; SADIKI, M.; IBNSOUDA, S. K. Methods for in vitro evaluating antimicrobial activity: A review. **Journal of pharmaceutical analysis**, v. 6, n. 2, p. 71-79, 2016.

BONATERRA, A. et al. Prospects and limitations of microbial pesticides for control of bacterial and fungal pomefruit tree diseases. **Trees**, v. 26, n. 1, p. 215-226, 2012.

BRAGA JUNIOR, G. M. B. et al. Controle biológico de fitopatógenos por *Bacillus subtilis* in vitro. **Biota Amazônia (Biote Amazonie, Biota Amazonia, Amazonian Biota)**, v. 7, n. 3, p. 45-51, 2017.

CHEN, Q. et al. Effect of citronella essential oil on the inhibition of postharvest *Alternaria alternata* in cherry tomato. **Journal of the Science of Food and Agriculture**, v. 94, n. 12, p. 2441-2447, 2014.

DE TOLEDO, L. G. et al. Essential oil of Cymbopogon nardus (L.) Rendle: A strategy to combat fungal infections caused by *Candida* species. **International Journal of Molecular Sciences**, v. 17, n. 8, p. 1252, 2016.

DUKARE, A. S. et al. Exploitation of microbial antagonists for the control of postharvest diseases of fruits: a review. **Critical reviews in food science and nutrition**, v. 59, n. 9, p. 1498-1513, 2019.

EUROPEAN FOOD SAFETY AUTHORITY (EFSA) et al. Updated review of the existing maximum residue levels for imazalil according to Article 12 of Regulation (EC) No 396/2005 following new toxicological information. **EFSA Journal**, v. 16, n. 10, p. 5453, 2018.

FAO, Food and Agriculture Organization. FAOSTAT - Food and agriculture data, 2018. Available at: <http://www.fao.org/faostat/es/#rankings/countries_by_commodity>. Acessed: 23 dec. 2020.

FERRAZ, D. M. M. et al. Efeito do cloreto de cálcio sobre a antracnose e características de frutos de goiaba em pós-colheita. **Agrotropica**, v. 28, n. 2, p. 311-318, 2016.

FISCHER, I. H. et al. Effect of conventional and alternative products on postharvest disease control in avocados. **Revista Brasileira de Fruticultura**, v. 40, n. 1, 2018.

GADELHA, J. C. **Controle preventivo e curativo da podridão pós-colheita de frutos de melão com produto alternativo**. Dissertação (Mestrado em Fitotecnica). Ceará. Universidade Federal do Ceará, 2002.

KIST, B. B. et al. Anuário brasileiro de Horti & Fruti. Santa Cruz do Sul: Editora Gazeta Santa Cruz, 2019. Available at: <http://www.editoragazeta.com.br/sitewp/wp-content/uploads/2019/07/HortiFruti_2019_DUPLA.pdf>. Accessed: 15 jul. 2020.

LI, Y. G. et al. Occurrence of Fruit Rot of Cantaloupe Caused by *Fusarium equiseti* in China. **Plant Disease**, v. 103, n. 10, p. 2683-2683, 2019.

NUANGMEK, W. et al. First report of fruit rot on cantaloupe caused by *Fusarium equiseti* in Thailand. **Journal of General Plant Pathology**, v. 85, n. 4, p. 295-300, 2019.

SEOW, Y. X. et al. Plant essential oils as active antimicrobial agents. **Critical reviews in food science and nutrition**, v. 54, n. 5, p. 625-644, 2014.

SIVAKUMAR, D.; FALLIK, E. Influence of heat treatments on quality retention of fresh and fresh-cut produce. **Food Reviews International**, v. 29, n. 3, p. 294-320, 2013.

SUI, Y. et al. Reduction of *Fusarium* rot and maintenance of fruit quality in melon using eco-friendly hot water treatment. **Environmental Science and Pollution Research**, v. 21, n. 24, p. 13956-13963, 2014.

TERAO, D. et al. Integração de fungicidas à refrigeração no controle de podridão pós-colheita em frutos de meloeiro. **Fitopatologia Brasileira**, v. 31, n. 1, p. 89-93, 2006.

USALL, J. et al. Physical treatments to control postharvest diseases of fresh fruits and vegetables. **Postharvest Biology and Technology**, v. 122, p. 30-40, 2016.

YADAV, E. et al. Tea tree oil: a promising essential oil. **Journal of Essential Oil Research**, v. 29, n. 3, p. 201-213, 2017.

CHAPTER II

PEDUNCULAR ROT OF MELON IS CAUSED BY SEVERAL *Fusarium* SPECIES

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Abstract

Peduncular rot of melon, caused by species of the genus *Fusarium*, has become an important postharvest disease for many Brazilian producers. Due to the symptoms starting belatedly, this disease can frequently be detected only when fruits arrive at the importer country, thus generating economic loss for the exportation of the fruit. This study was developed with the aim to investigate which *Fusarium* species cause postharvest fruit rot in melon and to evaluate if there are differences in aggressiveness and development of symptoms. Species were identified through phylogenetic analysis of two loci and morphological markers. The 28 isolates obtained from diseased melon fruits of different commercial cultivars were identified as *Fusarium falciforme* (FSSC), *F. sulawesiense*, *F. pernambucanum* (FIESC) and *F. kalimantanense* (FOSC). Three isolates belong to a new phylogenetic lineage within the *Fusarium fujikuroi* species complex (FFSC). All isolates were tested for pathogenicity, and two days after inoculation, first symptoms of rot in Canary melon were observed. Isolates of *F. falciforme* and *F. sulawesiense* showed to be more aggressiveness. Our results extend information on *Fusarium* species that cause peduncular rot in melon and support the development of management strategies, once there is currently no efficient control for this disease. To our knowledge, this is the first report of occurrence of species of the FSSC, FOSC and FFSC from muskmelon fruits in Brazil.

Keywords: Cucumis melo, Molecular Phylogeny, Pathogenicity, Postharvest disease.

1 INTRODUCTION

Melon (*Cucumis melo* L.) is the most important fresh fruit in volume for exportation from Brazil. The largest melon production is in the Northeast region, with the states of Rio Grande do Norte (RN) and Ceará (CE) holding for 95% of the national total production (Kist et al., 2019). In 2019, together they exported 248.4 thousand tons, generating revenue of US$ 158.4 mi (Agrostat, 2020). In addition to the economic importance for Brazil, the culture of melon represents an important source of income for many families, because small farmers associate with larger farmers to sell their production and improve their product value in the market, creating about 25,000 job opportunities every season (Abrafrutas, 2018).

The increment in cultivated areas and the low rotation with other crops have contributed to increase the incidence of diseases in the field, which also reflects on the quality in the postharvest phase. According to the producers, about 15% of the exported melon was lost in recent harvest seasons due to postharvest diseases, accounting for an average loss of US$ 22 million per season (Oster et al., 2018). Shipment of fruits for exportation is done under refrigeration and, most of the time, symptoms are only seen when fruits arrive in the importer country.

Postharvest rots caused by *Fusarium* species have been reported in different producing areas worldwide and are considered one of the most important diseases limiting commercialization of melon (Mahdikhani & Davoodi, 2016). Species of the *Fusarium incarnatum-equiseti* species complex (FIESC) have been recently reported as the causal agent of postharvest rot in melon from different parts of the world, like Thailand (Wonglom & Sunpapao, 2020; Nuangmek et al., 2019) and China (Li et al., 2019). In Brazil, isolates collected from fruits with initial symptoms like water-soaked lesions were identified as members of the FIESC based on the evaluation of morphological characters and recently *F. sulawesiense* as well as another phylogenetic species have been identified (Dias & Terao, 2006; Lima et al., 2020).

The process of fruit infection of such pathogens has not been well understood yet. Penetration of the fungus may occur before or even during the harvest procedures, which hampers the adoption of preventive control methods. For this reason, understanding the diversity of species causing peduncular rot in melon allows a more accurate diagnosis of the causal agent. In consequence, this will enable the development of adequate management practices, since each species may show distinct behavior upon different control methods. Given the information on postharvest rot in melons, there are indications that this disease is

caused by a diversity of *Fusarium* species, not previously reported on melons. Therefore, the aims of this study were to (i) identify *Fusarium* species associated with postharvest fruit rot of melon fruits from different production areas in the states Ceará and Rio Grande do Norte (Brazil), using a two loci molecular phylogeny and morphological markers, and (ii) to evaluate the pathogenicity of identified isolates.

2 MATERIAL AND METHODS

2.1 Sampling and isolation

Melon fruits of different commercial varieties, like Canary, Cantaloupe, Galia and Piel de Sapo, were collected in the 2018 harvest season from the main producing areas of Brazil, with historical incidence of peduncular rot (Figure S1). Fruits were sent to the Plant Pathology and Microbiology laboratory of the Universidade Federal Rural do Semi-Árido (UFERSA), where they were kept in moist chamber for up to 10 days to induce the vegetative growth of the fungus and development of symptoms.

Fungal isolation was performed by two different methods. Direct isolation was made by transferring the growing mycelium from the peduncle to PDA medium (Himedia Laboratories, Mumbai, India) (added with 0.05 g of tetracycline per L). In the indirect method, fragments of the symptomatic tissue were surface-sterilized in 70% ethanol (30 s) and 1% sodium hypochlorite (1 min), rinsed in distilled sterile water and placed on PDA. Plates were maintained in a Bio-Oxygen Demand (BOD) incubator for seven days at 28 ± 2 °C, under photoperiod of 12 h.

Fusarium isolates were obtained and initially grouped based on their morphological characters, such as color of colony and microscopic characteristics. Single conidial cultures were prepared and preserved with Castellani's method (Castellani, 1939) in the mycological collection of the Plant Pathology and Microbiology laboratory of the UFERSA. Duplicates are deposited in the Mycological Collection of Lavras, Department of Phytopathology, Federal University of Lavras (UFLA), stored with Castellani's method at 10 °C in the dark and cryopreserved in 15%-glycerol spore suspension at -80 °C (Table 1).

Table 1. Isolates of *Fusarium* obtained from melons with peduncular rot symptoms.

Species complexes[a]/Lineages[b]	Species	Code[c]	Melon variety	Geographic origin[d]	GenBank access number	
					EF-1α	***RPB2***
FSSC 3+4	*F. falciforme*	CFM 02A	Galia	Icapuí, CE	MT476598	-
		CFM 05B	Canary	Mossoró, RN	MT476599	-
		CML 4171	Canary	Mossoró, RN	MT476600	MT461683
		CFM 07B	Canary	Mossoró, RN	MT476601	-
		CML 4172	Canary	Baraúna, RN	MT476602	-
		CFM 09A	Cantaloupe	Mata Fresca, RN	MT476603	-
		CML 4173	Cantaloupe	Mata Fresca, RN	MT476604	MT461684
		CML 4174	Cantaloupe	Baraúna, RN	MT476605	-
		CFM 12A	Canary	Afonso Bezerra, RN	MT476606	-
		CFM 13A	Canary	Afonso Bezerra, RN	MT476607	-
		CFM 14B	Piel de Sapo	Afonso Bezerra, RN	MT476608	-
		CML 4204	Piel de Sapo	Jandaíra, RN	MT476609	MT461685
		CML 4205	Galia	Mossoró, RN	MT476610	MT461686
		CML 4175	Piel de Sapo	Jandaíra, RN	MT476611	MT461687
FIESC 16	*F. sulawesiense*	CML 4176	Canary	Baraúna, RN	MT476613	MT461677
		CML 4177	Canary	Mossoró, RN	MT476614	MT461678
		CML 4178	Canary	Mossoró, RN	MT476615	MT461679
		CML 4179	Canary	Mossoró, RN	MT476616	MT461680
		CML 4180	Canary	Baraúna, RN	MT476617	MT461681
FIESC 17	*F. pernambucanum*	CML 4181	Galia	Icapuí, CE	MT476612	MT461676
		CML 4182	Piel de Sapo	Jandaíra, RN	MT476618	MT461682
FOSC	*F. kalimantanense*	CML 3183	Canary	Apodí, RN	MT461672	MT461688

Table 1. (Continued).

Species complexes[a]/Lineages[b]	Species	Code[c]	Melon variety	Geographic origin[d]	GenBank access number	
					EF-1α	***RPB2***
		CML 4184	Canary	Baraúna, RN	MT461673	MT461689
		CML 4185	Cantaloupe	Mata Fresca, RN	MT461674	MT461690
		CML 4186	Canary	Upanema, RN	MT461675	MT461691
FFSC	*Fusarium* sp.	CML 4187	Canary	Icapuí, CE	-	-
		CML 4188	Canary	Baraúna, RN	-	-
		CML 4189	Canary	Mossoró, RN	-	-

[a]FSSC, *Fusarium solani* species complex; FIESC, *F. incarnatum-equiseti* species complex; FOSC, *F. oxysporum* species complex; FFSC, *F. fujikuroi* species complex.

[b]Arabic numerals to identify phylogenetic species within FSSC and FIESC designated by O'Donnell et al. (2008, 2009).

[c]CML, Coleção Micológica de Lavras, Departamento de Fitopatologia, Universidade Federal de Lavras, Minas Gerais, Brazil; CFM, Coleção *Fusarium* Meloeiro, Universidade Federal Rural do Semi-Árido, Rio Grande do Norte, Brazil.

[d]Brazilian states: CE, Ceará; RN, Rio Grande do Norte.

2.2 DNA extraction, PCR amplification and DNA sequencing

The isolates were cultured for 3 days in 2%-malt extract broth (Himedia Laboratories, Mumbai, India) under constant stirring at 100 rpm at 25 ± 2 °C. The fungal biomass was harvested by filtration and macerated under liquid nitrogen. DNA extraction was conducted following the CTAB protocol (Leslie & Summerell, 2006). DNA concentration and purity were determined using a Nanodrop 2000 (Thermo Fisher Scientific Inc., Waltham, USA), and sample concentrations were standardized to 50 ng.

Fragments of the translation elongation factor 1-alpha (*EF-1α*) and the second largest subunit of RNA polymerase (*RPB2*) genes were amplified by using primers EF3/EF22 (O'Donnell et al., 2008) and 5F2/7cR (Liu et al., 1999; Sung et al., 2007), respectively. The amplifications were performed using the GoTaq® Colorless Master Mix kit (Promega, Madison, WI, USA) in My Cycler Thermocycler (Bio-Rad, Hercules, CA, USA) as described by O'Donnell et al. (2008). The PCR products were analyzed by electrophoresis on 1% agarose gel, stained with GelRed dye (Biotium®, Hayward, CA, USA), comparing band size with a 1 kb ladder (New England BioLabs, Ipswich, MA, USA). The amplified fragments were purified with a Wizard® SV Gel and PCR Clean-up System kit (Promega) and sequenced in both directions by a commercial service.

2.3 Phylogenetic analysis

Consensus sequences were assembled using SeqAssem (Hepperle, 2004). Reference sequences of *Fusarium* species from both *EF-1α* and *RPB2* were imported from the GenBank database and added to the alignments (Table S1). The multiple alignments of nucleotide sequences were built using the MUSCLE algorithm, implemented in the MEGAX (Kumar et al., 2018). Alignments with the *EF-1α* gene sequences consisted of 119 informative positions for parsimony in 493 bp for the *F. solani* species complex (FSSC), 58/497 bp for the *F. incarnatum-equiseti* species complex (FIESC), 28/453 bp for *F. oxysporum* species complex (FOSC) and 106/667 bp for the *F. fujikuroi* species complex (FFSC). Alignments of *RPB2* gene were 179/847 bp for FSSC, 29/876 bp for FIESC, 18/860 bp for FOSC and 104/902 bp for FFSC. The alignments were deposited at TreeBASE (accession number S26976, http://purl.org/phylo/treebase/phylows/study/TB2:S26976). Maximum likelihood (ML) and maximum parsimony (MP) phylogenetic analyses were performed in MEGAX for each gene partition and combined dataset. Clade support was inferred from 1000 bootstrap replications.

The GTR+I+G model of nucleotide substitution for ML analyses was estimated with the jModelTest (Darriba et al., 2012).

2.4 Morphological characterization

A mycelial disc of 5-mm diameter was transferred from the edge of a 5-days-old colony to the center of a PDA plate (four repetitions) and incubated in the dark in the BOD incubator, at 25 ± 2 °C to evaluate the colony growth. The diameter was measured after four days of incubation using a caliper, in two perpendicular directions and the colony color was evaluated after 14 days of growth.

Micromorphological characters were assessed on colonies grown on synthetic low nutrient agar (SNA), incubated in the BOD incubator at 25 ± 2 °C under photoperiod of 12 h of fluorescent white light/12 h dark. The evaluation was performed between 10 and 14 days of incubation after preparing microscopic mounts. In order to assess the ability to produce sporodochia, isolates were cultured on carnation leaf agar (CLA) and incubated under the same conditions as previously mentioned (Leslie and Summerell, 2006).

2.5 Pathogenicity tests

Fruits at the stage of physiological maturity (65 days after planting, ≥ 10° Brix) were used for the pathogenicity test (one fruit per isolate). Variety 'Canary' was chosen because of its importance in commercialization worldwide. The fruits were surface-sterilized with 70% ethanol (30 s) and 1% sodium hypochlorite (1 min), and rinsed with distilled sterile water. Five holes of 5-mm diameter and 3-mm in depth were perforated with a sterile cork borer on the surface of each melon. Same-sized discs of each isolate's colony (n=19) were transferred to the holes, with the mycelium facing the interior of the fruit. As a control, fruits were mock-inoculated with PDA only. Fruits were kept in moist chambers for seven days at 25 ± 2 ºC.

Development of symptoms was checked daily, by assessing the period of incubation of each isolate (days) and the diameter of the lesions (mm) after seven days, measured in two perpendicular directions with a caliper. The symptoms were described and documented. The fungi were re-isolated and their morphology was compared with those initially inoculated. The experiment was conducted in a complete randomized design, with five replicates each represented by a single hole. The normality of the data was tested and values were submitted to analysis of variance (ANOVA) and the means of the diameter of the lesions were compared

with the Scott-Knott test ($p \leq 0.05$) using the R statistical software, version 3.6.1 (R Core Team, 2019).

3 RESULTS

3.1 Phylogenetic analysis

The phylogenetic analyses of sequences from the EF-1α and RPB2 genes allowed the identification of the 28 Fusarium isolates in five species grouped in the *F. solani* (FSSC), *F. incarnatum-equiseti* (FIESC), *F. oxysporum* (FOSC) and *F. fujikuroi* species complexes (FFSC) (Table 1). Most isolates (n=14) are members of the FSSC and were identified as F. falciforme based on EF-1α sequences (Figure S2). A subset of five isolates was selected to represent this group in the combined tree (EF-1α + RPB2), resulting in a more robust analysis with 94 (ML) and 99% (MP) of bootstrap support (Figure 1).

Within the FIESC, five isolates were identified as *F. sulawesiense* (FIESC 16) and other two as *F. pernambucanum* (FIESC 17) (Figure 2). Four other Fusarium isolates revealed a well-defined clade within the FOSC, grouping together with sequences of type material of *F. kalimantanense* (Figure 3). In the FFSC, sequences of three isolates clustered within a well-supported clade with 100% of bootstrap, distinct from all other known phylogenetic species (Figure 4), suggesting a new species.

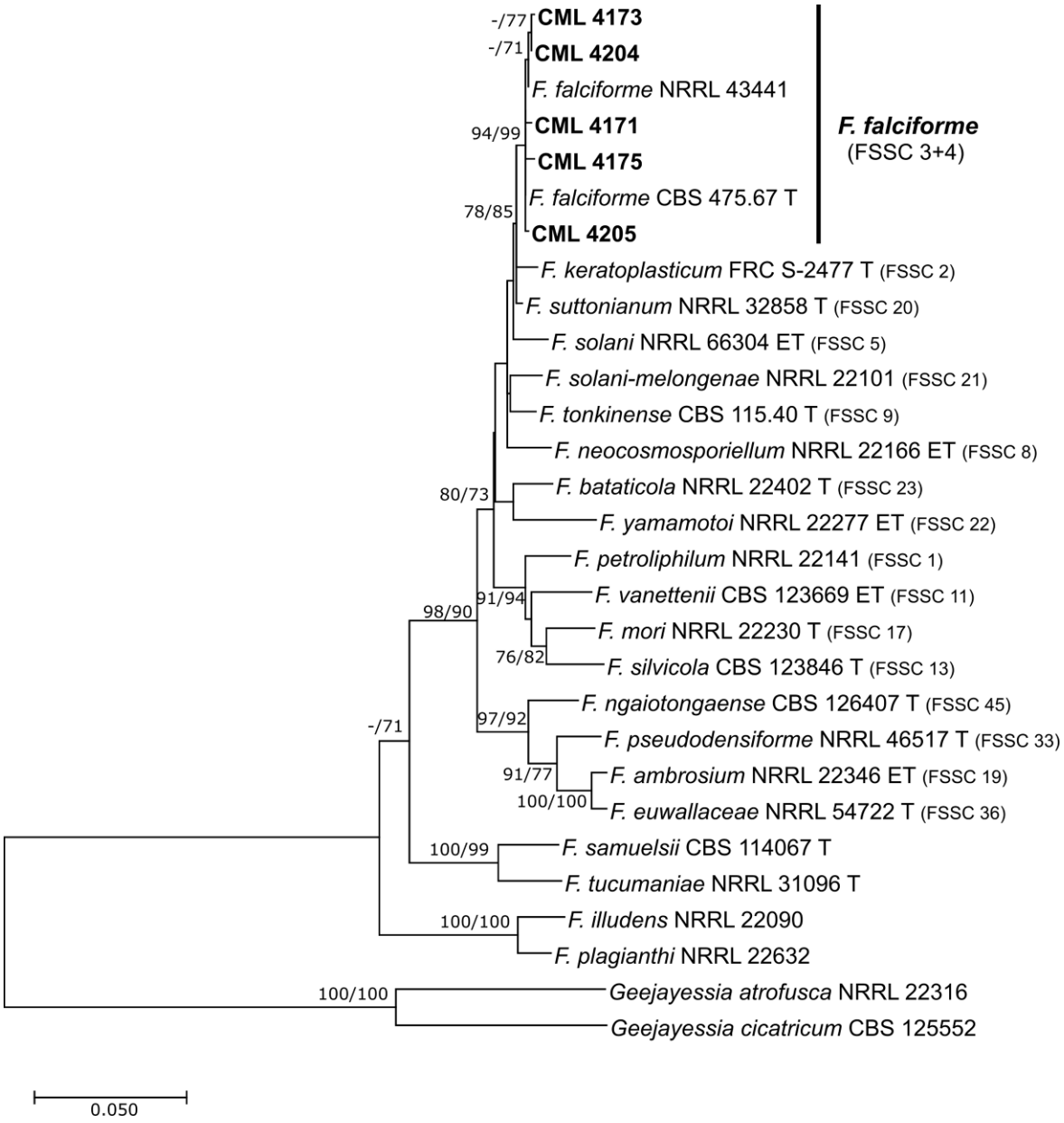

Figure 1. Maximum likelihood phylogram inferred from the sequences of the *EF-1α* and *RPB2* gene fragments, with reference sequences from *Fusarium solani* species complex. The codes in bold refer to the isolates from this study. Other codes concern to the reference sequences from the GenBank. The numbers of the phylogenetic lineages of the FSSC species are in parentheses. Bootstrap values ≥ 70% (1000 replications) for maximum likelihood and maximum parsimony are indicated in the internodes, respectively. Sequences from *Geejayessia atrofusca* (NRRL 22316) and *G. cicatricum* (CBS 125552) were used as outgroup. T = ex-type strain; ET = ex-epitype strain.

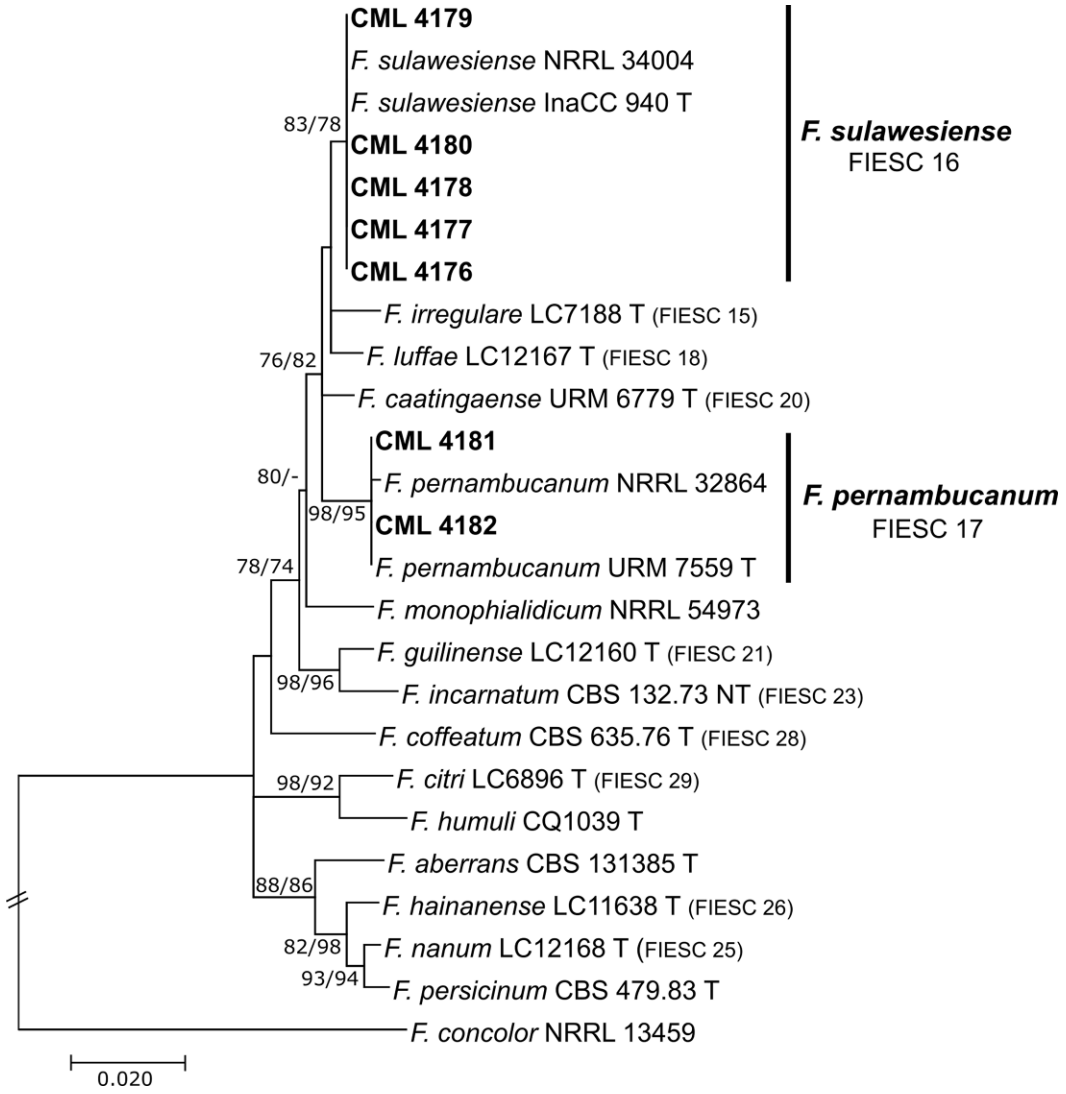

Figure 2. Maximum likelihood phylogram inferred from the sequences of the *EF-1α* and *RPB2* gene fragments of isolates from the *Fusarium incarnatum-equiseti* species complex. The codes in bold refer to the isolates from this study. Other codes concern to the reference sequences from the GenBank. The numbers of the phylogenetic lineages of the FIESC species are in parentheses. Bootstrap values ≥ 70% (1000 replications) for maximum likelihood and maximum parsimony are indicated in the internodes, respectively. Sequences from *F. concolor* (NRRL 13459) were used as outgroup. T = ex-type strain; NT = ex-neotype strain.

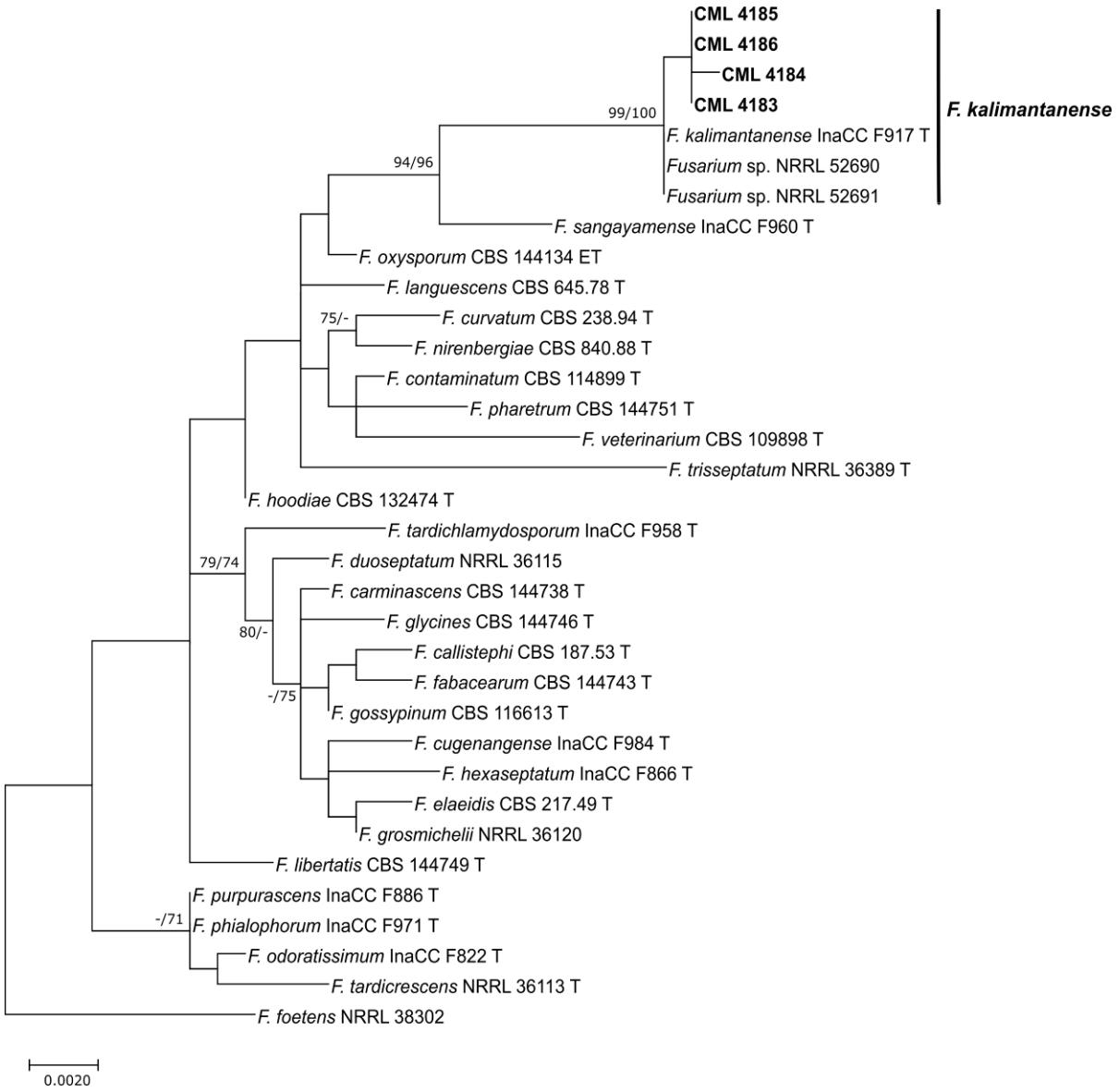

Figure 3. Maximum likelihood phylogram inferred from the sequences of the *EF-1α* and *RPB2* gene fragments of isolates from the *Fusarium oxysporum* species complex. The codes in bold refer to the isolates from this study. Other codes concern to the reference sequences from the GenBank. Bootstrap values ≥ 70% (1000 replications) for maximum likelihood and maximum parsimony are indicated in the internodes, respectively. Sequences from *F. foetens* (NRRL 38302) were used as outgroup. T = ex-type strain; ET = ex-epitype strain.

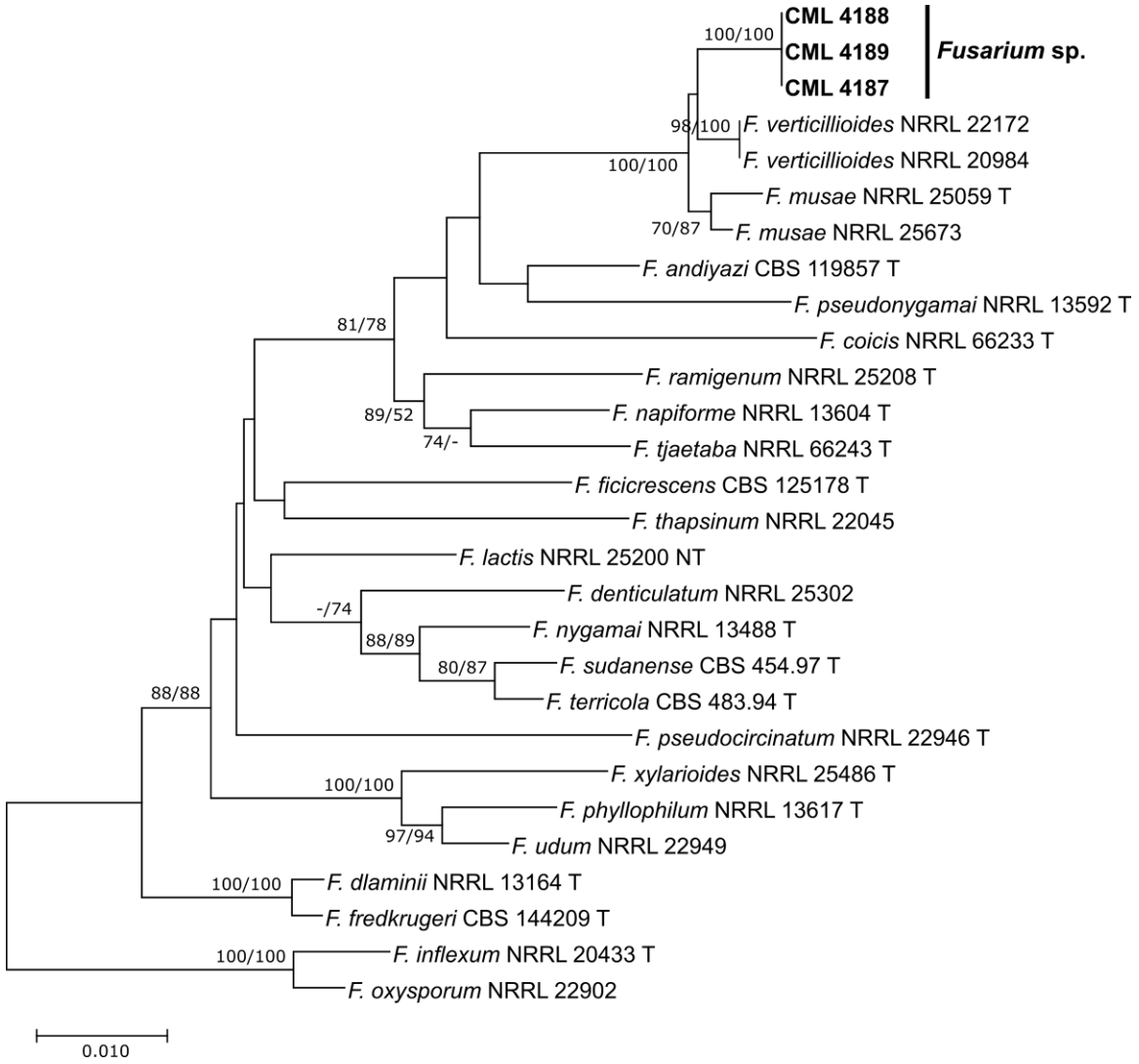

Figure 4. Maximum likelihood phylogram inferred from the sequences of the *EF-1α* and *RPB2* gene fragments of isolates from the *Fusarium fujikuroi* species complex. The codes in bold refer to the isolates from this study. Other codes concern to the reference sequences from the GenBank. Bootstrap values ≥ 70% (1000 replications) for maximum likelihood and maximum parsimony are indicated in the internodes, respectively. Sequences from *F. inflexum* and *F. oxysporum* were used as outgroup. T = ex-type strain; NT = ex-neotype strain.

3.2 Morphological characterization

The morphological characters of the isolates agree with the original descriptions of each species (Table 2). All isolates of this study produced conidia in aerial mycelium and sporodochium with color ranging from white, cream or pale orange on carnation leaves, with abundant production of macroconidia in CLA medium.

Table 2. Morphological characteristics of *Fusarium* isolates obtained from melons with peduncular rot.

Species	Sporodochial conidia			Aerial conidia		
	Shape	Septa	Size (µm)*	Shape	Septa	Size (µm)*
F. falciforme	falcate	3–4	36.6 (44.1) 49.3 × 4.6 (5.1) 6.1	oval/fusiforme	0–3	10.4 (16.6) 20.5 × 2.0 (2.5) 2.9
F. sulawesiense	falcate	3–8	22.7 (31.3) 36.7 × 2.8 (3.5) 4.0	falcate	0–7	18.8 (25.6) 25.6 × 3.7 (4.1) 4.5
F. pernambucanum	falcate	2–5	22.6 (30.2) 33.6 × 2.8 (3.3) 3.8	falcate	0–5	17.5 (24.7) 30.2 × 2.7 (3.3) 3.3
F. kalimantanense	straight/falcate	3–7	32.1 (36.6) 41.2 × 3.1 (3.4) 3.9	oval/ellipsoid	0	9.0 (13.8) 27.9 × 2.8 (3.5) 4.8
Fusarium sp.	curved	1–3	22.4 (38.3) 46.6 × 2.3 (3.1) 3.6	oval/obovoid	0	7.1 (9.4) 13.3 × 2.3 (2.6) 3.0

* Values in parentheses refer to the average length and width of the conidia.

Fusarium falciforme presented average colony diameter of 33 mm (± 9) at four days of incubation, colony initially white progressing to pale yellow and reverse in sulfur- to honey-yellow. Abundant production of globose or oval chlamydospores, located terminally or interspersed in hyphae, single or in chains (Figure 5).

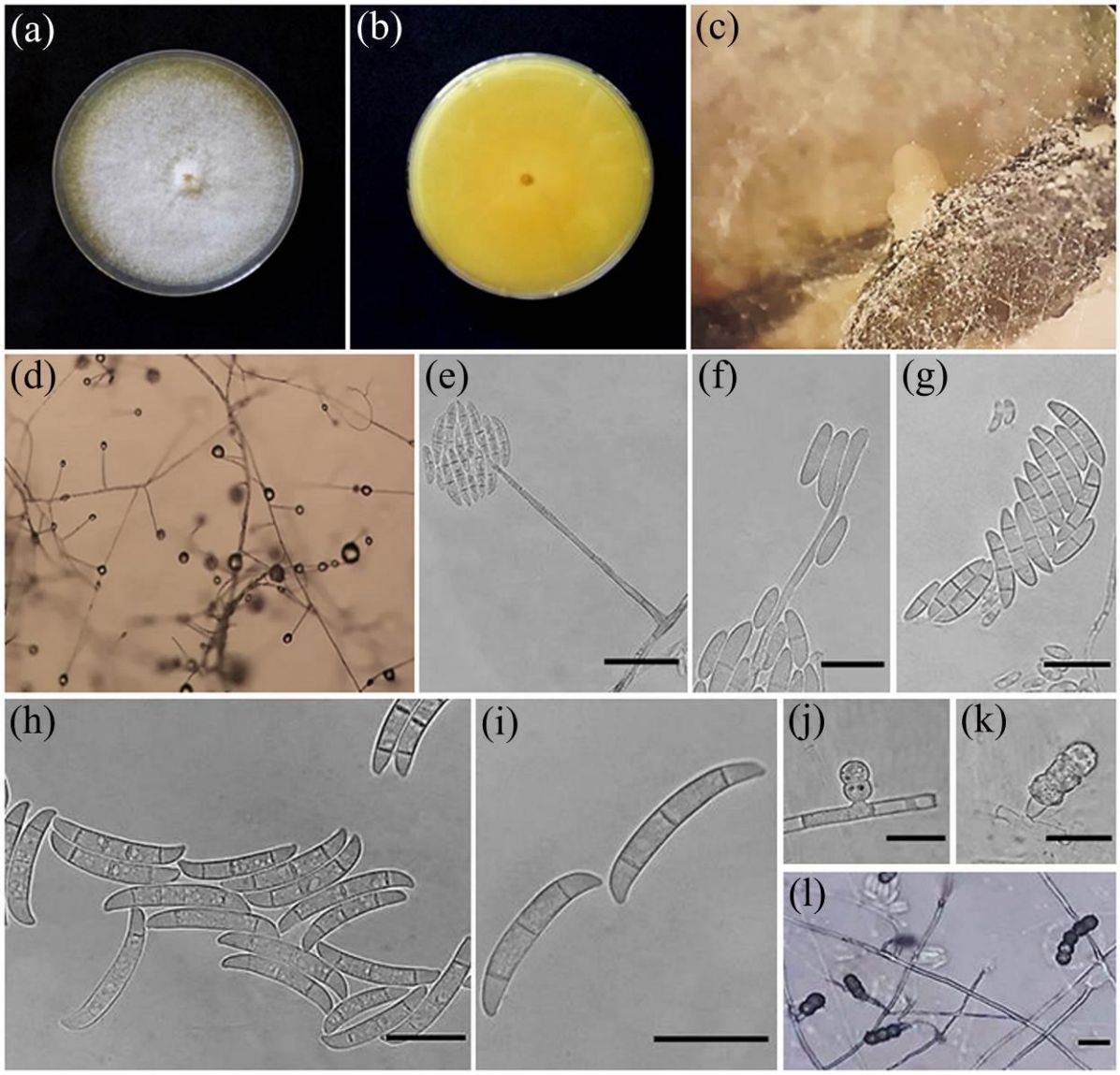

Figure 5. Morphological characteristics of *Fusarium falciforme*. (a-b) colony on PDA; (c) sporodochium on carnation leaf; (d) aerial mycelium; (e-f) monophyialides and microconidia (e: in false heads); (g) conidia from aerial mycelium; (h-i) sporodochial conidia; (j-l) chlamydospores. Scale bars f, g, j-l = 10 μm and e, h, i = 20 μm.

All FIESC isolates presented similar colonies of greenish-yellow color (center) and white or cream (towards the edges), reverse in amber-yellow progressing to honey-yellow towards the edge. Isolates of *F. sulawesiense* presented colony with average diameter of 40

mm (± 10) at four days of incubation, and the isolates *F. pernambucanum* presented average colony diameter of 41 mm (± 7). There was no production of chlamydospores by the isolates (Figure 6).

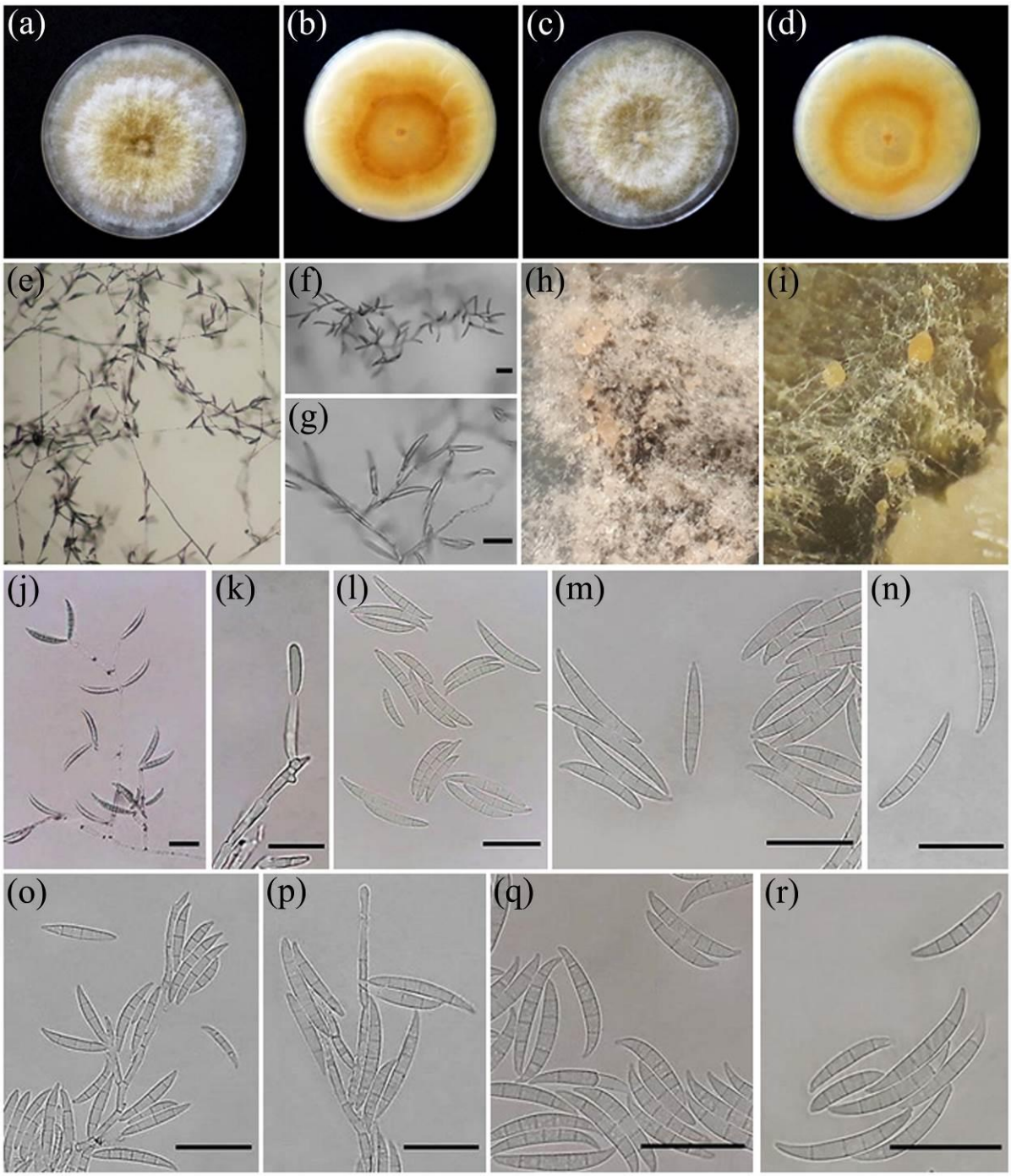

Figure 6. Morphological characteristics of *Fusarium sulawesiense* and *F. pernambucanum*. (a-b) *F. sulawesiense* on PDA; (c-d) *F. pernambucanum* on PDA; (e-g) aerial mycelium; (h-i) sporodochia in carnation leaf; (j-n) *F. sulawesiense*: conidiophore (j), conidiogenous cell (k), conidia from aerial mycelium (l) and sporodochial conidia (m-n); (o-r) *F. pernambucanum*:

conidiophores and conidia from aerial mycelium (o-p), sporodochial conidia (q-r). Scale bars = 20 μm.

The morphology of isolates from FOSC was similar to *F. kalimatanense*. Colony with cottony texture and abundant aerial mycelium in violet to lilac, becoming whitish towards the edge, and reverse with center in purple and edges in yellow, with average diameter of the colony of 39 mm (± 0.1) at four days of incubation. Production of chlamydospores was observed in SNA medium (Figure 7).

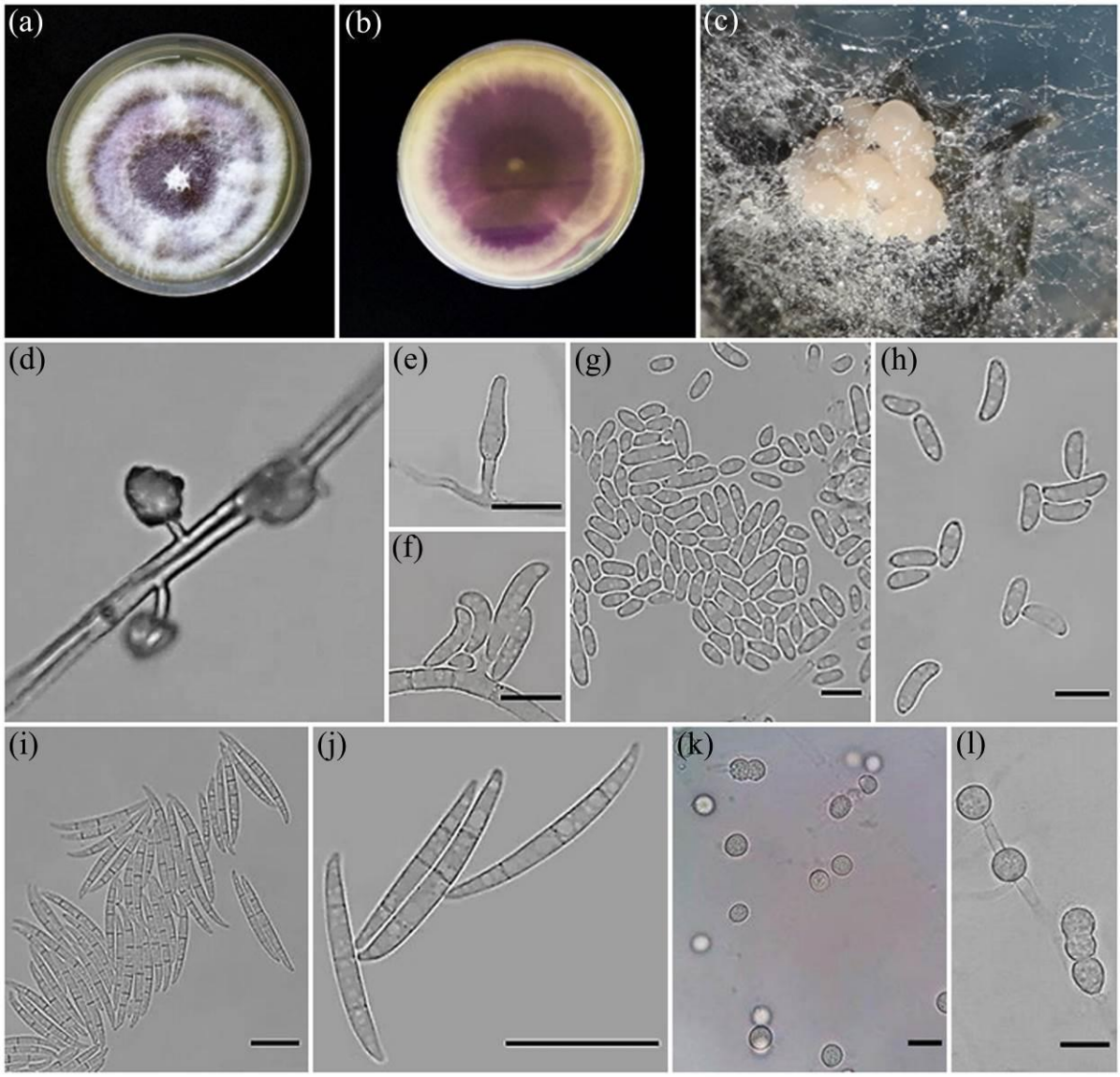

Figure 7. Morphological characteristics of *Fusarium kalimantanense*. (a-b) colony on PDA; (c) sporodochium on carnation leaf; (d) short monophialides from aerial mycelium; (e-f) conidiogenous cell; (g-h) conidia from aerial mycelium; (i-j) sporodochial conidia; (k-l) chlamydospores. Scale bars e-h, k, l = 10 μm and i-j = 20 μm.

The *Fusarium* sp. isolates from FFSC presented morphological markers similar to *F. verticillioides*, like the production of microconidia in the aerial mycelium with a truncate base, produced in long chains from monophialides. Colonies on PDA are purple, lilac to scarlet and white towards the edge, reverse in purple, violet or reddish in the center and lilac in the edges. Colony diameter measured 33 mm (± 3) at four days of incubation. A particular feature is the production of macroconidia which are long, slender and curved. The presence of chlamydospores was not observed.

3.3 Pathogenicity tests

The pathogenicity of the isolates was confirmed after observation of the symptoms in inoculated fruits and comparison with the control treatment (absence of symptoms) (Figure 8). All isolates were pathogenic to Canary melon, however, we verified that isolates of *F. falciforme* (CML 4171, CML 4175) and *F. sulawesiense* (CML 4176) showed greater aggressiveness (Figure 9). Symptoms started two days after inoculation, as superficial necrosis developing into water-soaked or dry lesions in light brown, cracks from the site of inoculation and abundant mycelial growth. Internally, pulp depression with spongy to watery texture was observed. Microscopic analyzes showed that the same *Fusarium* species inoculated in the pathogenicity test were re-isolated from symptomatic fruits, fulfilling Koch's postulates.

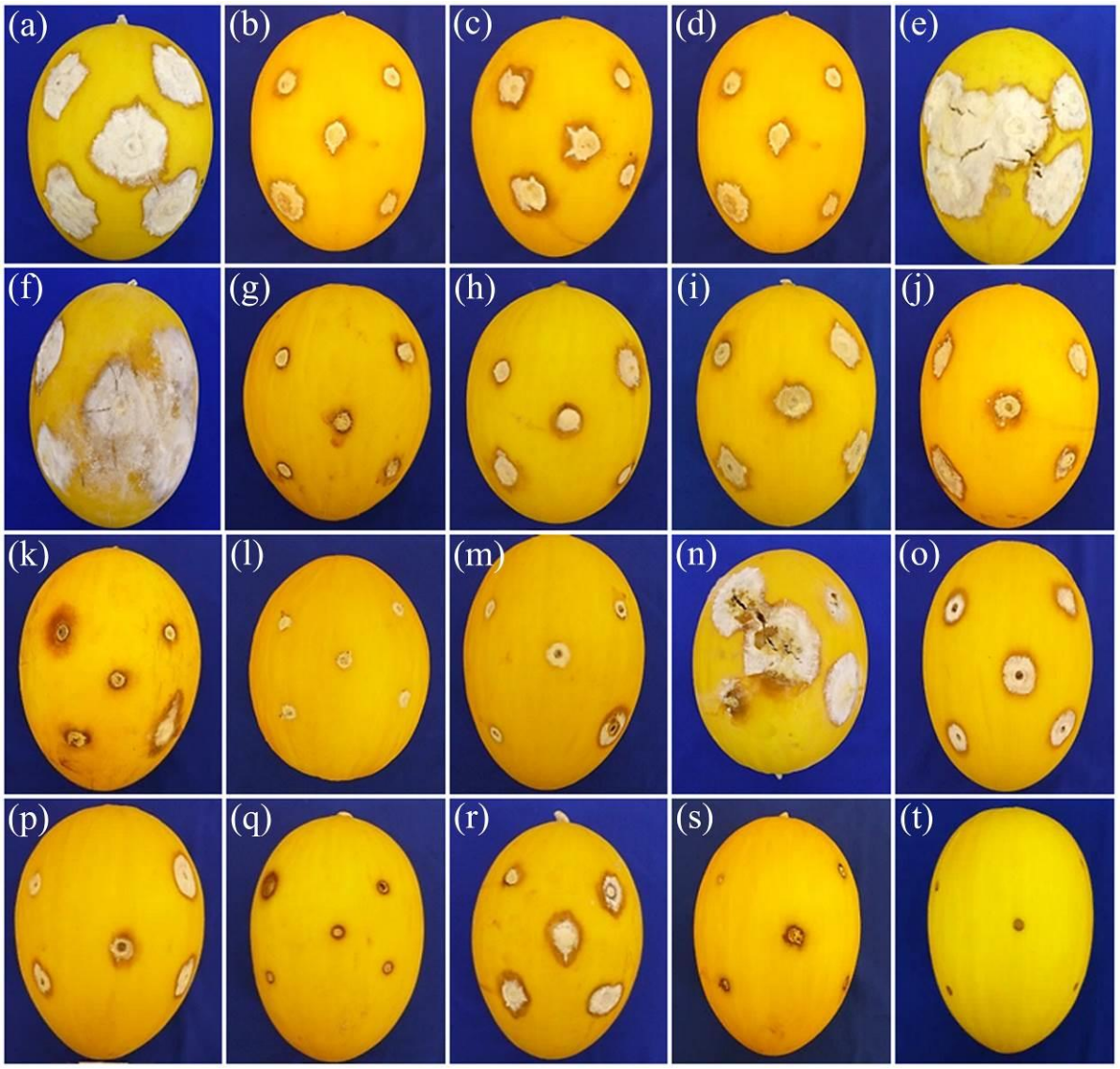

Figure 8. Pathogenicity test of *Fusarium* spp. in Canary melon. (a-e) *Fusarium falciforme* isolates: CML 4171 (a), CML 4173 (b), CML 4204 (c), CML 4205 (d) and CML 4175 (e); (f-j) *Fusarium sulawesiense* isolates: CML 4176 (f), CML 4177 (g), CML 4178 (h), CML 4179 (i) and CML 4180 (j); (k-l) *Fusarium pernambucanum* isolates: CML 4181 (k) and CML 4182 (l); (m-p) *Fusarium kalimantanense* isolates: CML 4183 (m), CML 4184 (n), CML 4185 (o) and CML 4186 (p); (q-s) *Fusaium* sp. isolates: CML 4187 (q), CML 4188 (r) and CML 4189 (s); (t) Control.

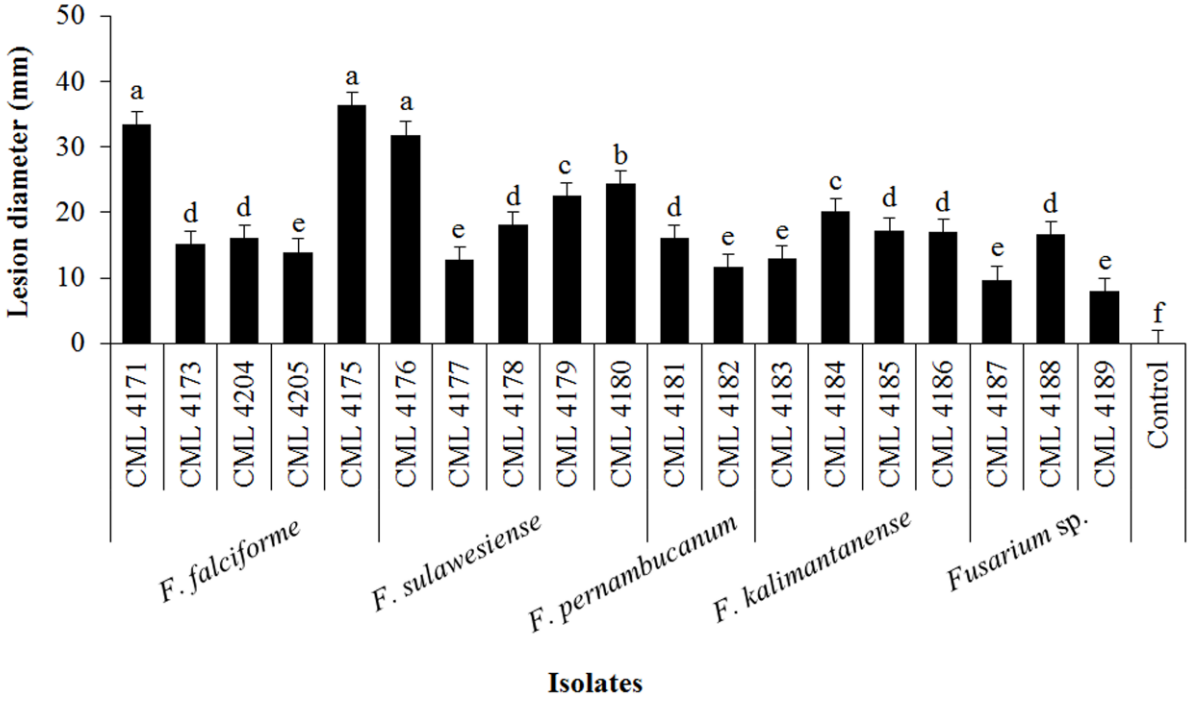

Figure 9. Diameter (mm) of lesions in Canary melon inoculated with *Fusarium* isolates. Means with the same letter are not significantly different by the Scott-Knott test ($p \leq 0.05$).

4 DISCUSSION

In this study, we characterized species of *Fusarium* collected at the main melon producing regions of Brazil, which have been facing difficulties due to damages and losses caused by the peduncular rot in the crop. Molecular phylogeny, morphological evaluation and pathogenic analyses allowed us to identify *F. falciforme* (FSSC), *F. sulawesiense*, *F. pernambucanum* (FIESC), *F. kalimantanense* (FOSC) and *Fusarium* sp. (FFSC) as the causal agents of the disease in different types of melon (Canary, Cantaloupe, Galia and Piel de Sapo). The diversity of species found draws the attention for the knowledge of the biologic, genetic and pathogenic relations of *Fusarium* species occurring on melon crop, therefore, our study may largely contribute to a precise diagnosis of the etiological agents of the peduncular rot and, consequently, for the adoption of management practices against this group of pathogens.

Although members of the FIESC are often reported associated with peduncular rot in melons, most of the isolates in our survey clustered within the FSSC, a complex that includes saprophyte and pathogenic fungi frequently isolated from the soil and plant materials. Species

of the clade 3 in FSSC, including *F. falciforme,* present relatively faster growth than other species, and, consequently, have a higher probability of dissemination and infection (O'Donnell et al., 2008; Sandoval-Denis et al., 2018). In this work, *F. falciforme* was the species that occurred most frequently associated with peduncular rot of the different melon cultivars. Recently, González et al. (2020) reported for the first time the occurrence of *F. falciforme* in melon plants in Spain. In Brazil, this species was identified for the first time causing wilting and root rot in melon plants cultivated in Rio Grande do Norte, Brazil (Silva, 2019). However, peduncular rot in the postharvest of melon caused by *F. falciforme* has not been previously reported and, to our knowledge, this is the first report of this species causing damage in melon fruits in Brazil.

Frequently, members of the FIESC are identified as causal agents of rot in melon (Dias & Terao, 2006; Li et al., 2019; Wonglom & Sunpapao, 2020; Lima et al., 2020). In our study, we found the species *F. sulawesiense*, isolated from Canary melon, and *F. pernambucanum*, from Galia and Piel de Sapo. In Brazil, *F. sulawesiense* was also isolated from Canary melon in the Northeast region, together with another phylogenetic species, close to *F. lacertarum* (Lima et al., 2020). The species *F. pernambucanum* was described by Santos et al. (2019), after reporting its occurrence on insects, and until now its potential to cause disease in plants was unknown, aside from its association with rice grains in Brazil (Avila et al., 2019). The species *F. sulawesiense* was initially described as a causal agent of rot in the pseudostem of banana plants (*Musa acuminata* var. Pisang Cere) (Maryani et al., 2019b), and later in postharvest causing crown rot (Wang et al., 2019). In general, fungi from the FIESC have been reported in different crops, especially in cereals, where they are related with mycotoxin contamination (Villani et al., 2016; O'Donnell et al., 2018).

Four isolates from Canary and Cantaloupe melons were identified as *F. kalimantanense*. The species has so far been reported in Indonesian banana plants (pseudostem), being described by Maryani et al. (2019a) as a new phylogenetic species within the FOSC, which, differently from other species in the complex, shows fast growth development and aseptate microconidia. The FOSC comprises the main soilborne pathogens causing vascular wilt, rot and plant death of several crops (Lombard et al., 2019). From this complex, *Fusarium oxysporum* f. sp. *melonis* has been reported causing wilting in melon plants (Lee et al., 2018), but sequences of this *forma specialis* did not group with *F. kalimantanense* (result not shown). The production of mycotoxin like beauvericin and fusaric acid have been considered as a virulence factor for the pathogenic species of the FOSC (López-Díaz et al., 2018).

The FFSC isolates were obtained from Canary melon and represent a novel phylogenetic species within this complex. The FFSC comprehends pathogenic species, mainly to grass hosts, in addition to others like fruit crops (Aoki et al., 2014). In melon, no report has described infections with species from this complex, which makes this the first case of a probable novel species occurring in this globally important crop. Lima et al. (2012) theorized that the expansion of crops to areas previously constituted of native vegetation contributed to the emergence of new diseases caused by locally adapted fungal species from the FFSC.

From the analysis performed, it was found that among the most aggressive isolates, two were *F. falciforme* (CML 4171 and CML 4175) and one was *F. sulawesiense* (CML 4176). The resulting symptoms were similar to those ones reported by melon farmers during the postharvest of the fruit, and other researchers (Dias & Terao, 2006; Oster et al., 2018), who identified the causal agent of such fruit rot as *F. pallidoroseum*, a member of the FIESC, based on morphological characters. Lesions were water-soaked, further developing a pale coloration, with abundant mycelial growth. The more aggressive isolates provoked cracking of the skin at the point of inoculation, seven days after inoculation. Except for the aggressiveness, there was no difference among species regarding the symptoms induced in inoculated fruits.

The origin of new *Fusarium* species in the fields of melon cultivation in the states CE and RN has not yet been elucidated, which makes it necessary to understand how these fungi were introduced in these areas. One hypothesis is the jump from an unknown host, for example, from native plant species to the culture of the melon or through cultural remains as a source of inoculum. Thus, the diversity of *Fusarium* species occurring in the postharvest of the melon can be a reflection of the occurrence of these fungi in the field. Once the cultivation becomes more intense, with low or none turnover with other crops, the population of opportunistic microorganisms increases and, consequently, new diseases arise, which may require the use of new management methods. Other assumptions are the endophytic association of such fungi, which colonize internal tissues of the fruit and, at some point, become pathogenic and cause disease, requiring a better understanding of the pathogen-host relationship, or these species were transmitted and introduced by seeds. Wonglom and Sunpapao (2020), evaluating the pathogenicity of *Fusarium* isolates from melon, verified that the infection only occurs after the fruit has been injured, inferring that the cut made in the peduncle during harvest procedures may promote the infection in the field. This may also indicate infection through natural openings present in some types of melon with a net-like

texture in the skin (Galia, Cantaloupe, Piel de Sapo), where symptoms may appear in the whole body of the fruit.

In the last two years, it has been observed that a constant increase in peduncular fruit rot of melon at the main producing areas of Brazil, with just a few cases where the symptoms could already be observed in the packing house. By contrast, most of the time, symptoms are frequently noted when fruits are in the importer market (Europe), bringing large economic losses. The studies reporting the actual species and causal agent of the problem are of great importance to melon producers and researchers of the crop, aiming at finding new strategies to minimize the damages. Given the information acquired with our study, we can suggest that there is an increase in the population of different *Fusarium* species in the field. This is certainly due to intensive cultivation of the crop, in addition to the adaptation of these fungi to infect different types of melon, due to their opportunistic flexibility to adapt to adverse environmental conditions, thus causing severe damage in postharvest.

The results from our study provide important information to producers of melon worldwide, as well as to future studies on the pathogen-host relationship, the development of less-resistant melon cultivars to the mentioned *Fusarium* spp. and the proper management of the disease. The knowledge about the causal agent is important to determine its susceptibility to chemical and natural fungicides and to develop management strategies to reduce the incidence of postharvest diseases in melon. Thus, our survey favors both melon production and exportation and contributes to maintain or even increase the employability of the agricultural sector, which is of great importance for the Brazilian Northeast region, considering the socio-economic aspects.

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Data Availability Statement

The data that support the findings of this study are openly available in GenBank at www.ncbi.nlm.nih.gov/genbank/

REFERENCES

Abrafrutas, 2018. Cenário Hortifruti Brasil 2018. https://abrafrutas.org/wp-content/uploads/2019/09/Relatorio-Hortifruti.pdf. [Acessed 10 March 2020].

Agrostat, 2020. Estatísticas de Comércio Exterior do Agronegócio Brasileiro. http://indicadores.agricultura.gov.br/agrostat/index.htm. [Acessed 10 September 2020].

Aoki, T., O'Donnell, K., Geiser, D. M. (2014). Systematics of key phytopathogenic *Fusarium* species: current status and future challenges. *Journal General Plant Pathology* **80**, 189–201.

Avila, C. F., Moreira, G. M., Nicolli, C. P. et al. (2019). *Fusarium incarnatum-equiseti* species complex associated with Brazilian rice: Phylogeny, morphology and toxigenic potential. *International Journal of Food Microbiology* **306**, 108267.

Castellani, A. (1939). Viability of somepathogenic fungi in distilled water. *Journal of Tropical Medicine and Hygiene* **42**, 225-226.

Darriba, D., Taboada, G. L., Doallo, R., Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**, 772.

Dias, R. C. S., Terao, D. (2006). Doenças das cucurbitáceas. In: *Patologia pós-colheita frutas, olerícolas e ornamentais tropicais.* Brasilia, DF: Embrapa Informação Tecnológica; Fortaleza: Embrapa Agroindústria Tropical, 595–627.

González, V., García-Martínez, S., Ruiz, J. J., Flores-León, A., Picó, B., Garcés-Claver, A. (2020). First report of *Neocosmospora falciformis* causing wilt and root rot of muskmelon in Spain. *Plant Disease* **104**, 1256.

Hepperle, D. 2004. SeqAssem©. Win32–Version. A sequence analysis tool contig assembler and trace data visualization tool for molecular sequences. Available at: http://www.sequentix.de.

Kist, B. B. et al. Anuário brasileiro de Horti & Fruti. Santa Cruz do Sul: Editora Gazeta Santa Cruz, 2019. [http://www.editoragazeta.com.br/sitewp/wp-content/uploads/2019/07/HortiFruti_2019_DUPLA.pdf]. Accessed 15 July 2020.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **35**, 1547–49.

Lee, S. M., Lee, W. J., Jang, K. S., Choi, Y. H., Kim, H., Choi, G. J. (2018). Resistance of commercial melon cultivars to isolates of *Fusarium oxysporum* f. sp. *melonis*. *Korean Journal of Horticultural Science and Technology* **36**, 577–84.

Leslie JF, Summerell BA, 2006. *The* Fusarium *Laboratory Manual*. Ames, Iowa, USA: Blackwell Publishing.

Li, Y. G., Zhang, R., Meng, L. et al. (2019). Occurrence of fruit rot of Cantaloupe caused by *Fusarium equiseti* in China. *Plant Disease* **103**, 2683.

Liu, Y. J., Whelen, S., Hall, B. D. (1999). Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Molecular Biology and Evolution* **16**, 1799–1808.

Lima, C. S., Pfenning, L. H., Costa, S. S., Abreu, L. M., Leslie, J. F. (2012). *Fusarium tupiense* sp. nov., a member of the *Gibberella fujikuroi* complex that causes mango malformation in Brazil. *Mycologia* **104**, 1408–14.

Lima, E. N., Oster, A. H., Bordallo, P. N., de Araújo, A. A. C., da Silva, D. E. M., Lima, C. S. (2020). A novel lineage in the *Fusarium incarnatum-equiseti* species complex is one of the causal agents of *Fusarium* rot on melon fruits in northeast Brazil. *Plant Pathology*. doi: 10.1111/ppa.13271

Lombard, L., Sandoval-Denis, M., Lamprecht, S. C., Crous, P. W. (2019). Epitypification of *Fusarium oxysporum* – clearing the taxonomic chaos. *Persoonia - Molecular Phylogeny and Evolution of Fungi* **43**, 1–47.

López-Díaz, C., Rahjoo, V., Sulyok, M. et al. (2018). Fusaric acid contributes to virulence of *Fusarium oxysporum* on plant and mammalian hosts. *Molecular Plant Pathology* **19**, 440–53.

Mahdikhani, M., Davoodi, A. (2016). Evaluation of biocontrol potential of rhizosphere antagonist bacterial strains on *Fusarium* wilt and plant growth in muskmelon plants. *American-Eurasian Journal of Sustainable Agriculture* **10**, 15–24.

Maryani, N., Lombard, L., Poerba, Y. S., Subandiyah, S., Crous, P. W., Kema, G. H. J. (2019a). Phylogeny and genetic diversity of the banana *Fusarium* wilt pathogen *Fusarium oxysporum* f. sp. *cubense* in the Indonesian centre of origin. *Studies in Mycology* **92**, 155–94.

Maryani, N., Sandoval-Denis, M., Lombard, L., Crous, P. W., Kema, G. H. J. (2019b). New endemic *Fusarium* species hitch-hiking with pathogenic *Fusarium* strains causing Panama disease in small-holder banana plots in Indonesia. *Persoonia - Molecular Phylogeny and Evolution of Fungi* **43**, 48–69.

Nuangmek, W., Aiduang, W., Suwannarach, N., Kumla, J., Kiatsiriroat, T., Lumyong, S. (2019). First report of fruit rot on Cantaloupe caused by *Fusarium equiseti* in Thailand. *Journal of General Plant Pathology* **85**, 295–300.

O'Donnell, K., Sutton, D. A., Fothergill, A., McCarthy, D., Rinaldi, M. G., Brandt, M. E., Zhang, N. et al. (2008). Molecular phylogenetic diversity, multilocus haplotype nomenclature, and in vitro antifungal resistance within the *Fusarium solani* species complex. *Journal of Clinical Microbiology* **46**, 2477–90.

O'Donnell, K., Sutton, D. A., Rinaldi, M. G., Gueidan, C., Crous, P. W., Geiser, D. M. (2009). Novel multilocus sequence typing scheme reveals high genetic diversity of human pathogenic members of the *Fusarium incarnatum-F. equiseti* and *F. chlamydosporum* species complexes within the United States. *Journal of Clinical Microbiology* **47**, 3851–61.

O'Donnell, K., McCormick, S. P., Busman, M. et al. (2018). Marasas et al. (1984). "Toxigenic *Fusarium* Species: Identity and mycotoxicology" revisited. *Mycologia* **110**, 1058–80.

Oster, A. H., Silva, E. de O, Borballo, P. N. et al. (2018). Luz ultravioleta pulsada no controle de podridão pós-colheita e na qualidade de melão para exportação. *Embrapa Agroindústria Tropical - Boletim de Pesquisa e Desenvolvimento (INFOTECA-E)*. (Boletim de pesquisa, 173).

Sandoval-Denis, M., Guarnaccia, V., Polizzi, G., Crous, P. W. (2018). Symptomatic *Citrus* trees reveal a new pathogenic lineage in *Fusarium* and two new *Neocosmospora* species. *Persoonia - Molecular Phylogeny and Evolution of Fungi* **40**, 1–25.

Santos, A. C. S., Trindade, J. V. C., Lima, C. S. et al. (2019). Morphology, phylogeny, and sexual stage of *Fusarium caatingaense* and *Fusarium pernambucanum*, new species of the *Fusarium incarnatum-equiseti* species complex associated with insects in Brazil. *Mycologia* **111**, 244–59.

Silva, S. G. A. (2019). *Análise filogenética e reação de genótipos de meloeiro a linhagens do complexo de espécies* Fusarium solani. Thesis, Mossoró, Universidade Federal Rural do Semi-Árido.

Sung, G-H, Sung, J-M, Hywel-Jones, N. L., Spatafora, J. W. (2007). A multi-gene phylogeny of Clavicipitaceae (Ascomycota, fungi): Identification of localized incongruence using a combinational bootstrap approach. *Molecular Phylogenetics and Evolution* **44**, 1204–23.

R Core Team, 2019. R: a language and environment for statistical computing. *R Foundation for Statistical Computing website*. https://www.R-project.org/. [Accessed 20 August 2019].Villani A, Moretti A, De Saeger S *et al.*, 2016. A polyphasic approach for characterization of a collection of cereal isolates of the *Fusarium incarnatum-equiseti* species complex. *International Journal of Food Microbiology* **234**, 24–35.

Wang, M. M., Chen, Q., Diao, Y. Z., Duan, W. J., Cai, L. (2019). *Fusarium incarnatum-equiseti* complex from china. *Persoonia - Molecular Phylogeny and Evolution of Fungi* **43**, 70–89.

Wonglom, P., Sunpapao, A. (2020). *Fusarium incarnatum* is associated with postharvest fruit rot of muskmelon (*Cucumis melo*). *Journal of Phytopathology* **168**, 204–10.

ATTACHMENT – Supporting Information

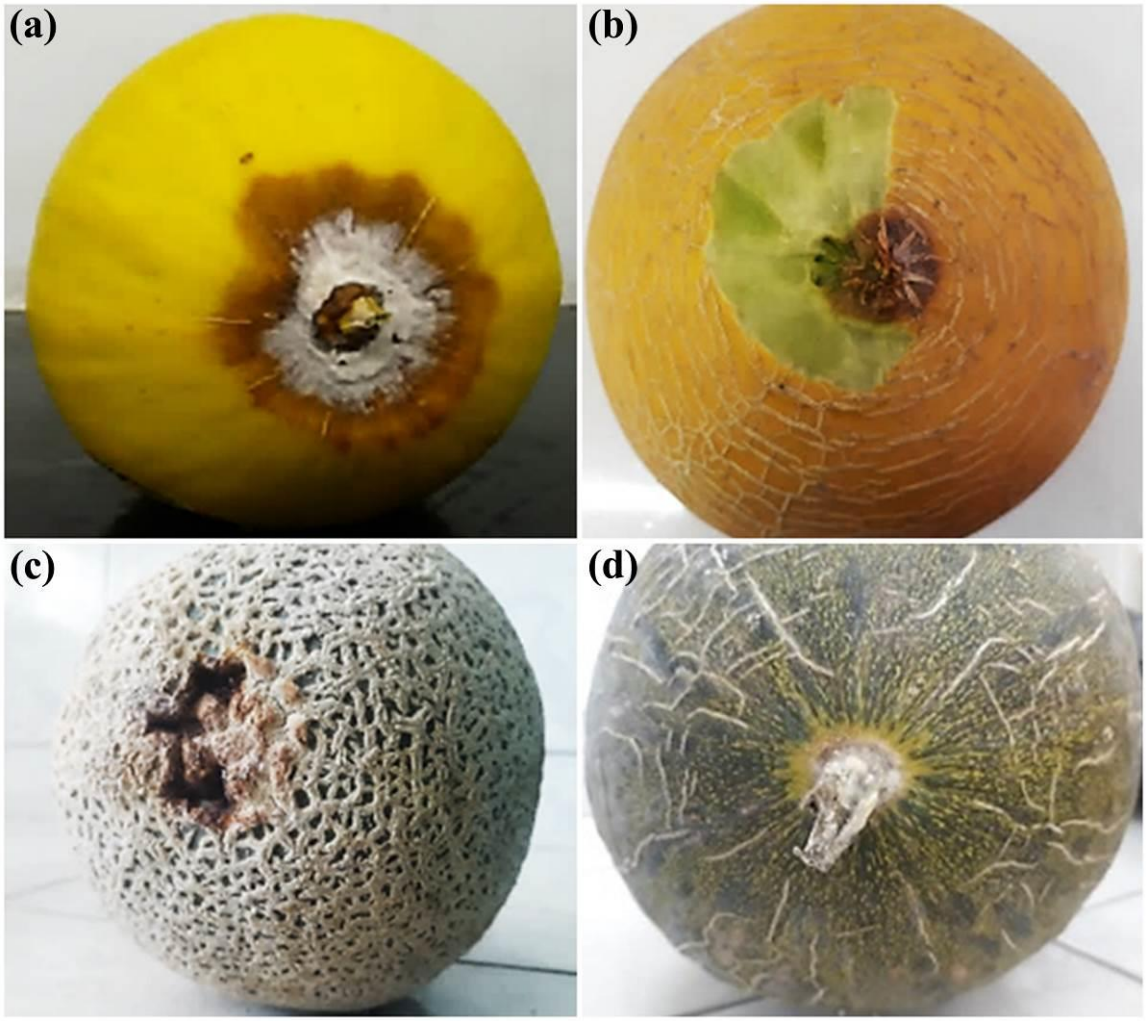

Figure S1. Melon fruits with symptoms of peduncular rot after harvest. Cultivars: (a) Canary; (b) Galia; (c) Cantaloupe and (d) Piel de Sapo.

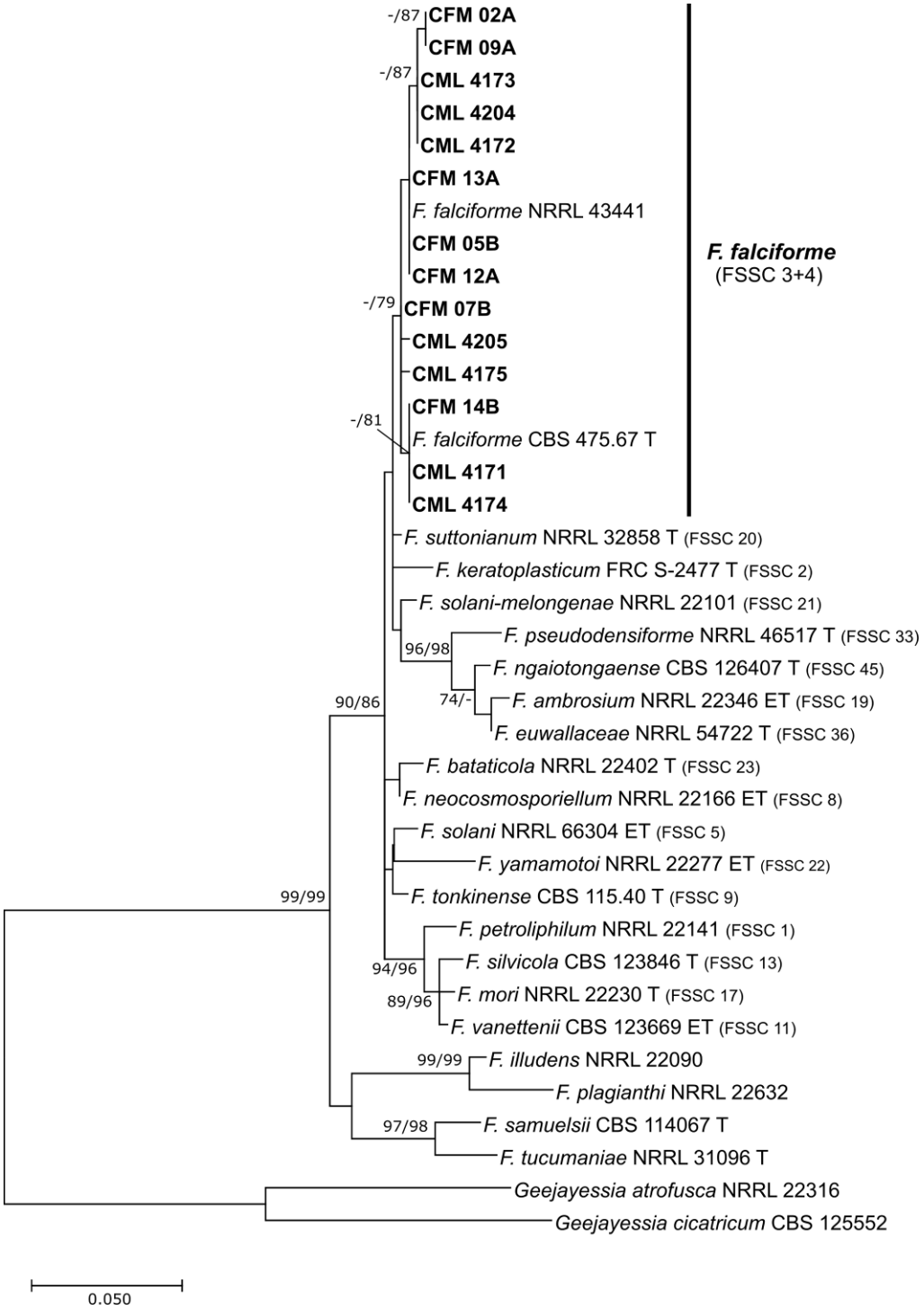

Figure S2. Maximum likelihood phylogram inferred from the sequences of the *EF-1α* gene fragment of *Fusarium solani* species complex. The codes in bold refer to the isolates from this study. Other codes concern to the reference sequences from the GenBank. Numbers of the phylogenetic lineages of the FSSC species are in parentheses. Bootstrap values ≥ 70% (1000 replications) for maximum likelihood and maximum parsimony are indicated in the internodes, respectively. Sequences from *Geejayessia atrofusca* (NRRL 22316) and *G. cicatricum* (CBS 125552) were used as outgroup. T = type strain; ET = epitype strain.

Table S1. GenBank sequence accession numbers of *Fusarium* strains included in the study to generate the phylograms.

Species complex[a] / Lineage	Species	Code[b]	Host / Substrate	Geographic origin	GenBank access number	
					EF-1α	***RPB2***
FCOSC	*F. concolor*	NRRL 13459	Plant debris	South Africa	GQ505674	GQ505852
FFSC	*F. andiyazi*	CBS 119857 T	*Sorghum bicolor*	South Africa	KR071718	KT154004
FFSC	*F. coicis*	NRRL 66233 T	*Coix gasteenii*	Australia	KP083251	KP083274
FFSC	*F. denticulatum*	NRRL 25302	*Ipomoea batatas*	USA	AF160269	LT996143
FFSC	*F. dlaminii*	NRRL 13164 T	Contact lens	South Africa	AF160277	KU171701
FFSC	*F. ficicrescens*	CBS 125178 T	*Ficus carica*	Iran	KP662899	KT154002
FFSC	*F. fredkrugeri*	CBS 144209 T	*Melhania acuminata* rhizosphere	South Africa	LT996097	LT996147
FFSC	*F. lactis*	NRRL 25200 NT	*Ficus carica*	USA	AF160272	LT996149
FFSC	*F. musae*	NRRL 25059 T	*Musa* sp.	Honduras	FN552086	FN552108
FFSC	*F. musae*	NRRL 25673	*Musa* sp.	Guatemala	FN552091	FN552113
FFSC	*F. napiforme*	NRRL 13604 T	*Pennisetum typhoides*	South Africa	AF160266	EF470117
FFSC	*F. nygamai*	NRRL 13448 T	*Sorghum bicolor*	Australia	AF160273	EF470114
FFSC	*F. phyllophilum*	NRRL 13617 T	*Dracaena deremensis*	Italy	AF160274	KF466410
FFSC	*F. pseudocircinatum*	NRRL 22946 T	*Solanum* sp.	Ghana	AF160271	LT996151
FFSC	*F. pseudonygamai*	NRRL 13592 T	*Pennisetum typhoides*	Nigeria	AF160263	LT996152
FFSC	*F. ramigenum*	NRRL 25208 T	*Ficus carica*	USA	AF160267	KF466412
FFSC	*F. sudanense*	CBS 454.97 T	*Striga hermonthica*	Sudan	KU711697	KU604266
FFSC	*F. terricola*	CBS 483.94 T	Soil	Australia	KU711698	KU604267
FFSC	*F. thapsinum*	NRRL 22045	*Sorghum bicolor*	South Africa	AF160270	JX171600
FFSC	*F. tjaetaba*	NRRL 66243 T	*Sorghum interjectum*	Australia	KP083263	KP083275
FFSC	*F. udum*	NRRL 22949	-	Germany	AF160275	LT996172
FFSC	*F. verticillioides*	NRRL 22172	*Zea mays*	Germany	AF160262	EF470122
FFSC	*F. verticillioides*	NRRL 20984	*Zea mays*	USA	FN552078	FN552100
FFSC	*F. xylarioides*	NRRL 25486 T	*Coffea robusta*	Uganda	AY707136	JX171630
FIESC	*F. aberrans*	CBS 131385 T	*Oryza australiensis*	Australia	MN170445	MN170378
FIESC	*F. humuli*	CQ1039 T	Humulus scandens	China	MK289570	MK289724
FIESC	*F. monophialidicum*	NRRL 54973	Rhinoceros eye	USA	MN170483	MN170416

Table S1. (Continued).

Species complex[a] / Lineage	Species	Code[b]	Host / Substrate	Geographic origin	GenBank access number	
					EF-1α	***RPB2***
FIESC	*F. persicinum*	CBS 479.83 T	Unknown	Unknown	MN170495	MN170428
FIESC 15	*F. irregulare*	LC7188 T	*Bamboo*	China	MK289629	MK289783
FIESC 16	*F. sulawesiensis*	InaCC F940 T	*Musa acuminata*	Indonesia	LS479443	LS479855
FIESC 16	*F. sulawesiensis*	NRRL 34004	Human BAL	USA	GQ505628	GQ505806
FIESC 17	*F. pernambucanum*	NRRL 32864	Human	USA	GQ505613	GQ505791
FIESC 17	*F. pernambucanum*	URM 7559 T	*Aleurocanthus woglumi*	Brazil	LS398489	LS398519
FIESC 18	*F. luffae*	LC12167 T	*Luffa aegyptiaca*	China	MK289601	MK289754
FIESC 20	*F. caatingaense*	URM 6779 T	*Dactylopius opuntiae*	Brazil	LS398466	LS398495
FIESC 21	*F. guilinense*	LC12160 T	*Musa nana*	China	MK289594	MK289747
FIESC 23	*F. incarnatum*	CBS 132.73 NT	*Trichosanthes dioica*	Malawi	MN170409	MN170476
FIESC 25	*F. nanum*	LC12168 T	*Musa nana*	China	MK289602	MK289755
FIESC 26	*F. hainanense*	LC11638 T	*Oryza* sp.	China	MK289581	MK289735
FIESC 28	*F. coffeatum*	CBS 635.76 T	*Cynodon lemfuensis*	South Africa	MN120755	MN120736
FIESC 29	*F. citri*	LC6896 T	*Citrus reticulata*	China	MK289617	MK289771
FOSC	*F. callistephi*	CBS 187.53 T	*Callistephus chinensis*	The Netherlands	MH484966	MH484875
FOSC	*F. carminascens*	CBS 144738 T	*Zea mays*	South Africa	MH485028	MH484937
FOSC	*F. contaminatum*	CBS 114899 T	Pasteurized chocolate milk	Germany	MH484992	MH484901
FOSC	*F. cugenangense*	InaCC F984 T	*Musa* sp.	Indonesia	LS479757	LS479308
FOSC	*F. curvatum*	CBS 238.94 T	*Beaucarnia* sp.	The Netherlands	MH484984	MH484893
FOSC	*F. duoseptatum*	NRRL 36115	*Musa sapientum*	Malaysia	MH484987	MH484896
FOSC	*F. elaeidis*	CBS 217.49 T	*Elaeis* sp.	Zaire	MH484961	MH484870
FOSC	*F. fabacearum*	CBS 144743 T	*Glycine max*	South Africa	MH485030	MH484939
FOSC	*F. foetens*	NRRL 38302	*Pinus radiata*	Chile	FJ985444	JX171652
FOSC	*Fusarium* sp.	NRRL 52690	*Zulia colombiana*	Colombia	JF740775	JF741101
FOSC	*Fusarium* sp.	NRRL 52691	*Zulia colombiana*	Colombia	JF740776	JF741102
FOSC	*F. glycines*	CBS 144746 T	*Glycine max*	South Africa	MH485033	MH484942
FOSC	*F. gossypinum*	CBS 116613 T	*Gossypium hirsutum*	Ivory Coast	MH485000	MH484909
FOSC	*F. grosmichelii*	NRRL 36120	*Musa sapientum*	Thailand	FJ985331	LS479222

Table S1. (Continued).

Species complex[a] / Lineage	Species	Code[b]	Host / Substrate	Geographic origin	GenBank access number	
					EF-1α	***RPB2***
FOSC	*F. hexaseptatum*	InaCC F866 T	*Musa acuminata*	Indonesia	LS479805	LS479359
FOSC	*F. hoodiae*	CBS 132474 T	*Hoodia gordonii*	South Africa	MH485020	MH484929
FOSC	*F. inflexum*	NRRL 20433 T	*Vicia faba*	Germany	AF008479	JX171583
FOSC	*F. kalimantanense*	InaCC F917 T	*Musa acuminata*	Indonesia	LS479690	LS479241
FOSC	*F. languescens*	CBS 645.78 T	*Solanum lycopersicum*	Morocco	MH484971	MH484880
FOSC	*F. libertatis*	CBS 144749 T	Rock surface	South Africa	MH485035	MH484944
FOSC	*F. nirenbergiae*	CBS 840.88 T	*Dianthus caryophyllus*	The Netherlands	MH484978	MH484887
FOSC	*F. odoratissimum*	InaCC F822 T	*Musa* sp.	Indonesia	LS479828	LS479386
FOSC	*F. oxysporum*	NRRL 22902	*Pseudotsuga menziesii*	USA	AF160312	LT575065
FOSC	*F. oxysporum*	CBS 144134 ET	*Solanum tuberosum*	Germany	MH485044	MH484953
FOSC	*F. pharetrum*	CBS 144751 T	*Aliodendron dichotomum*	South Africa	MH485042	MH484951
FOSC	*F. phialophorum*	InaCC F971 T	*Musa* sp.	Indonesia	LS479741	LS479292
FOSC	*F. purpurascens*	InaCC F886 T	*Musa* sp.	Indonesia	LS479827	LS479385
FOSC	*F. sangayamense*	InaCC F960 T	*Musa* sp.	Indonesia	LS479732	LS479283
FOSC	*F. tardichlamydosporum*	InaCC F958 T	*Musa acuminata*	Indonesia	LS479729	LS479280
FOSC	*F. tardicrescens*	NRRL 36113 T	*Musa* sp.	Malawi	LS479665	LS479217
FOSC	*F. triseptatum*	NRRL 36389 T	*Ipomoea batatas*	USA	FJ985352	MH484873
FOSC	*F. veterinarium*	CBS 109898 T	Shark peritoneum	The Netherlands	MH484990	MH484899
FSSC	*F. illudens*	NRRL 22090	*Beilschmiedia tawa*	New Zealand	AF178326	JX171601
FSSC	*F. plagianthi*	NRRL 22632	*Hoheria glabrata*	New Zealand	AF178354	JX171614
FSSC	*F. tucumaniae*	NRRL 31096 T	*Glycine max*	Argentina	AY220181	GU170600
FSSC	*F. samuelsii*	CBS 114067 T	Bark	Guyana	LR583644	LR583874
FSSC 1	*F. petroliphilum*	NRRL 22141	*Cucurbita* sp.	New Zealand	AF178329	EU329491
FSSC 2	*F. keratoplasticum*	FRC S-2477 T	Indoor plumbing	USA	JN235712	JN235897
FSSC 3+4	*F. falciforme*	CBS 475.67 T	Human mycetoma	Puerto Rico	LT906669	LT960558
FSSC 3+4	*F. falciforme*	NRRL 43441	Human cornea	USA	DQ790478	DQ790566
FSSC 5	*F. solani*	NRRL 66304 ET	*Solanum tuberosum*	Slovenia	KT313611	KT313623
FSSC 8	*F. neocosmosporiellum*	NRRL 22166 ET	*Heterodera glycines*	USA	EU329497	EU329497

Table S1. (Continued).

Species complex[a] / Lineage	Species	Code[b]	Host / Substrate	Geographic origin	GenBank access number	
					EF-1α	***RPB2***
FSSC 9	*F. tonkinense*	CBS 115.40 T	*Musa sapientum*	Vietnam	LT906672	LT960564
FSSC 11	*F. vanettenii*	CBS 123669 ET	*Pisum sativum* and soil	USA	LR583636	LR583862
FSSC 13	*F. silvicola*	CBS 123846 T	*Liriodendron tulipifera*	USA	LR583646	LR583876
FSSC 17	*F. mori*	NRRL 22230 T	*Morus alba*	Japan	AF178358	EU329499
FSSC 19	*F. ambrosium*	NRRL 22346 ET	*Euwallacea fornicatus*	India	FJ240350	EU329503
FSSC 20	*F. suttonianum*	NRRL 32858 T	Human wound	USA	DQ247163	EU329630
FSSC 21	*F. solani-melongenae*	NRRL 22101	Cotton cloth	Panama	AF178333	EU329490
FSSC 22	*F. yamamotoi*	NRRL 22277 ET	*Xanthoxylum piperitum*	Japan	AF178336	FJ240380
FSSC 23	*F. bataticola*	NRRL 22402 T	*Ipomoea batatas*	USA	AF178344	FJ240381
FSSC 33	*F. pseudodensiforme*	NRRL 46517 T	Dead tree	Sri Lanka	DQ247512	KC691645
FSSC 36	*F. euwallaceae*	NRRL 54722 T	*Euwallacea* sp.	Israel	JQ038007	JQ038028
FSSC 45	*F. ngaiotongaense*	CBS 126407 T	Tree bark	New Zealand	LR583621	LR583846
	Geejayessia atrofusca	NRRL 22316	*Staphylea trifolia*	USA	AF178361	EU329502
	Geejayessia cicatricum	CBS 125552	Dead twig	Slovenia	HM626644	HQ728153

[a]FCOSC = *Fusarium concolor* species complex; FFSC = *F. fujikuroi* species complex; FIESC = *F. incarnatum-equiseti* species complex; FOSC = *F. oxysporum* species complex.

[b]CBS = Westerdijk Fungal Biodiverity Institute, Utrecht, The Netherlands; CQ = Working Collection of Q. Chen, State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, P.R. China; FRC = Fusarium Research Center, Penn State University, Pennsylvania, USA; InaCC = Indonesian Culture Collection, Research Center for Biology, Indonesian Institute of Science, Cibinong, Indonesia; LC = working collection of Lei Cai, State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, P.R. China; NRRL = ARS Culture Collection, Peoria, Illinois, USA; URM = Universidade do Recife Micologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil. T = type strain; ET = epitype strain; NT = neotype strain.

CHAPTER III

ALTERNATIVE DISEASE MANAGEMENT OF PEDUNCULAR ROT IN MELON, CAUSED BY *Fusarium falciforme*

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Abstract

Peduncular rot (PR), caused by several species of the genus *Fusarium*, is one of the most challenging postharvest diseases faced by melon producers in Brazil. Symptoms appear late and, consequently, hinders disease management. Currently, there is no effective method to control the disease. In the present study different commercial products were assessed, such as currently used agricultural products, essential oils (EOs), and calcium chloride ($CaCl_2$), in combination with thermotherapy in the management of PR in Canary melon, in fruits inoculated with *Fusarium falciforme* and non-inoculated fruits. The experiments were conducted twice, with the second as a repetition of the first. Fruits, from both experiments, treated only with hot water at 58 °C for 30 seconds had lower incidence and severity of PR, compared to non thermo-treated fruits. Under conditions of high humidity (average precipataion of 265 mm during the period of production), the combination of thermotherapy with Copper Crop® (8 mL/L) or with Citronella EO (25 v/v) was efficient to control peduncular rot in melon for up to 30 days, and thermotherapy with Serenade® (10 mL/L) or $CaCl_2$ controlled the disease for 40 days in storage. In the absence of thermotherapy, Compost Aid® and Citronella EO controlled PR for 30 and 40 days, respectively, under the same conditions. Under low humidity (average precipitation of 4 mm during the period of

production), combination of thermotherapy with Compost Aid® (2 g/L) controlled the disease for 30 days, and the combination with Nem Out® (8 g/L), Enzimatic (10 mL/L), Copper Crop®, Citronella EO and Melaleuca EO (2.5% v/v) or $CaCl_2$, provided protection for up to 40 days in storage. At this same condition, the Enzimatic and Copper Crop® were efficient at controlling the disease for up to 30 and 40 days, respectively. Based on the results from this study it is possible to suggest for the managment of PR in melon, showing alternatives to replace imazalil, which is applied to control postharvest diseases in melon. No treatment has negatively affected fruit quality, keeping them free of any damage due to heat or phytotoxicity, with fruit firmness and soluble solids content remaining within the market standards.

Keywords: Alternative control. *Cucumis melo*. Postharvest disease. Thermotherapy.

1 INTRODUCTION

Melon (*Cucumis melo* L.) is one of the most appreciated vegetables worldwide, with a high productivity in Brazil, especially in the Northeast region. In 2018, 197.6 thousand tons of the fruit were exported, generating a revenue of US$ 136 mi, with the states of Ceará (CE) and Rio Grande do Norte (RN) producing together 423.8 thousand tons in this period, contributing to approximately 95% of the total national production, and more than 60% of the exportation of melon (IBGE, 2019). Exported fruits are mainly destinated to European countries like Spain, Netherlands, and the United Kingdom, however, the comercialization with the Chinese market has been initiated, due to the consumption potential of the country (Kist et al., 2019; Abrafrutas, 2020).

Among the major diseases affecting melon, fusariosis caused by several species of the genus *Fusarium*, causes root rot and wilting of plants. In addition to these damages in the field, postharvest losses due to *Fusarium* increase season after season. For instance, peduncular rot (PR), characterized by the darkening of the peduncular region and abundant mycelial growth, becomes one of the main obstacles for exportation of melons from Brazil, since first symptoms are often observed only 10 to 15 days after harvest (under refrigeration) when fruits have already been shipped overseas. Most of the time, symptoms are visible only when in the importer country, bringing major economic losses for producers (Terao et al., 2006). The process of fungal infection in the fruit is yet to be understood, however, those

postharvest pathogens survive in the soil and on plant residue as saprophytes. It is believed that infection takes place in the field, through wounds caused when cutting the peduncle of the fruit (Viana et al., 2001), but endophytic colonization of the fruits by the fungus should also be considered and a better understanding of such pathogen-host relationship is required.

The treatment of melon fruits with imazalil is considered an efficient measure to control PR caused by *Fusarium pallidoroseum* in Canary melons (Terao et al., 2009). Even though this chemical is registered for postharvest treatments, there is no registration for control of *Fusarium* in the database of the Brazilian Ministry of Agriculture, Livestock and Supply (Ministério da Agricultura, Pecuária e Abastecimento – MAPA). Since imazalil was put on the spotlight for its toxicological risk of residues in food, there is a major concern in case it is banned for application as a postharvest treatment in melon. Many alternatives to control PR are under investigation, aiming to control postharvest diseases. The interest in biocontrol increases with the demand of consumers concerned with their health and the environment, pushing the market towards new biocontrol agents in order to reduce the impacts caused by toxic chemicals.

Products which have antagonist microorganisms in their composition usually harbour bacteria from the genus *Bacillus* as biocontrol agent because of their many properties against plant pathogens, acting in different ways (Fira et al., 2018). The use of essential oils (EOs) is another efficient method in the control of plant pathogens. The essential oil (EO) of Citronella (*Cymbopogon nardus* (L.) Rendle) is used in the pharmaceutical industry due to its repellent properties, moreover, it has shown good activity in the control of bacteria and fungi (Ali et al., 2017). The Melaleuca EO (*Melaleuca alternifolia* Cheel), rich in terpinen-4-ol, is also a microbial suppressor due to its effect on the integrity of membranes and the inhibition of cellular respiration (Yadav et al., 2017). It is also important to point out the use of chemical compounds that boost the fruit resistance against pathogen infection as a treatment in postharvest, e.g. the Calcium chloride ($CaCl_2$), which improves fruit firmness, delays ripening and, consequently, hampers the early development of diseases (Ferraz et al., 2016).

The thermotherapy has been practiced by melon producing companies in Israel as a postharvest treatment, in which fruits are immersed in hot water, or in combination with brushing of fruits, to reduce contamination by *Fusarium* and *Alternaria* on fruit surface (Usall et al., 2016), however, in Brazil such practice is not yet implemented in the production lines. Sivakumar and Fallik (2013) observed that when Galia melons were treated in hot water (58 °C) for 15 seconds the postharvest loss reduced from 15 to 2%, suggesting an efficient

alternative for the managment of postharvest rots. Given the above, the combination of different control techniques might be more efficient than when used separately. Thus, the objective of the present work was to assess the effect of different commercial products (currently used in agriculture, EO, and $CaCl_2$) in combination with thermotherapy on the management of PR in melon.

2 MATERIAL AND METHODS

2.1 Pathogen

The pathogen *Fusarium falciforme* was obtained from our collection and previously identified by sequencing EF1-1α and RPB2 gene regions (GenBank accession No. MT476611/MT461687). It was isolated from melon fruits (Piel de Sapo) and showed high aggressiveness in Canary melon (unpublished data). The isolate was cultured on potato dextrose agar (PDA) for five days, and incubated in BOD at 25 ± 2 °C (12 h of photoperiod) to conduct the *in vitro* assays. For the *in vivo* assays, a spore suspension of the isolate was prepared using seven-days-old cultures (prepared under the same conditions as previously mentioned), for a final concentration of 10^6 conidia/mL, determined using a hemocytometer (Yushu et al., 2017).

2.2 *In vitro* experiment

Firstly, we conducted an *in vitro* assay in the lab, to test the effect of different commercial products on the growth of *Fusarium falciforme* (Table 1). The following treatments were tested: control (PDA only); Magnate®, following manufacturer's recommended dose (2 mL/L); Compost Aid® (2, 4, 8 and 10 g/L); Nem Out® (2, 4, 8 and 10 g/L); Serenade® (2, 4, 8 and 10 mL/L); Enzimatic (2, 4, 8 and 10 mL/L); Copper Crop® (2, 4, 8 and 10 mL/L); Citronella EO (1, 2 and 2.5% v/v); Melaleuca EO (1, 2 and 2.5% v/v); Tween 20 (1% v/v) and $CaCl_2$ (2% v/v). Each material was amended with molten PDA (about 50 °C) and poured onto Petri dishes. One disc of 5 mm-diameter was transferred from the edge of a 5-days-old colony to the center of each plate (mycelium facing down). The plates were stored for seven days in BOD at 25 ± 2 ºC (12 h photoperiod). A completely randomized

experimental design was used, with 30 treatments and four replicates, where each Petri dish corresponded to one replicate.

Table 1

Composition of products used for control of peduncular rot in melon.

Product	Formulation*	Active ingredients / Composition
Magnate®	EC	Imazalil 500 g/L
Compost Aid®	WP	*Lactobacillus plantarum* ($1,5x10^6$ CFU/g) + *Enterococcus faecium* ($1,5x10^6$ CFU/g) + *Bacillus subtilis* ($1,5x10^6$ CFU/g) + Enzymes (amylase, cellulase, protease)
Nem Out®	WP	*Bacillus licheniformis* ($3,75x10^8$ CFU/g) + *Bacillus subtilis* ($3,75x10^8$ CFU/g) + Enzymes (amylase, cellulase, protease)
Serenade®	SC	*Bacillus subtilis* ($1,05x10^{12}$ CFU/L)
Enzimatic	SC	Secondary metabolites from the controlled microbial multiplication + Copper + Enzymes (xylanase, hemicellulase, lignase)
Copper Crop®	Liquid	Copper + Nitrogen + Amino acids
Citronella oil	Liquid	Citronella extract (geraniol, citronelol, citronelal)
Melaleuca oil	Liquid	Melaleuca extract (terpinen-4-ol)
Calcium chloride	Powder	Calcium chloride ($CaCl_2$)

*EC, emulsifiable concentrate; WP, wettable powder; SC, suspension concentrate.

Treatments in which EO was applied used Tween-20 as an emulsifier (at a rate of 1% of the oil volume). Tween-20 was also tested to verify any effect on fungal growth. Plates with PDA only and Magnate® were used as absolute and positive controls, respectively. Although calcium chloride is classified as a resistance inductor in plants, it was also tested *in vitro* in order to assess any potential effect on the pathogen growth.

The growth inhibition was assessed after the 7^{th} day of incubation, calculating the Percentage of Mycelial Growth Inhibition (PMGI), given by PMGI (%) = [CG – TG / CG] x 100, where CG is the growth of the absolute control (mm) and TG is the growth of a given treatment (mm). Data were transformed according to the Aligned Rank Transform (ART) methodology for non-parametric analysis with the ARTool software (Wobbrock et al., 2011). The analysis of variance (ANOVA) was performed with the SAS PROC GLM (version 9.2) (SAS Institute Inc., Cary, NC) (Durner, 2019) to study the effect of the mycelial growth

inhibition of each treatment and the means were compared using the Scott-Knott test ($p \leq 0.05$).

2.3 *In vivo* experiment (I)

The *in vivo* experiment consisted of an evaluation of the control of PR, combining the best *in vitro* treatments with and without thermotherapy of fruits inoculated with *F. falciforme* and non-artificially inoculated fruits (Supplementary Fig. S1). The experiment *in vivo* I was conducted in April 2019 (fruits produced under high humidity condition, average precipitation of 265 mm) in Icapuí, Brazil (4º51'27"S, 37º20'56"W). One hundred Canary melon fruits were used for each assay (inoculated and non-inoculated fruits), hence 200 fruits in total, harvested in the commercial maturity stage. The fruits were sanitized in chlorine water (150 ppm, pH 5.5 ± 0.5) and dried at room temperature. Each melon was punctured with a set of three needles of 0.5 mm diameter each at four equidistant points in the base of the peduncle, at a depth of 3 mm.

Fruits were inoculated with 5 µL of the spore suspension in each of the four punctured sites (20 µL of spore suspension per fruit). In order to assure fungal penetration, fruits were kept standing for two hours before treatment application (adapted from Oliveira et al., 2014). For non-inoculated fruits, sterile distilled water (SDW) was used instead of spore suspension. Half of the non-inoculated and inoculated fruits were submitted to thermotherapy in a steel (AISI 304) heating tank with capacity for 500 L (KR Hidrojat), through immersion in hot water (58 ºC) for 30 s. After drying at room temperature (28 ± 2 °C), fruits were treated: control (SDW); Magnate® (2 ml/L); Compost Aid® (2 g/L); Nem Out® (8 g/L); Serenade® (10 mL/L); Enzimatic (10 mL/L); Copper Crop® (8 mL/L); Citronella EO (2% v/v); Melaleuca EO (2.5% v/v) and $CaCl_2$ (2% v/v). The application was made by soaking a wad of sterile cotton in the solution and wetting the peduncle region of the fruit (0.5 mL per fruit). Tween-20 was added to the solutions of essential oils (1% of the oil volume used). Fruits were let to dry, packed in paper boxes, and stored in cold room at 10 ± 2 ºC and relative humidity of 85 ± 2% for 40 days. The experiments were conducted under a completely randomized experimental design in a 2x10 (thermotherapy x products + control) factorial scheme, with five replicates.

The evaluations took place at 30 and 40 days of storage (DS), simulating the length of time for shipment of fruits to Europe (up to 30 days) and Asia (maximum of 40 days), with

individual statistical analysis for each period of evaluation. The following parameters were assessed: disease incidence (INC), as of absence or presence of lesions in the peduncular region, in percentage, and disease severity (SEV), according to a rating scale from 0 to 5, from Terao et al. (2006), where: (0) no lesions; (1) lesions of up to 10 mm; (2) lesions between 11 and 20 mm; (3) lesions between 21 and 40 mm; (4) lesions between 41 and 60 mm, and (5) lesions larger than 60 mm in diameter.

Data were transformed according to the Aligned Rank Transform (ART) methodology for non-parametric factorial analysis with the ARTool software (Wobbrock et al., 2011). The analysis of variance (ANOVA) was performed with the SAS PROC GLM (version 9.2) (SAS Institute Inc., Cary, NC) (Durner, 2019) to study the effect of temperature, products and their interactive effect on the peduncular rot of fruits, and the means were compared using the T-test ($p \leq 0.05$).

2.4 *In vivo* experiment (II)

The second experiment, considered as a repetition of the first, was conducted in July 2019 (fruits produced under low humidity condition, average precipitation of 4 mm) in Mossoró, Brazil (4º54’29”S, 37º24’05”W) (Supplementary Fig. S1). The *in vivo* II was conducted and evaluated in the same conditions as detailed before, adding one extra step for the assessment of fruit quality at 40 DS: external and internal quality, fruit firmness, and soluble solids content were recorded. For the assessment of internal and external quality, lesions in the peduncular region, patches, depression, physical damage, internal pulp collapse, and loose seeds were noted, classifying them according to a rating scale: (1) extremely severe damage (> 50% of the fruit); (2) severe damage (31-50%); (3) moderate damage (11-30%); (4) mild defect (1-10%), and (5) no lesions. Fruits with a score of 3.0 or less were considered as unmarketable (Aroucha et al., 2012). The internal quality was recorded as presence (1) or absence (0) of internal rot (Fig. 1).

Fig. 1. Rating score for external quality (A-E) and internal quality (F-H) of melon in fruits treated for control of peduncular rot, at 40 days of storage in cold room. (A) Score 1 = extremely severe damage; (B) Score 2 = severe damage; (C) Score 3 = moderate damage; (D) Score 4 = mild defect; (E) Score 5 = no lesions; (F e G) Fruits showing internal deterioration in the peduncular region, and (H) Healthy fruit, no lesions, adapted from Aroucha et al. 2012.

To assess fruit firmness, fruits were lengthwise sliced in two halves and the readings were taken in four spots in the equatorial region of the fruit, using a penetrometer (Instrutherm®, PTR-100) pressure of 7.9 mm diameter, drilling to 10 mm deep in the pulp. The values obtained were converted into Newton (N) using a conversion factor of 9.80665. The soluble solids content was measured with the aid of a portable refractometer (Instruthem®, RT-30ATC), expressing results in degree Brix (°Brix). Data from external and internal quality were transformed using the Aligned Rank Transform (ART) methodology for non-parametric factorial analysis, using the ARTool software (Wobbrock et al., 2011). The analysis of variance (ANOVA) was performed with the SAS PROC GLM (version 9.2) (SAS Institute Inc., Cary, NC) (Durner, 2019) and the means were compared using the T-test ($p \leq 0.05$). The fruit firmness and soluble solids content data showed normal distribution and were submitted to ANOVA using the R statistical software (version 3.6.1) (R Development Core Team, 2019) and means were compared using the T-test ($p \leq 0.05$).

3 RESULTS

3.1 *In vitro* experiment

There was a significant difference ($p \leq 0.05$) among treatments on the PMGI of the fungus. The greatest effects on inhibition of mycelial growth were observed on treatments Enzimatic (10 mL/L), Copper Crop® (8 and 10 mL/L) and Citronella EO (2 and 2.5%), not differing from the control with Magnate®, which fully inhibited the mycelial growth of *F. falciforme* until the 7th day of incubation (Table 2).

Table 2

Minimum inhibitory concentration of different products on mycelial growth of *Fusarium falciforme*.

Product	PMGI (%)*	Product	PMGI (%)*
Control	N/A	Enzimatic 4 mL/L	9.9 g
Magnate® 2 ml/L	100.0 a	Enzimatic 8 mL/L	51.6 e
Compost Aid® 2 g/L	70.2 b	Enzimatic 10 mL/L	100.0 a
Compost Aid® 4 g/L	60.1 d	Copper Crop® 2 mL/L	26.1 f
Compost Aid® 8 g/L	47.6 e	Copper Crop® 4 mL/L	95.9 a
Compost Aid® 10 g/L	69.8 b	Copper Crop® 8 mL/L	100.0 a
Nem Out® 2 g/L	55.4 d	Copper Crop® 10 mL/L	100.0 a
Nem Out® 4 g/L	51.2 e	Citronella oil 1%	59.8 d
Nem Out® 8 g/L	73.5 b	Citronella oil 2%	100.0 a
Nem Out® 10 g/L	67.3 c	Citronella oil 2.5%	100.0 a
Serenade® 2 mL/L	63.9 c	Melaleuca oil 1%	15.9 f
Serenade® 4 mL/L	57.1 d	Melaleuca oil 2%	28.0 f
Serenade® 8 mL/L	71.7 b	Melaleuca oil 2.5%	40.1 e
Serenade® 10 mL/L	75.8 b	Tween-20 1%	9.2 g
Enzimatic 2 mL/L	5.1 g	Calcium chloride 2%	6.2 g

*Percentage of Mycelial Growth Inhibition (PMGI); Not applicable (N/A). Different letters indicate the significant differences within column, according to Scott-Knott test ($p \leq 0.05$).

The Compost Aid® had the greatest inhibitory effect when applied at a rate of 2 g/L, inhibiting 70% of mycelial growth. Nem Out® at a rate of 8 g/L showed the greatest effect, with 73% of inhibition, while Serenade® (10 mL/L) inhibited the pathogen growth by 76%. Although the treatments with Melaleuca EO (2.5% v/v), calcium chloride (2% v/v), and Tween-20 (1% v/v) showed low inhibitory potential, there was still some inhibitory action of 40, 6 and 9%, respectively, regarding the control (Supplementary Fig. S2).

3.2 *In vivo* experiment (I)

A significant effect ($p \leq 0.05$) was observed for interaction of thermotherapy and products for INC and SEV, for both non-inoculated and fruits inoculated with *F. falciforme.* The symptoms in fruits stored in cold room started between 15 and 30 DS, with progressive increase of lesions in the peduncle. Fruits treated with thermotherapy showed lower INC and SEV, in most treatments.

3.2.1 *Fruits artificially inoculated with F. falciforme*

At 30 DS, significant difference was observed between treatments with and without thermotherapy for INC, where the thermotherapy in combination with Copper Crop® and with Citronella EO promoted the greatest reduction of INC, regrading the control. The absolute values show absence of PR in such treatments (0% of incidence), and also in treatments treated with Compost Aid® alone, which differed from the other treatments without thermotherapy. By analysing the isolated effect of thermotherapy, from the absolute values, a reduction of 60% in INC of treated fruits was observed when comparing to non-thermo treated fruits. Greater reduction of INC was observed when thermotherapy was used with Cooper Crop®, Citronella EO and Melaleuca EO, in comparison to the same treatments without thermotherapy. There was no difference among treatments with thermotherapy for SEV at 30 DS, however, Cooper Crop® and Citronella EO reduced SEV when compared to the same treatments without thermotherapy (Table 3 and Supplementary Fig. S3).

For INC and SEV at 40 DS, there was no difference between fruits under thermotherapy, but thermotherapy in combination with Copper Crop® had 80% less INC, and the largest reduction in SEV, in comparison to the same treatments without thermotherapy. There was no difference in the INC of treatments without thermotherapy, but Compost Aid® statistically differed from the other treatments for SEV, with less PR injuries (Table 3 and supplementary Fig. S3).

Table 3

Disease incidence and severity of peduncular rot in melon fruits inoculated with *F. falciforme*, with or without thermotherapy, in combination with different products, at 30 and 40 days of storage in cold room (*in vivo* experiment I).

Treatments*	30 days of storage				40 days of storage			
	Average rank of incidence		Average rank of severity		Average rank of incidence		Average rank of severity	
	TT	WT	TT	WT	TT	WT	TT	WT
Control	37.5 (20) abA	67.5 (80) bcB	38.3 (1) aA	70.7 (4) bB	62.0 (80) aA	62.0 (80) aA	56.7 (4) aA	68.4 (5) bA
Magnate®	37.5 (20) abA	47.5 (40) abA	38.3 (1) aA	54.9 (3) abA	32.0 (20) aA	42.0 (40) aA	33.6 (2) aA	45.2 (3) abA
Compost Aid®	57.5 (60) bcB	27.5 (0) aA	51.4 (2) aA	27.5 (0) aA	52.0 (60) aA	42.0 (40) aA	56.0 (4) aA	35.6 (2) aA
Nem Out®	47.5 (40) abcA	57.5 (60) bcA	45.7 (2) aA	45.7 (3) bA	42.0 (40) aA	62.0 (80) aA	45.2 (3) aA	68.4 (5) bA
Serenade®	67.5 (80) cA	67.5 (80) bcA	55.4 (3) aA	76.5 (4) bA	62.0 (80) aA	62.0 (80) aA	58.8 (4) aA	68.4 (5) bA
Enzimatic	37.5 (20) abA	47.5 (40) abA	34.9 (1) aA	52.0 (3) abA	42.0 (40) aA	42.0 (40) aA	33.2 (1) aA	45.2 (3) abA
Copper Crop®	27.5 (0) aA	77.5 (100) cB	27.5 (0) aA	72.5 (4) bB	32.0 (20) aA	72.0 (100) aB	27.4 (1) aA	73.8 (5) bB
Citronella Oil	27.5 (0) aA	57.5 (60) bcB	27.5 (0) aA	59.4 (2) bB	52.0 (60) aA	52.0 (60) aA	37.5 (2) aA	56.8 (3) abA
Melaleuca Oil	47.5 (40) abcA	77.5 (100) cB	45.7 (2) aA	71.3 (3) bA	42.0 (40) aA	72.0 (100) aA	39.0 (2) aA	70.4 (5) bA
$CaCl_2$	37.5 (20) abA	57.5 (60) bcA	38.3 (1) aA	62.8 (3) bA	32.0 (20) aA	52.0 (60) aA	33.6 (2) aA	56.8 (4) abA

*Thermotherapy (TT); Without thermotherapy (WT). Values in brackets are absolute values of disease incidence (%) and severity [rating scale: (0) no lesions; (1) lesions of up to 10 mm; (2) lesions between 11 and 20 mm; (3) lesions between 21 and 40 mm; (4) lesions between 41 and 60 mm, and (5) lesions larger than 60 mm in diameter]. Different lowercase letters indicate the significant differences within thermotherapy (with or without) treatments, and uppercase letters between thermotherapy and without thermotherapy, according to T-test ($p \leq 0.05$).

3.2.2 *Fruits non-artificially inoculated with F. falciforme*

No significant difference was observed among treatments under thermotherapy for INC at 30 DS, however the absolute values show that fruits treated with thermotherapy together with Serenade®, Copper Crop® and $CaCl_2$ presented no symptoms (0% of PR incidence). In fruits not treated with thermotherapy, Citronella EO had the lowest INC (0%) in comparison to other treatments. Also, Serenade® and $CaCl_2$ induced the largest reduction of INC when applied after thermotherapy, with significant difference between thermo- and non thermo-treated fruits. For SEV, there was a significant difference among treatments under thermotherapy, with Cooper Crop® and Citronella EO showing the best results, and absolut values also showing absence of PR in fruits treated with Serenede® and $CaCl_2$, similarly to what was observed for INC. Among the fruits not treated with thermotherapy, Compost Aid® had the greatest average for SEV, differing from other treatments, although fruits treated with Citronella EO did not present any symptoms of the disease. When comparing both thermo- and non thermo-treated fruits, treatments with Copper Crop®, Citronella EO and Melaleuca EO had the best performance when combined with thermotherapy, and the opposite is true for Compost Aid®, which had lower SEV when applied without thermotherapy (Table 4 and Supplementary Fig. S4).

At 40 DS, there was significant difference between treatments with and without thermotherapy. Fruits treated with thermotherapy in association with Serenade® and $CaCl_2$ had a reduction of 60% in INC and lower SEV, in comparison to the control, with absolute values showing absence of PR on such fruits. Significant difference was also observed in these treatments when combined or not with the thermotherapy, with 100% of reduction in INC and SEV in fruits treated with thermotherapy. Among fruits without thermotherapy, those ones treated with Citronella EO presented the lowest INC and SEV, with a reduction of 40% in INC when compared to the control (Table 4 and Supplementary Fig. S4).

Table 4

Disease incidence and severity of peduncular rot in non-inoculated melons, with or without thermotherapy, in combination with different products, at 30 and 40 days of storage in cold room (*in vivo* experiment I).

Treatments*	30 days of storage				40 days of storage			
	Average rank of incidence		Average rank of severity		Average rank of incidence		Average rank of severity	
	TT	WT	TT	WT	TT	WT	TT	WT
Control	52.0 (40) aA	62.0 (60) bcA	37.5 (2) abA	67.5 (3) bcB	53.0 (60) bcA	53.0 (60) abcA	45.7 (3) abcA	52.6 (3) abcA
Magnate®	42.0 (20) aA	62.0 (60) bcA	37.5 (1) abA	47.5 (2) abA	53.0 (60) bcA	63.0 (80) bcA	45.7 (3) abcA	62.9 (3) bcA
Compost Aid®	52.0 (40) aA	52.0 (40) abA	57.5 (2) bcB	27.5 (3) aA	63.0 (80) bcA	53.0 (60) abcA	58.5 (4) cA	61.4 (4) bcA
Nem Out®	42.0 (20) aA	52.0 (40) abA	47.5 (2) abcA	57.5 (3) bcA	43.0 (40) abcA	63.0 (80) bcA	41.7 (3) abcA	60.4 (4) abcA
Serenade®	32.0 (0) aA	72.0 (80) bcB	67.5 (0) cA	67.5 (4) bcA	23.0 (0) aA	73.0 (100) cB	23.0 (0) aA	82.6 (5) cB
Enzimatic	52.0 (40) aA	52.0 (40) abA	37.5 (3) abA	47.5 (2) abA	53.0 (60) cA	53.0 (60) abcA	57.0 (3) bcA	52.6 (3) abcA
Copper Crop®	32.0 (0) aA	52.0 (40) abA	27.5 (0) aA	77.5 (3) cB	33.0 (20) abA	43.0 (40) abA	27.8 (1) abA	48.6 (3) abA
Citronella Oil	42.0 (20) aA	32.0 (0) aA	27.5 (1) aA	57.5 (0) bcB	43.0 (40) abcA	33.0 (20) aA	37.3 (2) abcA	27.8 (1) aA
Melaleuca Oil	52.0 (40) aA	62.0 (60) bcA	47.5 (2) abcA	77.5 (3) cB	53.0 (60) bcA	63.0 (80) bcA	57.0 (4) bcA	69.8 (5) bcA
$CaCl_2$	32.0 (0) aA	82.0 (100) cB	37.5 (0) abA	57.5 (4) bcA	23.0 (0) aA	73.0 (100) cB	23.0 (0) aA	74.6 (5) bcB

*Thermotherapy (TT); Without thermotherapy (WT). Values in brackets are absolute values of disease incidence (%) and severity [rating scale: (0) no lesions; (1) lesions of up to 10 mm; (2) lesions between 11 and 20 mm; (3) lesions between 21 and 40 mm; (4) lesions between 41 and 60 mm, and (5) lesions larger than 60 mm in diameter]. Different lowercase letters indicate the significant differences within thermotherapy (with or without) treatments, and uppercase letters between thermotherapy and without thermotherapy, according to T-test ($p \leq 0.05$).

3.3 *In vivo* experiment (II)

At 30 and 40 DS, there was significant interaction ($p \leq 0.05$) of thermotherapy and products for INC and SEV, for both non-inoculated and fruits inoculated with *F. falciforme*. As reported in the first *in vivo* experiment, most fruits treated with thermotherapy showed lower INC and SEV. The symptoms in fruits stored in a cold room started between 20 and 30 DS, with progressive increase of lesions throughout the storage period.

3.3.1 *Fruits artificially inoculated with F. falciforme*

There was no significant difference at 30 DS among treatments under thermotherapy for INC and SEV, however, the absolute values show absence of PR in fruits treated with thermotherapy in combination with Magnate®, Compost Aid®, Nem Out®, Copper Crop®, Melaleuca EO and the $CaCl_2$ (0% of incidence). When applied after thermotherapy, the Compost Aid®, Nem Out®, Enzimatic and Copper Crop® presented greatest reduction in INC and SEV, when compared to the same treatments without thermotherapy. Among fruits without thermotherapy, Magnate® and $CaCl_2$ differed from the other treatments, with 40 and 20% of reduction of INC, respectively, in comparison to the control, and the absolute value of severity in fruits treated with Magnate® showed absence of PR at 30 DS (Table 5 and Supplementary Fig. S5).

In fruits under thermotherapy at 40 DS, no significant difference was registered for INC and SEV, however, absolute data show absence of PR in fruits treated with thermotherapy in combination with Nem Out®, Copper Crop®, Melaleuca EO and $CaCl_2$. As previously observed, thermotherapy in combination with Compost Aid®, Nem Out®, Enzimatic and Copper Crop®, at 40 DS, promoted lower INC and SEV, in comparison to the same treatments without thermotherapy. Among fruits without thermotherapy, Magnate® was the best treatment, with the greatest reduction of INC and SEV (40%) in comparison to the control (absence of PR at 40 DS) (Table 5 and Supplementary Fig. S5).

Table 5

Disease incidence and severity of peduncular rot in melon fruits inoculated with *F. falciforme*, with or without thermotherapy, in combination with different products, at 30 and 40 days of storage in cold room (*in vivo* experiment II).

Treatments*	30 days of storage				40 days of storage			
	Average rank of incidence		Average rank of severity		Average rank of incidence		Average rank of severity	
	TT	WT	TT	WT	TT	WT	TT	WT
Control	36.0 (0) aA	56.0 (40) abA	36.0 (0) aA	59.6 (3) bcB	34.5 (0) aA	54.5 (40) abcA	34.0 (0) aA	55.5 (4) abcA
Magnate®	36.0 (0) aA	36.0 (0) aA	36.0 (0) aA	36.0 (0) aA	44.5 (20) aA	34.5 (0) aA	45.0 (2) aA	34.5 (0) aA
Compost Aid®	36.0 (0) aA	86.0 (100) cB	36.0 (0) aA	86.5 (4) dB	44.5 (20) aA	84.5 (100) dB	41.4 (1) aA	87.0 (5) cdB
Nem Out®	36.0 (0) aA	76.0 (80) bcB	36.0 (0) aA	74.7 (3) cdB	34.0 (0) aA	74.5 (80) cdB	34.5 (0) aA	76.5 (5) cdB
Serenade®	46.0 (20) aA	56.0 (40) abA	43.5 (1) aA	55.3 (2) abcA	44.5 (20) aA	64.5 (60) bcdA	42.1 (1) aA	59.4 (3) bdA
Enzimatic	46.0 (20) aA	76.0 (80) bcB	44.5 (1) aA	77.0 (4) cdB	44.5 (20) aA	74.5 (80) cdB	45.0 (2) aA	76.5 (5) cdB
Copper Crop®	36.0 (0) aA	76.0 (80) bcB	36.0 (0) aA	75.1 (3) cdB	34.5 (0) aA	74.5 (80) cdB	34.5 (0) aA	76.5 (5) cdB
Citronella Oil	46.0 (20) aA	56.0 (40) abA	44.5 (1) aA	59.6 (3) bcA	44.5 (20) aA	54.5 (40) abcA	42.1 (2) aA	55.5 (3) abcA
Melaleuca Oil	36.0 (0) aA	56.0 (40) abA	36.0 (0) aA	55.8 (3) abcA	34.5 (0) aA	54.5 (40) abcA	34.5 (0) aA	55.5 (3) abcA
$CaCl_2$	36.0 (0) aA	46.0 (20) aA	36.0 (0) aA	45.9 (1) abA	34.5 (0) aA	44.5 (20) abA	34.5 (0) aA	45.0 (2) abA

*Thermotherapy (TT); Without thermotherapy (WT). Values in brackets are absolute values of disease incidence (%) and severity [rating scale: (0) no lesions; (1) lesions of up to 10 mm; (2) lesions between 11 and 20 mm; (3) lesions between 21 and 40 mm; (4) lesions between 41 and 60 mm, and (5) lesions larger than 60 mm in diameter]. Different lowercase letters indicate the significant differences within thermotherapy (with or without) treatments, and uppercase letters between thermotherapy and without thermotherapy, according to T-test ($p \leq 0.05$).

3.3.2 *Fruits non-artificially inoculated with F. falciforme*

There was no significant difference in INC and SEV for treatments with thermotherapy at 30 DS, however from the absolute values we observe no incidence of PR on fruits treated with Magnate®, Nem Out®, Enzimatic, Citronela EO, Melaleuca EO and $CaCl_2$. Nem Out® and Melaleuca EO promoted the greatest reduction in INC when applied after thermotherapy, reducing PR in up to 60 and 80%, respectively, more than the same treatments without thermotherapy, and for SEV, both products and the Compost Aid® also performed better when combined with thermotherapy. Among fruits treated without thermotherapy, at 30 DS, those with Enzimatic and Copper Crop® did not show PR symptoms, with a reduction in INC and SEV of 60%, in comparison to the control (Table 6 and Supplementary Fig. S6).

For INC and SEV of PR at 40 DS, no significant difference was observed for treatments with thermotherapy, but as mentioned previously, fruits treated with Magnate®, Nem Out®, Enzimatic, Citronella EO, Melaleuca EO and $CaCl_2$ had no indicence of PR. Treatments with Compost Aid®, Nem Out® and both essential oils performed better at 40 DS when combined with thermotherapy, for reduction of INC and SEV of PR, in comparison to the same treatments without thermotherapy. Among treatments without thermotherapy, Copper Crop® was again the best treatment against the PR, reducing in 60% the INC and in 80% the SEV, with no diseased fruits (0% of incidence). Thermotherapy alone promoted a reduction of asymptomatic fruits of up to 60% when compared to fruits without thermotherapy (absolute values), up to 40 DS (Table 6 and Supplementary Fig. S6).

Table 6

Disease incidence and severity of peduncular rot in non-inoculated melons, with or without thermotherapy, in combination with different products, at 30 and 40 days of storage in cold room (*in vivo* experiment II).

Treatments*	30 days of storage				40 days of storage			
	Average rank of incidence		Average rank of severity		Average rank of incidence		Average rank of severity	
	TT	WT	TT	WT	TT	WT	TT	WT
Control	39.0 (0) aA	69.0 (60) bcB	39.0 (0) aA	69.0 (4) bcB	36.5 (0) aA	66.5 (60) bcB	36.5 (0) aA	65.7 (4) bcB
Magnate®	39.0 (0) aA	59.0 (40) abcA	39.0 (0) aA	56.2 (2) abcA	36.5 (0) aA	56.5 (40) abcA	36.5 (0) aA	55.1 (3) abcA
Compost Aid®	49.0 (20) aA	69.0 (60) bcA	47.6 (1) aA	72.4 (3) bcB	46.5 (20) aA	76.5 (80) cB	47.1 (2) aA	78.9 (5) cB
Nem Out®	39.0 (0) aA	69.0 (60) bcB	39.0 (0) aA	66.4 (3) abcB	36.5 (0) aA	76.5 (80) cB	36.5 (0) aA	75.7 (4) cB
Serenade®	49.0 (20) aA	69.0 (60) bcA	47.6 (1) aA	69.4 (3) abcA	46.5 (20) aA	66.5 (60) bcA	47.1 (2) aA	68.3 (4) bcA
Enzimatic	39.0 (0) aA	39.0 (0) aA	39.0 (0) aA	39.0 (0) aA	36.5 (0) aA	46.5 (20) abA	36.5 (0) aA	44.5 (1) abA
Copper Crop®	49.0 (20) aA	39.0 (0) aA	49.2 (1) aA	39.0 (0) aA	46.5 (20) aA	36.5 (0) aA	47.1 (2) aA	36.5 (0) aA
Citronella Oil	30.0 (0) aA	49.0 (20) abA	39.0 (0) aA	47.6 (1) abA	36.5 (0) aA	66.5 (60) bcB	36.5 (0) aA	62.5 (3) bcB
Melaleuca Oil	39.0 (0) aA	79.0 (80) cB	39.0 (0) aA	82.6 (4) cB	36.5 (0) aA	76.5 (80) cB	36.5 (0) aA	78.9 (5) cB
$CaCl_2$	39.0 (0) aA	49.0 (20) abA	39.0 (0) aA	50.6 (2) abcA	36.5 (0) aA	46.5 (20) abA	36.5 (0) aA	47.1 (2) abA

*Thermotherapy (TT); Without thermotherapy (WT). Values in brackets are absolute values of disease incidence (%) and severity [rating scale: (0) no lesions; (1) lesions of up to 10 mm; (2) lesions between 11 and 20 mm; (3) lesions between 21 and 40 mm; (4) lesions between 41 and 60 mm, and (5) lesions larger than 60 mm in diameter]. Different lowercase letters indicate the significant differences within thermotherapy (with or without) treatments, and uppercase letters between thermotherapy and without thermotherapy, according to T-test ($p \leq 0.05$).

3.3.3 *Fruit quality assessment*

The thermotherapy in both inoculated and non-inoculated fruits showed better results for external and internal quality, regarding the presence of patches, depressions, symptoms of rot in the peduncular region, internal rot and loose seeds, which influenced in the fruit firmness, with a significant difference between fruits treated with and without thermotherapy.

3.3.3.1 *Fruits artificially inoculated*

A significant difference ($p \leq 0.05$) was observed for the interactive effect between thermotherapy and products on the external quality of fruits. Even with no difference among fruits treated with thermotherapy, it is possible to observe that Compost Aid®, Nem Out® and Copper Crop® had better results for external quality when combined with thermotherapy. In fruits without thermotherapy, the Melaleuca EO provided the best results for external quality. Regarding the internal quality, there was no difference between treatments with and without thermotherapy, but the fruits treated with Compost Aid®, Nem Out®, Enzimatic, Copper Crop® and the Citronella EO, showed best values when treated with thermotherapy, compared to non-thermo treated fruits (Fig. 2).

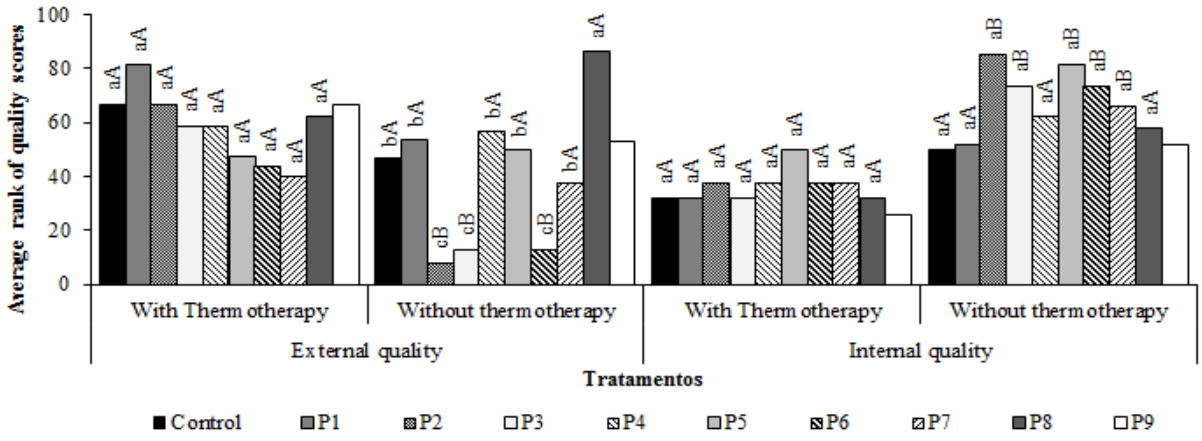

Fig. 2. External and internal quality of melon fruits inoculated with *F. falciforme*, with or without thermotherapy, in combination with different products, at 40 days of storage in cold room. Magnate® (P1); Compost Aid® (P2); Nem Out® (P3); Serenade® (P4); Enzimatic (P5); Copper Crop® (P6); Citronella essential oil (P7); Melaleuca essential oil (P8); $CaCl_2$ (P9). Different lowercase letters indicate the significant differences within thermotherapy (with or without) treatments, and uppercase letters between thermotherapy and without thermotherapy, according to Scott-Knott test ($p \leq 0.05$).

For fruit firmness, a significant difference ($p \leq 0.05$) was observed with the treatment combination of thermotherapy and products. Treatments in fruits with thermotherapy showed no significant difference, unlike the non-thermo treated fruits, which showed better results when Serenade® and the Melaleuca EO were applied, reporting 37 N and 33.4 N, respectively. A significant difference was noticed between Nem Out® and Enzimatic in comparison to the thermotherapy, occurring better results under thermotherapy relative to the non-thermo treated fruits. No noticeable differences were observed in soluble solids content among treatments with and without thermotherapy (Table 7).

Table 7

Fruit firmness and soluble solids content of melon inoculated with *Fusarium falciforme* under the effect of thermotherapy, or not, and different products, at 40 days of storage in cold room.

Treatment*	Fruit firmness (N)		Soluble solids content (° Brix)	
	With Thermotherapy	Without Thermotherapy	With Thermotherapy	Without Thermotherapy
Control	30.6 aA	19.8 bB	10.9 aA	11.3 aA
Magnate®	30.2 aA	20.6 bA	11.2 aA	11.4 aA
Compost Aid®	26.0 aA	16.8 bA	12.2 aA	11.7 aA
Nem Out®	33.6 aA	19.4 bB	10.1 aA	11.6 aA
Serenade®	29.8 aA	37.0 aA	11.6 aA	11.2 aA
Enzimatic	31.4 aA	8.6 bB	11.7 aA	11.8 aA
Copper Crop®	26.6 aA	18.0 bA	11.5 aA	11.6 aA
Citronella oil	25.8 aA	21.2 bA	11.8 aA	11.9 aA
Melaleuca oil	28.2 aA	33.4 aA	12.0 aA	10.8 aA
$CaCl_2$	24.6 aA	22.2 bA	10.8 aA	10.7 aA

*Different letters (lowercase within columns, uppercase within rows) indicate the significant differences, according to Scott-Knott test ($p \leq 0.05$).

3.3.3.2 *Non-artificially inoculated fruits*

There was a significant difference ($p \leq 0.05$) in the interactive effect of thermotherapy and products on the external and internal quality of fruits. For the external quality, there was no difference among the treatments with thermotherapy, however the thermotherapy combined with Compost Aid®, Nem Out®, and Serenade® showed better results when compared to the same non-thermo treated treatments. Fruits in which no thermotherapy treatment was applied showed best external quality with $CaCl_2$. For internal quality, no difference was verified between treatments with thermotherapy, but the Magnate®, Compost

Aid®, Nem Out®, Serenade® and both EOs (Citronella and Melaleuca) had better results after thermotherapy in comparison to the same non-thermo treatments. Among fruits without thermotherapy, the Magnate®, Compost Aid®, Enzimatic, Copper Crop® and $CaCl_2$ had the best results for internal appearance (Fig. 3).

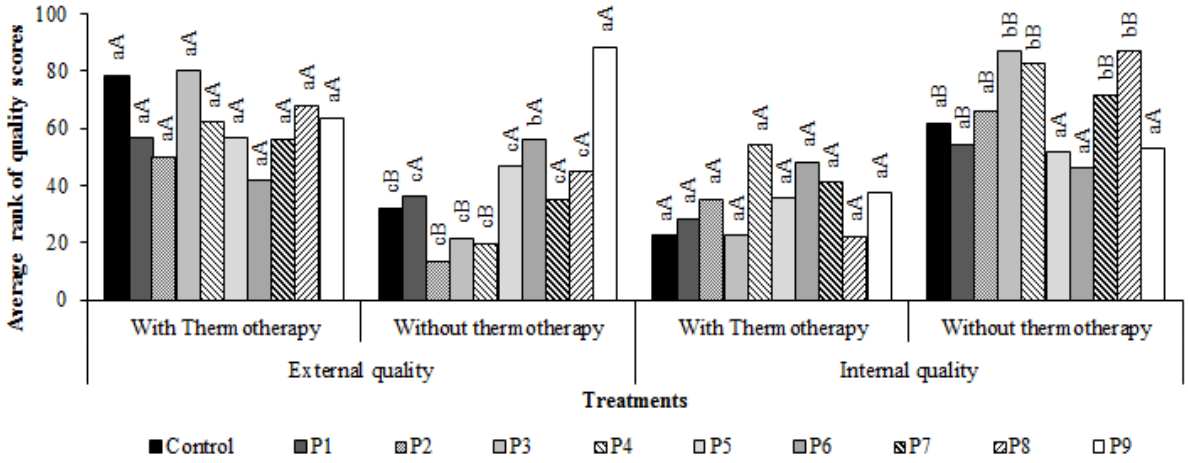

Fig. 3. External and internal quality of non-inoculated melon, with or without thermotherapy, in combination with different products, at 40 days of storage in cold room. Magnate® (P1); Compost Aid® (P2); Nem Out® (P3); Serenade® (P4); Enzimatic (P5); Copper Crop® (P6); Citronella essential oil (P7); Melaleuca essential oil (P8); $CaCl_2$ (P9). Different lowercase letters indicate the significant differences within thermotherapy (with or without) treatments, and uppercase letters between thermotherapy and without thermotherapy, according to Scott-Knott test ($p \leq 0.05$).

For the fruit firmness, the interactive effect of both factors (thermotherapy and products) showed a significant difference ($p \leq 0.05$). The thermotherapy combined with the application of Compost Aid® (27 N), Nem Out® (27.6 N), Enzimatic (30.4 N), Melaleuca EO (34.2 N) and $CaCl_2$ (28.4 N) showed the best results at 40 DS for the firmness. For fruits without thermotherapy, there was no difference among treatments, but the thermotherapy combined with Nem Out®, Enzimatic, and Melaleuca EO promoted greater firmness of fruits, in comparison to the same treatments only with products mentioned. No noticeable differences were observed in soluble solids content among treatments with and without thermotherapy (Table 8).

Table 8

Fruit firmness and soluble solids content of melon non-inoculated with *Fusarium falciforme* under the effect of thermotherapy, or not, and different products, at 40 days of storage in cold room.

Treatment*	Fruit firmness (N)		Soluble solids content (° Brix)	
	With Thermotherapy	Without Thermotherapy	With Thermotherapy	Without Thermotherapy
Control	25.0 bA	22.2 aA	11.1 aA	11.3 aA
Magnate®	21.8 bA	22.8 aA	11.8 aA	12.2 aA
Compost Aid®	27.0 aA	20.4 aA	11.3 aA	12.3 aA
Nem Out®	27.6 aA	15.8 aB	12.0 aA	13.6 aA
Serenade®	21.6 bA	15.0 aA	12.1 aA	13.3 aA
Enzimatic	30.4 aA	20.4 aB	11.8 aA	13.1 aA
Copper Crop®	19.4 bA	25.6 aA	12.3 aA	12.8 aA
Citronella Oil	23.4 bA	15.6 aA	12.9 aA	13.0 aA
Melaleuca Oil	34.2 aA	16.6 aB	11.1 aA	11.9 aA
$CaCl_2$	28.4 aA	20.2 aA	11.8 aA	12.9 aA

*Different letters (lowercase within columns, uppercase within rows) indicate the significant differences, according to Scott-Knott test ($p \leq 0.05$).

4 DISCUSSION

Not many studies are focused on the management of melon diseases caused by the genus *Fusarium,* although there is an increasing demand for efficient measures against rot caused by such pathogens. Moreover, there is a lack of choices when looking for chemicals registered in the MAPA for control of *Fusarium* in postharvest. The present study addressed the use of thermotherapy and different products to manage the PR in melon, simulating the time needed for fruits to reach markets in Europe and Asia, since the largest share of the Brazilian production is shipped to Europe and takes up to 30 days traveling by sea and, after a recent deal, the exportation to China has started, demanding at least 40 days for fruits to be available in the shelves of Chinese retailers. The first experiment (*in vivo* I), conducted during the period of higher humidity in the Brazilian semiarid region, showed higher incidence of PR than the second experiment (*in vivo* II), which took place during the season of low humidity. It is known that rain favours the dissemination of the pathogen and, with the increase of umidity in high temperature, the environment became more favorable for disease

development. High humidity stimulates the process of infection of *Fusarium* and increases host susceptibility, affecting the disease progression and severity (Oliveira et al., 2014).

In both experiments, fruits treated only with thermotherapy at 58 °C for 30 seconds presented lower INC and SEV when compared to those treatments without thermotherapy, and in the experiment *in vivo* II, both inoculated and non-inoculated fruits had 0% of incidence when treated with thermotherapy. Fallik et al. (2000) observed that postharvest treatment with hot water affects directly fungi that colonize the external surface of fruits by reducing spore germination (weakening or killing) and restricting penetration by sealing natural openings, thus it maintains fruits firm and, consequently, delays disease development. This method is not commonly used on a commercial scale in production lines of melon in Brazil, but it is becoming a popular method to control postharvest melon diseases in other countries (Israel, Egypt, Marroco) given its easy application, not time spending, and for being an efficient control of diseases maintaining postharvest quality (Usall et al., 2016). In spite of its efficiency, there is a common concern with the costs of electricity and the amount of clean water required for the application of such products. Oster et al. (2018) reported that about 15% of the exported melon is lost due to postharvest rots, accounting for US$ 22 mi every season, showing the importance of a cost-benefit analysis on the use of thermotherapy in different melon producing regions around the world.

Overall, we observed that the combination of thermotherapy with most products (except for Serenade® and Enzimatic) has controlled PR up to 30 DS in fruits inoculated with *F. falciforme* and, for 40 DS, in fruits treated with thermotherapy combined with Nem Out®, Copper Crop®, Melaleuca EO and $CaCl_2$. In non-inoculated fruits, thermotherapy in association with the products inhibited 100% of disease for up to 30 DS (except for Compost Aid® and Copper Crop®). The majority of fruits in storage for 40 days also did not show any symptoms of PR, except for the ones treated with Magnate®, Compost Aid® and Copper Crop®. With these results, we observe that the thermotherapy alone is an efficient method to control the PR for up to 40 days, and in its absence, melon producers cultivating under high humidity levels, could opt to use the Compost Aid® (control for up to 30 DS) or Citronella EO, to market fruits within 40 days. During the melon season (period of low humidity), farmers may choose Cooper Crop® (control for up to 40 DS) as an alternative measure to replace imazalil applications. It is important mentioning that Enzimatic has controled disease for 30 DS and, although it is yet to be registered, its commercialization in the future will be another option in the management of peduncular rot of melon, free of chemical residues.

The demand for products using biocontrol agents as a mean to manage plant diseases is consistently increasing due to the concerns with pathogens developing resistance to chemicals, as a result of indiscriminate use (Nicot et al., 2016), as well as the tight restrictions on the use of convencial chemicals. The products Compost Aid®, Nem Out®, Serenade®, and Enzimatic are produced with microbial antagonists and/or using their metabolites, which have shown to be effective against the PR caused by *F. falciforme* in melon fruits in this study. According to the manufacturer, the Compost Aid® is an additive constituted by antagonists (*Lactobacillus plantarum*, *Enterococcus faecium* and *Bacillus subtilis*) and enzymes, which favors the composting process. *Lactobacillus plantarum* produces a compound with an antimicrobial activity called plantaricin, which may have inhibited the growth of *F. falciforme*, as observed by Baffoni et al. (2015), when using *L. plantarum* and *B. amyloliquefaciens*-based inoculants to control *F. culmorum* and *F. graminearum*. *Enterococcus faecium*, in turn, produces antimicrobial lactic acid, besides being recommended for use in postharvest processes to increase food security (Fhoula et al., 2013).

Bacillus subtilis, which is present in the composition of Compost Aid®, Nem Out® and Serenade®, is a strong suppressor of plant diseases due to the production of bactericide and fungitoxic compounds. The species is well studied as a biological control agent of a wide range of plant pathogens because of its many antagonist mechanisms (Braga Junior et al., 2017). Several studies have shown the activity of *B. subtilis* on *Fusarium* species, with positive results towards its control (Abdelmoteleb et al., 2017; De Senna and Lathrop, 2017; Zaim et al., 2018). Although both Nem Out® and Serenade® have *Bacillus* spp. in their formulation, the concentrations used in our study were not sufficient to suppress the PR in melon when they were used alone, but the disease was controlled for up to 40 days when they were used in combination with thermotherapy in both inoculated and non-inoculated fruits, contrasting with the Compost Aid®, which in the absence of thermotherapy it controlled the disease for up to 30 days in fruits inoculated with *F. falciforme*, in the experiment *in vivo* I.

It is important to elucidate that the Enzimatic does not consist of a commercial product but rather a compost of secondary metabolites from the fermentation of *Bacillus* and *Lactobacillus*, which has been shown satisfactory results against some pathogens, such as *Alternaria* sp., *Aspergillus* spp., *Colletotrichum* sp., *Fusarium* sp., *Lasiodiplodia* sp., on seeds (Nascimento et al., 2019) and in postharvest of papaya against anthracnose disease (unpublished data). In the present study, the thermotherapy in combination with Enzimatic was able to reduce disease incidence and severity of PR in both experiments, in inoculated and non-inoculated fruits for up to 40 DS and, in non-inoculated fruits in the experiment *in*

vivo II, when used alone it inhibited 100% of disease incidence for 30 days. According to Tyc et al. (2017), such metabolites (from antagonist and endophytic microorganisms) carry active compounds capable of inhibiting growth of other organisms. In addition to these metabolites, copper (Cu) is also part of the Enzimatic's formulation, as in the Copper Crop®, and has a strong antimicrobial activity.

According to the manufacturer, the Copper Crop® is a fertilizer obtained from the fermentation process of mixing macro- and micronutrients and amino acids, providing Cu in the form of an organic complex for better absorption and faster development of plants. The post-thermotherapy treatment with Copper Crop® had one of the best results in the control of PR in melon (non-inoculated and fruits inoculated with *F. falciforme*), with no apparent lesions for up to 40 DS. Alone, the Copper Crop® was better than the Magnate®, controlling disease for up to 40 DS in non-inoculated fruits. Banik and Luque (2017) reported inhibitory effect of Cu on growth and sporulation of plant pathogens but not harmful to *Trichoderma harzianum* and *Rhizobium*, i.e., it did not affect beneficial organimisms commonly found in the soil, surging as an alternative of in-field application to control of PR in melon.

When investigating the use of both essential oils tested in the study, it is noteworthy the lack of their characteristic odors after application. Citronella EO applied after fruit thermoterapy inhibited the indicence of PR in inoculated up to 30 DS (*in vivo* I) and non-inoculated fruits up to 40 DS (*in vivo* II), being just as effective as the Magnate® under the same conditions, and when applied without thermoterapy it suppresed the development of lesions for up to 30 DS in non-inoculated fruits (*in vivo* I). This EO has been showing satisfactory results since the *in vitro* experiment, which makes the oil a relevant alternative to the use of Imazalil. Some compounds found in the Cetronella EO such as geraniol, citral, citronella and the citronello (De Toledo et al., 2016) have antifungal activity with great potential for use in postharvest treatment as an alternative to synthetic chemicals (Chen et al., 2014). The inhibitory effect found in both *in vitro* and *in vivo* assays is in accordance with a previous report from Seixas et al. (2011), who were able to inhibit the growth of *F. subglutinans* using Citronella EO. The reported results show a potential product for the management of postharvest melon diseases. The treatment of fruits with the Melaleuca EO combined with thermotherapy showed no incidence (0%) of PR in inoculated and non-inoculated fruits up to 40 DS (*in vivo* II). The antifungal potential of the Melaleuca EO and its use as an alternative to replace synthetic products was reported by Sahab et al. (2014) when the oil inhibited the growth of *F. avenaceum, F. moniliforme, F. semitectum, F. solani, F. oxysporum* and *F. graminearum*.

Combination of $CaCl_2$ with thermotherapy has inhibited the PR in melons for up to 40 DS in both fruits inoculated with *F. falciforme* (*in vivo* II) and non-inoculated (*in vivo* I and II), showing to be an alternative postharvest treatment for melon. When used alone, it also reduced the INC and SEV in comparison to the other treatments (*in vivo* II). The $CaCl_2$ is already a well-used compound in postharvest of fruits and vegetables, ensuring better firmness. Its beneefits in postharvest has been reported in different fruits, as a result of reducing rind damage, such as chilling injuries, delaying ripening by the decrease in respiration rate and ethylene production, and maintaining pulp firmness, thus extending the shelf life of fruits (Werner et al., 2009; Silva et al., 2015).

Among the analyzed postharvest quality characteristics, fruit firmness is considered as one of the most important, since it is associated to the resistance of the fruits during processing and transportation, to their resistance against postharvest diseases, to the taste, and, consequently, influences the commercialization of the product (Martins Véras et al., 2019). We observed greater values of firmness in fruits treated with thermotherapy than in untreated fruits, in both inoculated and non-inoculated fruits. Sivakumar and Fallik (2013) comment about the effect of thermotherapy on the postharvest quality parameters, affirming that the thermal treatment prevents the early ripening as it inhibits ethylene synthesis and, consequently, keeps fruits firmer and resistant to diseases. According to Filgueiras et al. (2000), the standard fruit firmness for Canary melon aimed for exportation ranges from 24 to 40 N (depending on the cultivar), measured just after harvest. Considering such recommendations, the melons from the present study maintained firmness within the standards, showing that the thermotherapy did not interfere in such parameter, even after 40 DS under refrigeration. Analyzing the effect of the combination of thermotherapy and different products on fruit firmness, we observed that Nem Out®, Enzimatic and the Melaleuca EO, when applied after thermotherapty, have contributed to maintain a better fruit firmness than those fruits that were not treated with thermotherapy, showing results even better than Magnate® (with or without thermotherapy), in both inoculated and non-inoculated fruits.

For the internal and external quality of fruits, the thermotherapy resulted in fruits with better quality, as it reduced incidence of PR without causing any heat damage to the fruit. Although no significant difference was observed among treatments with thermotherapy, the Compost Aid®, Nem Out® and Copper Crop® had better results for the external quality after applying thermotherapy in inoculated fruit; and, for internal quality, in addition to the mentioned products, the Enzymatic and the Citronella EO also showed better results. The

soluble solids content is an important quality parameter required by the importers of melon. A desirable fruit should have good quality as regarding its sweetness (soluble solids content), with a minimum of 10% and fruit firmness of 22 N or higher (Tomaz et al., 2009). The soluble solids content of melon fruits inoculated with *Fusarium* and treated with thermotherapy did not show significant difference to the fruits without thermotherapy, with an average of 11.4 °Brix. Among non-inoculated, fruits without thermotherapy showed higher content of soluble solids of 12.5 °Brix on average, against 12 °Brix from the thermotherapy-treated fruits. A reasonable value for soluble solids in melon, from a commercial point of view, is situated between 10 and 12 °Brix for the foreign market (Filgueiras et al., 2000).

Even though the thermotherapy is still not a common practice in postharvest of melon in Brazil, we verified that such technique was efficient in controlling PR, and further methods to minimize costs of application can be evaluated, such as pressurized hot water or steam. The outcome of this study represents a contribution for the management of peduncular rot in melon, showing several promising curative measures as an alternative to the use of imazalil, free of chemical fungicide residue and favorable to maintain fruit quality, which can be chosen by the producers according to their costs and the market to which fruits will be commercialized.

5 CONCLUSIONS

Thermotherapy at 58°C for 30 seconds is an efficient treatment for the management of peduncular rot in melon caused by *F. falciforme*, in conditions of low relative humidity, inhibiting symptoms for up to 40 days after harvest and improving postharvest quality of fruits.

Under conditions of high humidity (average precipataion of 265 mm during the period of production), the combination of thermotherapy with Copper Crop® (8 mL/L) or with Citronella EO (25 v/v) was efficient to control peduncular rot in melon for up to 30 days, and thermotherapy with Serenade® (10 mL/L) or $CaCl_2$ controlled the disease for 40 days in storage. In the absence of thermotherapy, Compost Aid® and Citronella EO controlled the PR for 30 and 40 days, respectively, under the same conditions. Under low humidity (average precipitation of 4 mm during the period of production), combination of thermotherapy with Compost Aid® (2 g/L) controlled the disease for 30 days, and the combination with Nem Out® (8 g/L), Enzimatic (10 mL/L), Copper Crop®, Citronella EO and Melaleuca EO (2.5% v/v) or

$CaCl_2$ provided protection for up to 40 days in storage. Under the same condition, the Enzimatic and Copper Crop® were efficient at controlling the disease for up to 30 and 40 days, respectively.

None of the treatments have negatively affected fruit quality, keeping them free of any damage due to heat or phytotoxicity; fruit firmness, and soluble solids content remained within the market standards.

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REFERENCES

Abdelmoteleb, A., Troncoso-Rojas, R., Gonzalez-Soto, T., González-Mendoza, D. (2017). Antifungical activity of autochthonous *Bacillus subtilis* isolated from *Prosopis juliflora* against phytopathogenic fungi. Mycobiology. 45(4), 385-391. https://doi.org/10.5941/MYCO.2017.45.4.385

Abrafrutas, 2020. China recebe primeira carga de melão brasileiro após abertura de mercado. https://abrafrutas.org/2020/09/18/china-recebe-primeira-carga-de-melao-brasileiro-apos-abertura-de-mercado/ (Acessed 31 September 2020).

Ali, E. O. M., Shakil, N. A., Rana, V. S., Sarkar, D. J., Majumder, S., Kaushik, P., Kumar, J. (2017). Antifungal activity of nano emulsions of neem and citronella oils against phytopathogenic fungi, *Rhizoctonia solani* and *Sclerotium rolfsii*. Industrial crops and products. 108, 379-387. https://doi.org/10.1016/j.indcrop.2017.06.061

Aroucha, E. M. M., de Mesquita, H. C., de Souza, M. S., Torres, W. D. L., Ferreira, R. M. D. A. (2012). Vida útil pós-colheita de cinco híbridos de melão amarelo produzidos no agropólo Mossoró-Assu. Revista Caatinga. 25(3), 52-57.

Baffoni, L., Gaggia, F., Dalanaj, N., Prodi, A., Nipoti, P., Pisi, A., Biavati, B., Di Gioia, D. (2015). Microbial inoculants for the biocontrol of *Fusarium* spp. in durum wheat. BMC microbiology. 15(1), 242. https://doi.org/10.1186/s12866-015-0573-7.

Banik, S., Luque A. P. (2017). In vitro effects of copper nanoparticles on plant pathogens, beneficial microbes and crop plants. Spanish journal of agricultural research. 15(2), 23. https://doi.org/10.5424/sjar/2017152-10305.

Braga Junior, G. M., Junior, A. F. C., Chagas, L. F. B., de Carvalho Filho, M. R., de Oliveira Miller, L., e dos Santos, G. R. (2017). Controle biológico de fitopatógenos por *Bacillus subtilis* in vitro. Biota Amazônia. 7(3), 45-51.

Chen, Q., Xu, S., Wu, T., Guo, J., Sha, S., Zheng, X., Yu, T. (2014). Effect of citronella essential oil on the inhibition of postharvest *Alternaria alternata* in cherry tomato. Journal of the Science of Food and Agriculture. 94(12), 2441-2447. https://doi.org/10.1002/jsfa.6576.

De Senna, A., Lathrop, A. (2017). Antifungal screening of bioprotective isolates against *Botrytis cinerea*, *Fusarium pallidoroseum* and *Fusarium moniliforme*. Fermentation. 3(4), 53. https://doi.org/10.3390/fermentation3040053.

De Toledo, L. G., Ramos, M. A. D. S., Spósito, L., Castilho, E. M., Pavan, F. R., Lopes, É. D. O., Bauab, T. M. (2016). Essential oil of *Cymbopogon nardus* (L.) Rendle: A strategy to combat fungal infections caused by *Candida* species. International Journal of Molecular Sciences. 17(8), 1252. https://doi.org/10.3390/ijms17081252.

Durner, E. (2019). Effective analysis of interactive effects with non-normal data using the aligned rank transform, ARTool and SAS® University Edition. Horticulturae. 5(3), 57. https://doi.org/10.3390/horticulturae5030057.

Fallik, E., Aharoni, Y., Copel, A., Rodov, V., Tuvia-Alkalai, S., Horev, B., Regev, R. (2000). Reduction of postharvest losses of Galia melon by a short hot-water rinse. Plant Pathology, 49(3), 333-338. https://doi.org/10.1046/j.1365-3059.2000.00467.x.

Ferraz, D. M. M., Blum, L. E. B., Cruz, A. F., de Vasconcelos, T. M. M., Uesugi, C. H., Barreto, M. L. A. (2016). Efeito do cloreto de cálcio sobre a antracnose e características de frutos de goiaba em pós-colheita. Agrotropica. 28(2), 311-318. https://doi.org/10.21757/0103-3816.2016v28n3p311-318.

Fhoula, I., Najjari, A., Turki, Y., Jaballah, S., Boudabous, A., Ouzari, H. (2013). Diversity and antimicrobial properties of lactic acid bacteria isolated from rhizosphere of olive trees and desert truffles of Tunisia. BioMed Research International. 2013, 1-14. https://doi.org/10.1155/2013/405708.

Filgueiras, H. A., Menezes, J. B., Alves, R. E., Costa, F. D., PEREIRA, L. D. S., & Gomes Júnior, J. (2000). Colheita e manuseio pós-colheita. Melão pós-colheita, 23-41.

Fira, D., Dimkić, I., Berić, T., Lozo, J., Stanković, S. (2018). Biological control of plant pathogens by *Bacillus* species. Journal of Biotechnology. 285, 44-55. https://doi.org/10.1016/j.jbiotec.2018.07.044.

IBGE – Instituto Brasileiro de Geografia e Estatística, 2019. SIDRA – Sistema IBGE de Recuperação Automática. Rio de Janeiro, 2019. http://sidra.ibge.gov.br/tabela/5457#resultado. Accessed July 7, 2020.

Kist, B. B., Dos Santos, C. E., De Carvalho, C., Beling, R. R. (2019). Anuário brasileiro de Horti & Fruti. Santa Cruz do Sul: Editora Gazeta Santa Cruz, 2019. http://www.editoragazeta.com.br/sitewp/wp-content/uploads/2019/07/HortiFruti_2019_DUPLA.pdf. Accessed July 15, 2020.

Martins Véras, M. L., de Araújo, F. F., Junior, J. G., Finger, F. L. (2019). Enzymatic activity and post-harvest quality of 'Galia' melon under storage temperatures and modified atmosphere. Brazilian Journal of Agricultural Sciences/Revista Brasileira de Ciências Agrárias, 14(1). https://doi.org/10.5039/agraria.v14i1a5617.

Nascimento, L. V., Nogueira, G. A., Alves, T. R. C., Araújo, M. B. M., Dombroski, J. L. D., Machado, F. S., Ambrósio, M. M. Q. (2019). Sanitary quality in seeds from species of Caatinga biome and control methods for fungi. Emirates Journal of Food and Agriculture. 31, 945-950. https://doi.org/10.9755/ejfa.2019.v31.i12.2044.

Nicot, P. C., Stewart, A., Bardin, M., Elad, Y. (2016). Biological control and biopesticide suppression of *Botrytis*-incited diseases. In *Botrytis*–the fungus, the pathogen and its

management in agricultural systems. Springer, Cham. pp. 165-187. https://doi.org/10.1007/978-3-319-23371-0_9.

Oliveira, M. J. D., Laranjeira, D., Câmara, M. P. S., Laranjeira, F. F., Armengol, J., Michereff, S. J. (2014). Effects of wounding, humidity, temperature, and inoculum concentrations on the severity of corky dry rot caused by *Fusarium semitectum* in melon fruits. Acta Scientiarum. Agronomy. 36(3), 281-289. https://doi.org/10.4025/actasciagron.v36i3.17656.

Oster, A. H., Silva, E. O., Borballo, P. N., Zocolo, G. J., Silveira, M. R. S., Oiram Filho, F., De Araújo, A. A. C. (2018). Luz ultravioleta pulsada no controle de podridão pós-colheita e na qualidade de melão para exportação. Embrapa Agroindústria Tropical - Boletim de Pesquisa e Desenvolvimento (INFOTECA-E). (Boletim de pesquisa, 173).

R Development Core Team. (2019). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Sahab, A. F., Aly, S., Hathout, A. S., Ziedan, E. S. H., Sabry, B. A. (2014). Application of some plant essential oils to control *Fusarium* isolates associated with freshly harvested maize in Egypt. Journal of Essential Oil Bearing Plants, 17(6), 1146-1155. https://doi.org/10.1080/0972060X.2014.891447.

Santos, T. C., Aguiar, F. S., Rodrigues, M. L. M., Mizobutsi, G. P., da Silva Pinheiro, J. M. (2018). Quality of bananas harvested at different development stages and subjected to cold storage. Agricultural Research in the Tropics. 90-97. https://doi.org/10.1590/1983-40632018v4851297.

Seixas, P. T. L., Castro, H. C., Santos, G. R., Cardoso, D. P. (2011). Controle fitopatológico do *Fusarium subglutinans* pelo óleo essencial do capim-citronela (*Cymbopogon nardus* L.) e do composto citronela. Revista Brasileira de Plantas Medicinais. 13, 513-517. https://doi.org/10.1590/S1516-05722011000500003

Silva, W. B., Silva, G. M. C., Da Silva, L. R., Waldman, W. R., De Oliveira, J. G. (2015). Treatment with calcium chloride at postharvest delays the degreening and loss of firmness of papaya/uenf/caliman01. Revista Brasileira de Fruticultura. 37(3), 588-599. https://doi.org/10.1590/0100-2945-158/14

Sivakumar, D., Fallik, E. (2013). Influence of heat treatments on quality retention of fresh and fresh-cut produce. Food Reviews International, 29(3), 294-320.

Terao, D., Oliveira, S. M. A. D., Viana, F. M. P., Sá, C. R. L., Moura, R. D., Gondim, D. M. (2009). Manejo da podridão de melão pelo controle do amadurecimento através do 1-mcp, sob duas condições de armazenamento. Summa Phytopathologica. 35(2), 110-115. https://doi.org/10.1590/S0100-54052009000200005.

Terao, D., Oliveira, S., Viana, F. M. P., Rossetti, A. G., Souza, C. (2006). Integração de fungicidas à refrigeração no controle de podridão pós-colheita em frutos de meloeiro. Fitopatologia Brasileira. 31(1), 89-93. https://doi.org/10.1590/S0100-41582006000100016.

Tomaz, H. V. D. Q., Aroucha, E. M. M., Nunes, G. H. D. S., Bezerra Neto, F., Tomaz, H. V. D. Q., Queiroz, R. F. (2009). Qualidade pós-colheita de diferentes híbridos de melão-amarelo armazenados sob refrigeração. Revista Brasileira de Fruticultura. 31(4), 987-994. https://doi.org/10.1590/S0100-29452009000400011.

Tyc, O., Song, C., Dickschat, J. S., Vos, M., Garbeva, P. (2017). The ecological role of volatile and soluble secondary metabolites produced by soil bacteria. Trends in Microbiology. 25(4), 280-292. https://doi.org/10.1016/j.tim.2016.12.002.

Usall, J., Ippolito, A., Sisquella, M., Neri, F. (2016). Physical treatments to control postharvest diseases of fresh fruits and vegetables. 122 , 30-40. https://doi.org/10.1016/j.postharvbio.2016.05.002.

Viana, F. M. P., dos SANTOS, A. A., Freire, F., Cardoso, J. E., Vidal, J. C. (2001). Recomendações para o controle das principais doenças que afetam a cultura do melão na região Nordeste. Fortaleza: Embrapa Agroindústria.

Werner, E. T., Oliveira Junior, L. F. G. D., Bona, A. P. D., Cavati, B., Gomes, T. D. U. H. (2009). Efeito do cloreto de cálcio na pós-colheita de goiaba Cortibel. Bragantia. 68(2), 511-518. https://doi.org/10.1590/S0006-87052009000200026

Wobbrock, J. O., Findlater, L., Gergle, D., Higgins, J. J. (2011). The aligned rank transform for nonparametric factorial analyses using only anova procedures. In Proceedings of the SIGCHI conference on human factors in computing systems. p. 143-146. https://doi.org/10.1145/1978942.1978963

Yadav, E., Kumar, S., Mahant, S., Khatkar, S., Rao, R. (2017). Tea tree oil: a promising essential oil. Journal of Essential oil Research. 29(3), 201-213. https://doi.org/10.1080/10412905.2016.1232665.

Yushu, Z., Guo, Q., Yanan, Z., Yuting, L., Xuezheng, W. (2017). Controlling fusarium wilt disease in melon (Cucumis melo L.) Using tillered onion bulb extract. Pakistan Journal of Botany. 49(4), 1615-1623.

Zaim, S., Bekkar, A. A., Belabid, L. (2018). Efficacy of *Bacillus subtilis* and *Trichoderma harzianum* combination on chickpea *Fusarium* wilt caused by *F. oxysporum* f. sp. *ciceris*. Archives of Phytopathology and Plant Protection. 51(3-4), 217-226. https://doi.org/10.1080/03235408.2018.1447896

ATTACHMENT – Supporting Information

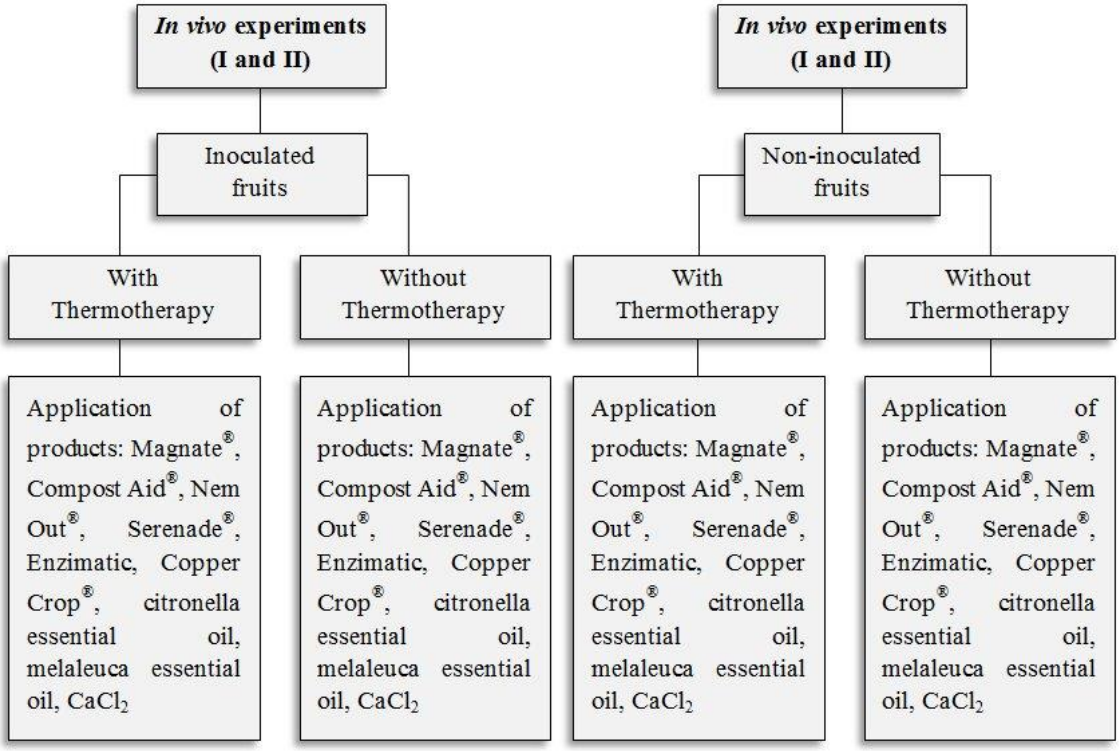

Fig. S1. Detailed scheme of *in vivo* experiments (I and II).

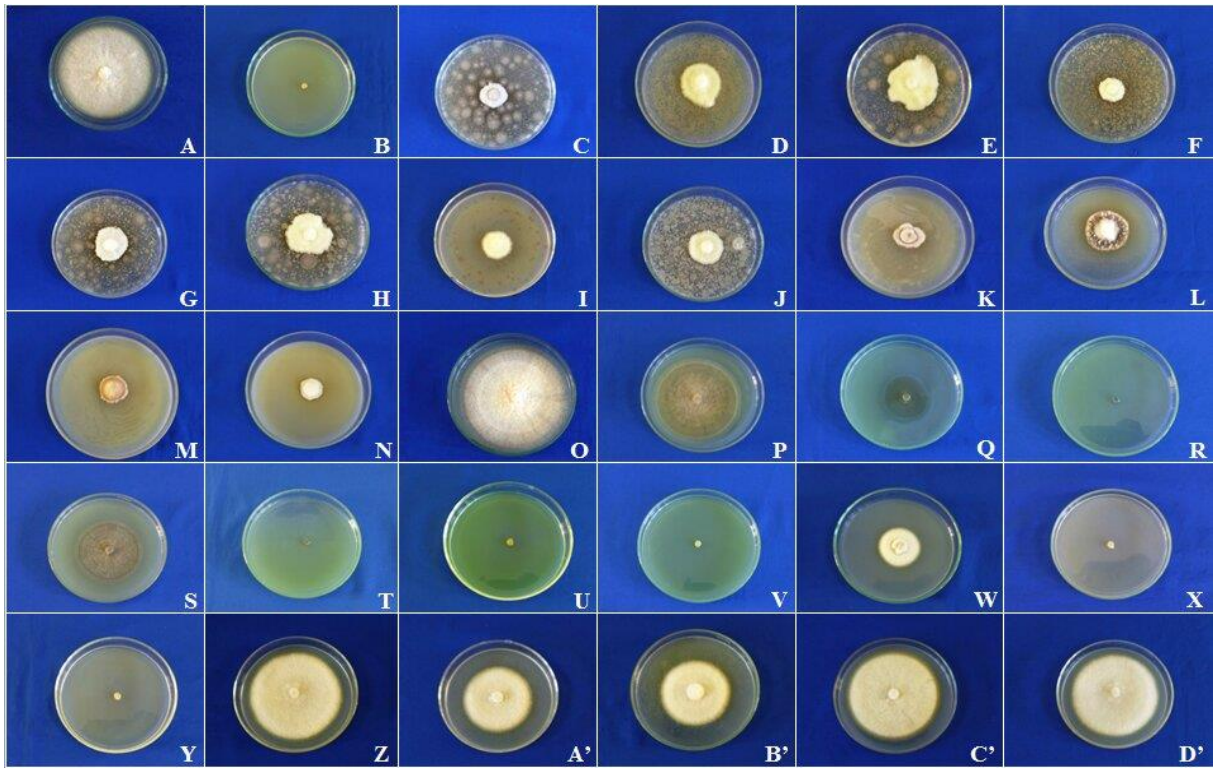

Fig. S2. *In vitro* growth of *Fusarium falciforme* under the effect of different products and concentrations. Control (A); Magnate® (B); Compost Aid® (C-F), rates of 2, 4, 8 and 10 g/L; Nem Out® (G-J), rates of 2, 4, 8 and 10 g/L; Serenade® (K-N), rates of 2, 4, 8 and 10 mL/L; Enzimatic (O-R), rates of 2, 4, 8 and 10 mL/L; Copper Crop® (S-V), rates of 2, 4, 8 and 10 mL/L; Citronella essential oil (W-Y), rates of 1, 2 and 2.5%; Melaleuca essential oil (Z-B'), rates of 1, 2 and 2.5%; Tween-20 at 1% (C'), and $CaCl_2$ at 2% (D').

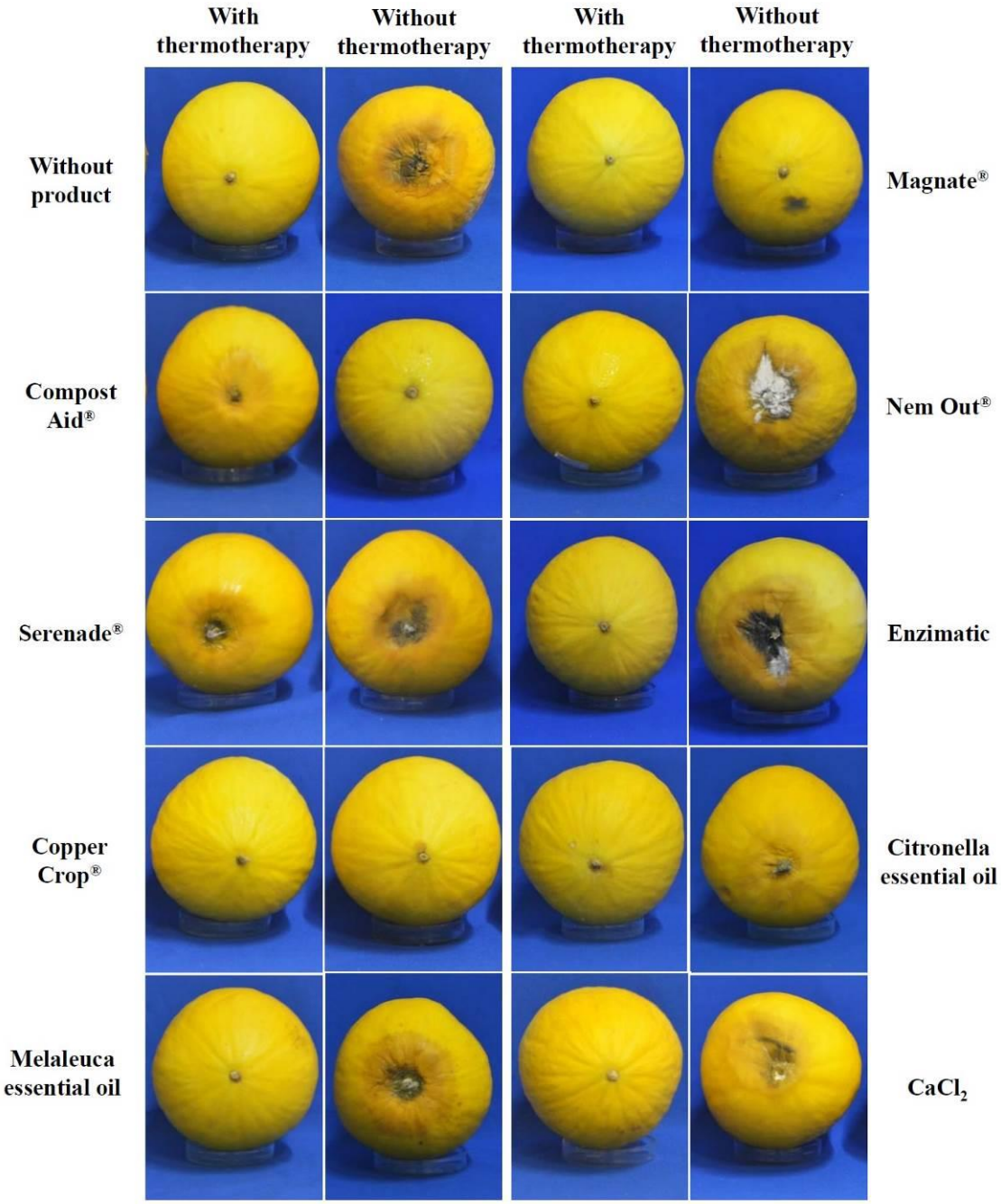

Fig. S3. Peduncular rot in 'Canary' melon fruits inoculated with *Fusarium falciforme*, treated or non-treated with thermotherapy, in combination with different products, at 40 days of storage in cold room (*in vivo* experiment [I]). The captions on the left and the captions on the right refer to the figures in the first and second columns, respectively.

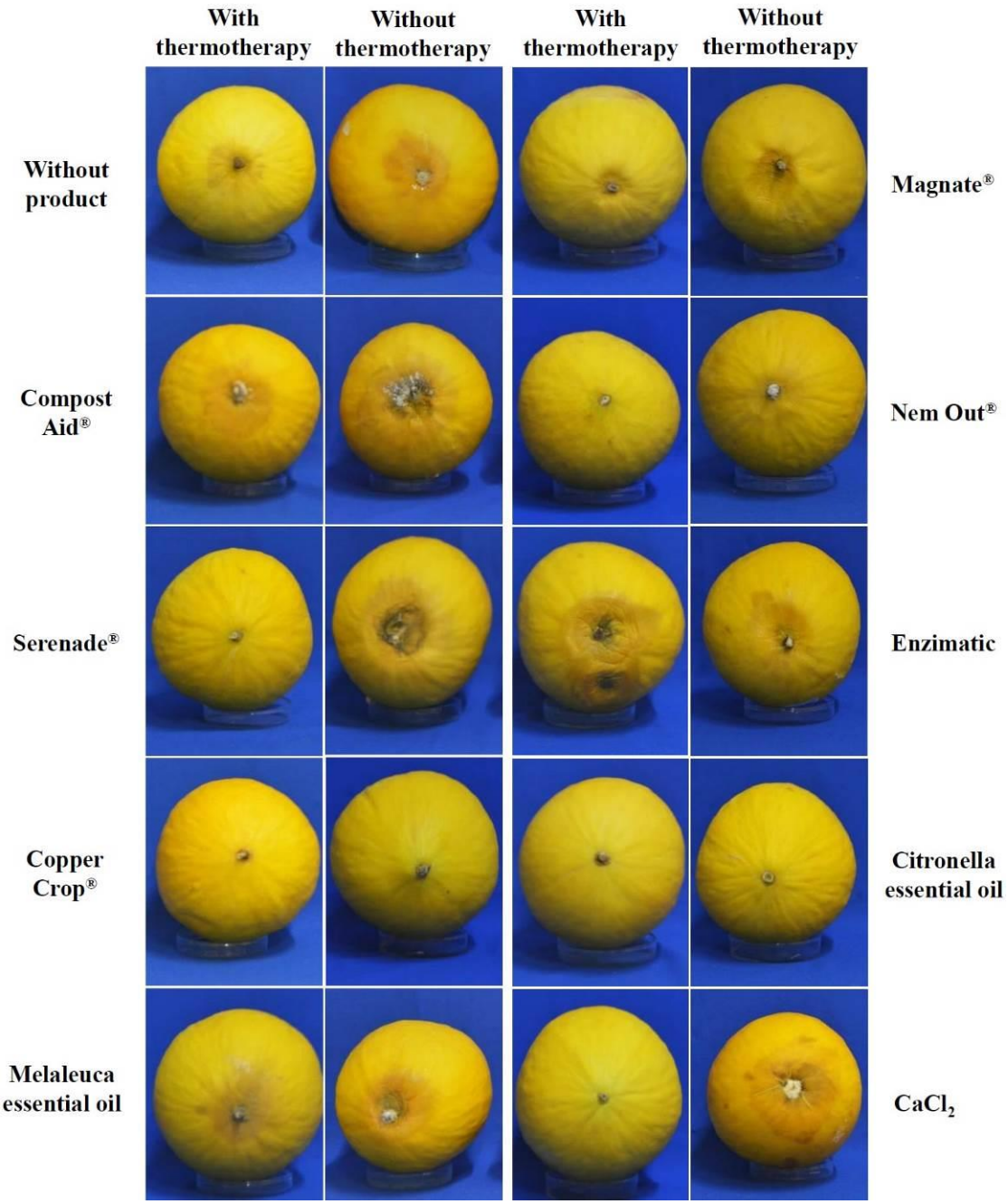

Fig. S4. Peduncular rot in non-inoculated 'Canary' melon fruits, treated or non-treated with thermotherapy, in combination with different products, at 40 days of storage in cold room (*in vivo* experiment [I]). The captions on the left and the captions on the right refer to the figures in the first and second columns, respectively.

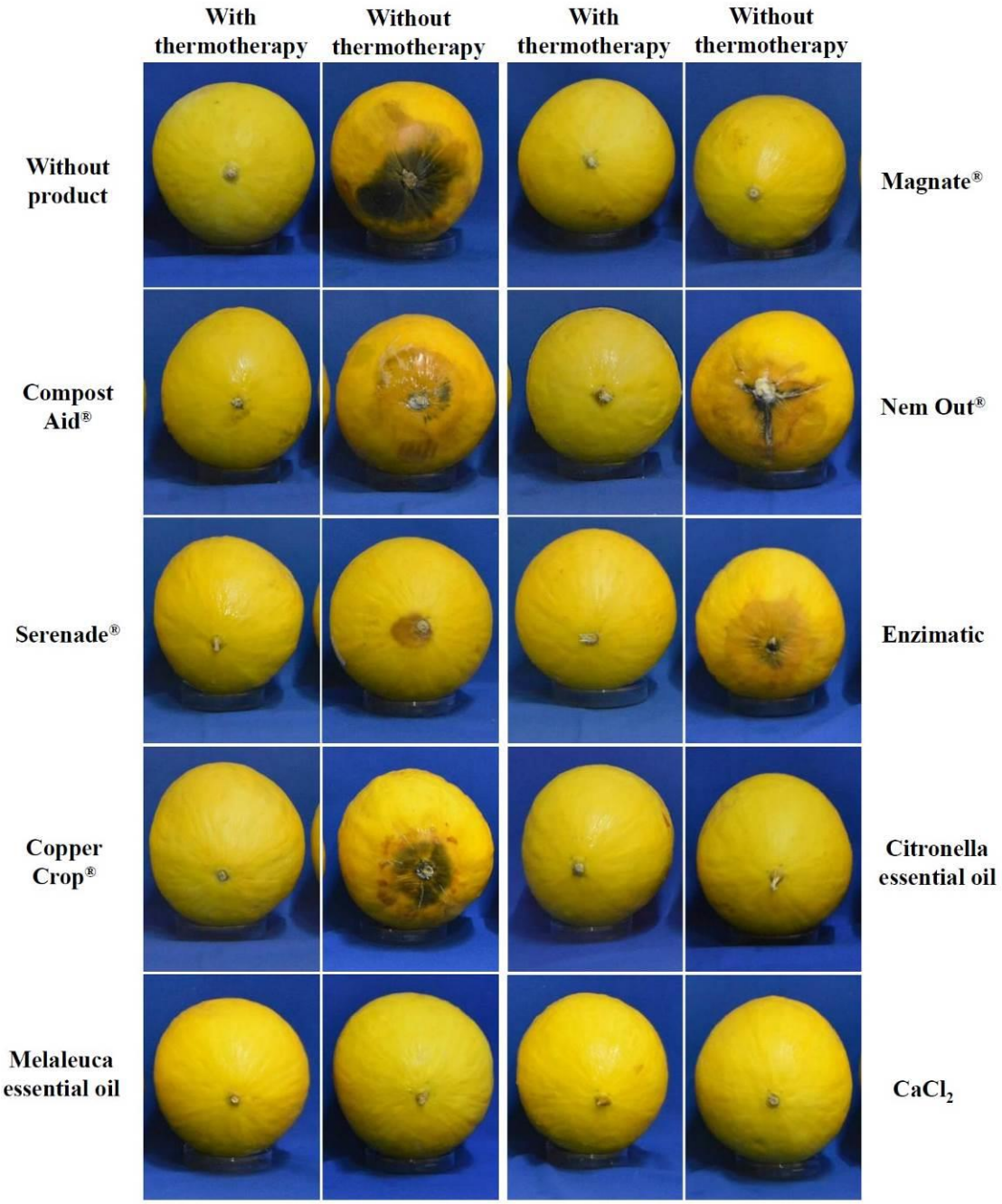

Fig. S5. Peduncular rot in 'Canary' melon fruits inoculated with *Fusarium falciforme*, treated or non-treated with thermotherapy, in combination with different products, at 40 days of storage in cold room (*in vivo* experiment [II]). The captions on the left and the captions on the right refer to the figures in the first and second columns, respectively.

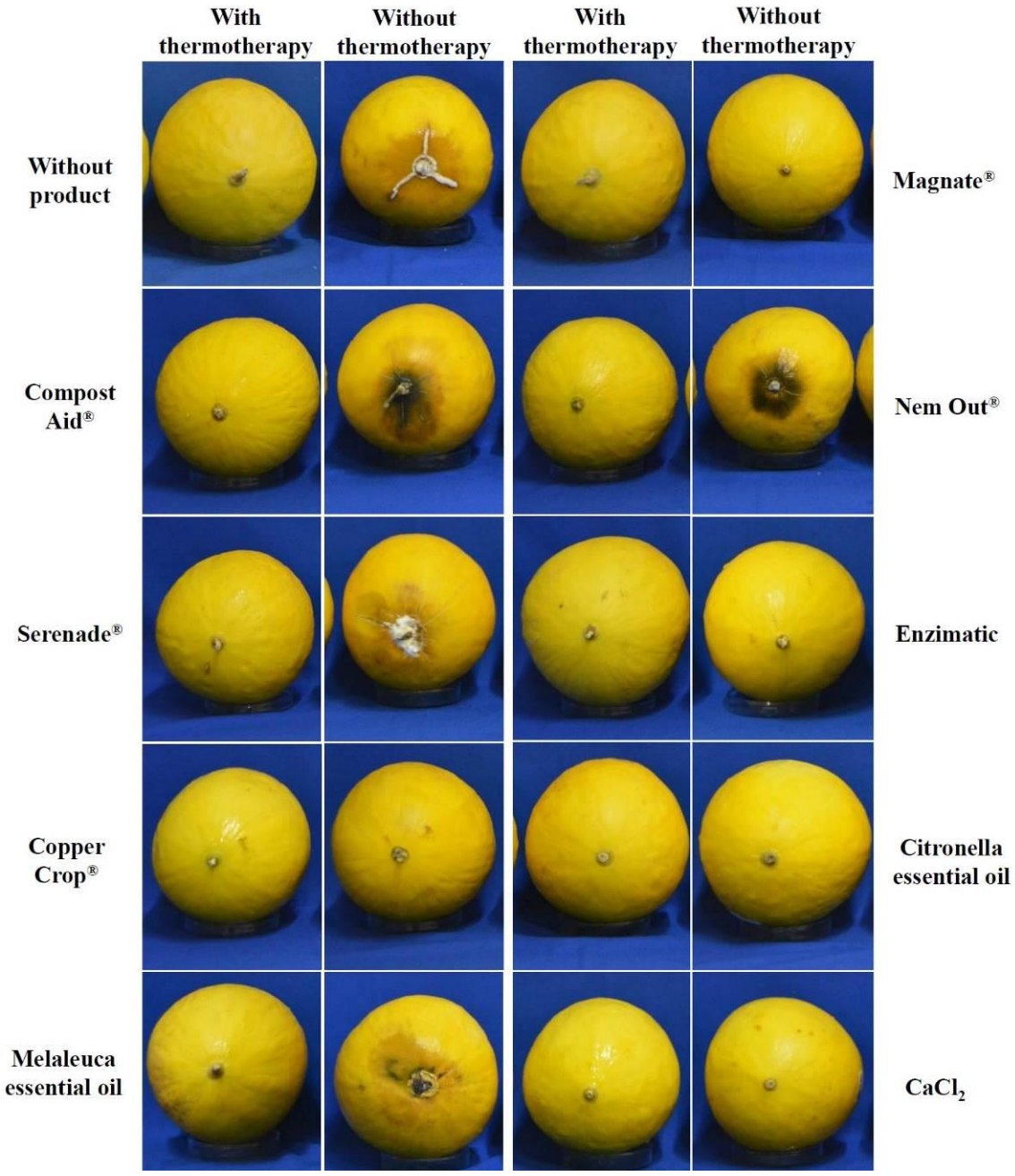

Fig. S6. Peduncular rot in non-inoculated 'Canary' melon fruits, treated or non-treated with thermotherapy, in combination with different products, at 40 days of storage in cold room (*in vivo* experiment [II]). The captions on the left and the captions on the right refer to the figures in the first and second columns, respectively.