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BRENO DE HOLANDA ALMEIDA

ECOPHYSIOLOGICAL AND SOIL SURVIVAL RESPONSES OF *Trichoderma* spp. IN THE BRAZILIAN SEMIARID AND POSTHARVEST MANAGEMENT OF FUSARIUM ROT IN MELON

MOSSORÓ
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Thesis advisor: Márcia Michelle de Queiroz Ambrósio, Prof. Dr.

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RESUMO

O meloeiro é uma das culturas de maior relevância no semiárido brasileiro, destacando-se pelo elevado valor de suas exportações e pela significativa contribuição para a geração de empregos na região. No entanto, essa cultura enfrenta limitações produtivas e de pós-colheita associadas à incidência de fitopatógenos agressivos, ao potencial de salinização do solo e elevadas temperaturas durante o cultivo. O gênero *Fusarium*, dentre muitos patógenos que infectam a cultura, possui a capacidade de infectá-lo em diferentes fases do desenvolvimento, desde a semeadura até o pós-colheita, causando podridões nas raízes e nos frutos, gerando perdas expressivas nas exportações. Diante da necessidade de estratégias sustentáveis e eficientes, e das limitações impostas aos fungicidas sintéticos, este trabalho objetivou investigar a plasticidade fisiológica e a sobrevivência de isolados de *Trichoderma* spp. no solo durante o plantio do melão, além de avaliar a eficácia da termoterapia associada a fitoreguladores de crescimento no controle de *Fusarium falciforme* e *F. sulawesiense* em frutos de melão. Para os isolados de *Trichoderma* spp., em laboratório, os limites de crescimento sob diferentes gradientes de pH, salinidade e temperatura foram avaliados. Em campo, a avaliação ocorreu em cultivos comerciais de melão 'Pele de Sapo,' submetido a *mulching* de polietileno, monitorando-se a sobrevivência fúngica e as alterações na temperatura e nas características químicas do solo. Em laboratório, os isolados de *Trichoderma* apresentaram crescimento micelial em ampla faixa de pH (2 a 10), alta salinidade (até 1000 mM de NaCl) e em uma ampla faixa de temperatura (10 ° a 35 °C). No campo, a sobrevivência no solo durante o período avaliado (65 dias) superou 74% para a maioria dos isolados, com destaque para *T. asperellum* URM 5911 (95%), que se destacou na redução da condutividade elétrica do solo rizosférico junto a cinco outros isolados. No pós-colheita, melões 'Gália' foram inoculados com duas espécies de *Fusarium* e submetidos a tratamentos isolados e combinados com termoterapia (água quente a 58 °C por 150 s), 1-MCP, giberelina e Graduate A+®, seguidos de armazenamento refrigerado (20 dias, 9 ± 2 °C). Melões sem nenhum tratamento e inoculados com as duas espécies de Fusarium consistiram no tratamento controle. Análises de severidade, pós-colheita e enzimáticas foram realizadas ao fim dos ensaios. Os tratamentos associados à termoterapia reduziram a severidade das lesões pedunculares. A combinação com 1-MCP preservou a firmeza da polpa dos frutos e reduziu o estresse oxidativo com o aumento da catalase (CAT) e fenilalanina amônia-liase (PAL), junto da redução de peróxido de hidrogênio (H_2O_2). Assim, os isolados de *Trichoderma* avaliados, consolidam-se como bioinoculantes resilientes e funcionais, capazes

de sobreviver e colonizar solos de regiões semiáridas do Brasil. De maneira complementar, a associação entre termoterapia e fitoreguladores constitui uma estratégia limpa, livre de resíduos e aplicável ao controle de podridão por *Fusarium* em melões. Juntas, as duas frentes de pesquisa fornecem uma abordagem fitossanitária contínua e integrada, conectando a proteção biológica do sistema radicular no campo à manutenção da integridade metabólica e *shelf-life* dos melões destinados ao mercado externo.

Palavras-chave: Plasticidade fisiológica, persistência fúngica, colonização rizosférica, tratamento térmico, estresse oxidativo.

ABSTRACT

Melon cultivation is one of the most relevant agricultural activities in the Brazilian semiarid region, standing out for the high value of its exports and its significant contribution to local job creation. However, this crop faces production and postharvest limitations associated with the incidence of aggressive phytopathogens, soil salinization potential, and high temperatures during cultivation. Among the numerous pathogens infecting the crop, the genus *Fusarium* is capable of causing infection at different developmental stages, from sowing to postharvest, leading to root and fruit rot and generating significant export losses. Given the need for sustainable and efficient strategies, as well as the limitations imposed on synthetic fungicides, this study aimed to investigate the physiological plasticity and soil survival of *Trichoderma* spp. isolates during melon cultivation, in addition to evaluating the efficacy of thermotherapy combined with plant growth regulators in controlling *Fusarium falciforme* and *F. sulawesiense* in melon fruits. For the *Trichoderma* spp. isolates, growth limits under different gradients of pH, salinity, and temperature were evaluated in the laboratory. In the field, the evaluation took place in commercial crops of 'Pele de Sapo' melons subjected to polyethylene mulching, monitoring fungal survival as well as soil temperature and chemical changes. In the laboratory, the *Trichoderma* isolates exhibited mycelial growth across a wide pH range (2 to 10), high salinity (up to 1000 mM NaCl), and a broad temperature range (10 °C to 35 °C). In the field, soil survival during the evaluated period (65 days) exceeded 74% for most isolates, notably *T. asperellum* URM 5911 (95%), which excelled in reducing the electrical conductivity of the rhizospheric soil alongside five other isolates. In the postharvest phase, 'Galia' melons were inoculated with both *Fusarium* species and subjected to individual and combined treatments involving thermotherapy (hot water at 58 °C for 150 s), 1-MCP, gibberellin, and Graduate A+®, followed by cold storage (20 days, 9 ± 2 °C). Untreated melons inoculated with the two *Fusarium* species served as the control treatment. Severity, postharvest quality, and enzymatic analyses were performed at the end of the trials. The treatments combined with thermotherapy reduced the severity of peduncular lesions. The combination with 1-MCP preserved fruit pulp firmness and reduced oxidative stress by increasing catalase (CAT) and phenylalanine ammonia-lyase (PAL) activities, alongside a reduction in hydrogen peroxide (H_2O_2). Thus, the evaluated *Trichoderma* isolates proved to be resilient and functional bioinoculants capable of surviving and colonizing soils in semi-arid regions of Brazil. Furthermore, the combination of thermotherapy and growth regulators constitutes a clean, residue-free, and applicable strategy for controlling *Fusarium* rot in

melons. Together, both research fronts provide a continuous and integrated phytosanitary approach, linking the biological protection of the root system in the field to the maintenance of metabolic integrity and the shelf life of melons destined for the foreign market.

Keywords: Physiological plasticity, fungal persistence, rhizospheric colonization, thermal treatment, oxidative stress.

FIGURE LIST

CHAPTER I

SUPPLEMENTARY MATERIAL

FIGURE LIST

CHAPTER II

TABLE LIST

CHAPTER I

CHAPTER II

LIST OF ABBREVIATIONS AND ACRONYMS

1-MCP	1-methylcyclopropene
4PL	Four-parameter logistic
ABRAFRUTAS	Associação Brasileira dos Exportadores de Frutas e Derivados
ANOVA	Analysis of variance
APX	Ascorbate peroxidase
BA	Bahia
BCOT	Biblioteca Central Orlando Teixeira
BOD	Biochemical oxygen demand
BRL	Brazilian Real
CAES	Connecticut Agricultural Experiment Station
CAT	Catalase
CE	Ceará
CEC	Cation exchange capacity
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
CRB	Regional Council of Librarianship
CRD	Completely randomized design
CT	Connecticut
DAT	Days after transplantation
EC	Electrical conductivity
EC_{50}	Half maximal effective concentration
EDTA	Ethylenediaminetetraacetic acid
et al.	And others (et alii)
FAO	Food and Agriculture Organization of the United Nations
GA	Gibberellin
GC	Knowledge Management
GRAD	Graduate A+
HCl	Hydrochloric acid
HWT	Hot water treatment
IB-APTA	Instituto Biológico - Agência Paulista de Tecnologia dos Agronegócios
IBGE	Instituto Brasileiro de Geografia e Estatística
LMC	Lavras Mycological Collection

LSL	Long Shelf Life
MAPA	Ministério da Agricultura, Pecuária e Abastecimento
MGR	Mycelial growth rate
MRL	Maximum residue level
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NFT	Nutrient Film Technique
NIFA	National Institute of Food and Agriculture
OC	Organic carbon
OpH	Optimum pH
OT	Optimum temperature
P	Phosphorus
PAL	Phenylalanine ammonia-lyase
PDA	Potato Dextrose Agar
PE	Pernambuco
PG&C	Perspectives in Management & Knowledge
POX	Peroxidase
PPO	Polyphenol oxidase
PVP	Polyvinylpyrrolidone
RCBD	Randomized complete block design
RN	Rio Grande do Norte
ROS	Reactive oxygen species
RPB2	RNA polymerase II second largest subunit
SCBGP	Specialty Crop Block Grant Program
SEBRAE	Serviço Brasileiro de Apoio às Micro e Pequenas Empresas
SEV	Disease severity
SIR	Information and Reference Sector
SOD	Superoxide dismutase
SOM	Soil organic matter
spp.	Species (plural)
Tmax	Maximum temperature
Tmed	Mean temperature
Tmin	Minimum temperature

TSM	Trichoderma semi-selective medium
UFERSA	Federal University of the Semi-Arid Region
UFLA	Universidade Federal de Lavras
UFPB	Universidade Federal da Paraíba
USD / US$	United States Dollar
USDA	United States Department of Agriculture
var	Variety

LIST OF SYMBOLS

@	At sign
©	Copyright
®	Registered trademark
%	Percent
$	Dollar sign
±	Plus or minus
<	Less than
>	Greater than
≤	Less than or equal to
=	Equal to
×	Multiplication sign
√	Square root
°C	Degrees Celsius
°C h^{-1}	Degrees Celsius per hour
µL	Microliter
Ah	Ampere-hour
Ca^{2+}	Calcium ion
cm	Centimeter
cm/d	Centimeters per day
cmolc/dm^3	Centimoles of charge per cubic decimeter
dS/m or dS·m^{-1}	DeciSiemens per meter
g	Gram
g/kg	Grams per kilogram
g/L	Grams per liter
h	Hour
H_2O	Water
H_2O_2	Hydrogen peroxide
K^+	Potassium ion
L	Liter
m^2	Square meters
Mg^{2+}	Magnesium ion

min	Minute
mL	Milliliter
M	Molar
mM	Millimolar
mm:	Millimeter
N	Newtonb
Na^+	Sodium ion
nL/L	Nanoliter per liter
nm	Nanometer
O_2	Oxygen
p	p-value / Probability
ppm	Parts per million
r	Pearson correlation coefficient
R^2	Coefficient of determination
s	Second
V	Volt
v/v	Volume per volume
W	Watt
w/w	Weight per weight
$\hat{y}$	Estimated value of y

SUMMARY

GENERAL INTRODUCTION

Melon (*Cucumis melo* L.), belonging to the Cucurbitaceae family, is a horticultural crop of great global importance and a cornerstone of Brazilian agribusiness, where Brazil ranks among the top five world producers (FAO, 2024). In the first half of 2026 alone, 113,753.16 tons of fresh fruit were exported, generating US$ 95,537,443 in revenue. Of this total, exports are heavily concentrated in Europe, which represents the destination for more than 94% of the exported volume, led by the Netherlands (40.42% of the volume; US$ 41,205,784), Spain (29.58%; US$ 25,081,848), and the United Kingdom (24.30%; US$ 24,536,947). Melons alone account for approximately 22.5% of the entire physical volume of fruit shipped abroad by the country, making it as the second most exported fruit by Brazil (ABRAFRUTAS, 2026).

In Brazil, the production of this commodity is highly concentrated in the Northeast region, which accounts for 98.35% of the national output (IBGE, 2024). According to the latest census, this region produced 816,939 tons, generating BRL 1.38 billion in revenue, with production predominantly led by the states of Rio Grande do Norte (RN; 61.84%), Pernambuco (PE; 12%), Bahia (BA; 11%), and Ceará (CE; 8.68%), while sustaining over 14,500 direct and 80,000 indirect jobs (IBGE, 2024; SEBRAE, 2025).

Commercially, melons produced in Brazil are divided into two main groups (Manchali et al., 2021): non-climacteric (*inodorus* type), such as 'Yellow' (Amarelo) and 'Pele de Sapo', and climacteric (*cantalupensis* type), such as 'Galia' and 'Cantaloupe'. The former comprises the most produced and exported fruits in Brazilian trade, characterized by smooth to slightly wrinkled rinds, extended shelf life, and a high nutritional profile (HABIBAH et al., 2026). The latter, despite its lower volume compared to the *inodorus* types, holds a significant share of the European market (ALMEIDA et al., 2026), offering spherical, sweet fruits with a characteristically shorter postharvest shelf life (DIAS et al., 2026).

The 'Pele de Sapo' melon, also known as Spanish melon, ranks as the second most produced and exported melon type in Brazil. Its market is almost exclusively European, driven by hybrids such as 'Sancho', 'BRS Açu', and 'Waikiki', which exhibit high yield potential and resilience to the semi-arid conditions of the Brazilian Northeast. Among the climacteric types, the 'Galia' melon holds a significant share of exports from the states of Rio Grande do Norte and Ceará. In these regions, hybrids like 'Doral', 'LSL' (Long Shelf Life), and 'Citrino' are particularly prominent due to their high productivity and extended postharvest shelf life compared to conventional hybrids (DIAS et al., 2026).

Sustaining this high productive capacity demands a detailed understanding of crop phenology within the dynamic agricultural systems of northeastern Brazil, where cultivation is strictly governed by both biotic and abiotic factors. The crop features a short cycle, spanning distinct developmental phases, including the vegetative stage (germination and growth) and the reproductive stage (flowering, fruiting, fruit development, and senescence) (WEN et al., 2022). It requires an average of 60 to 75 days from sowing to harvest, a period during which advancing developmental stages generate distinct and increasing nutritional and water requirements (DIAS et al., 2026).

Although adapted to the edaphoclimatic conditions of the northeastern semi-arid region, the crop requires specific abiotic parameters for optimal development, such as temperatures between 25 °C and 30 °C. Growth is severely restricted between 12 °C and 15 °C, and prolonged exposure to temperatures above 37 °C can impair fruit ripening at the end of the cycle. Other favorable factors include low to moderate relative humidity ranging from 55% to 75%, annual solar radiation of 2,000 to 3,000 hours, a soil pH of 6.4 to 7.2, and low rainfall concentrated in the first months of the year (January to April) (DIAS et al., 2026). Consequently, these environmental factors, combined with the continuous demand for precise irrigation and nutrient management during the rapid transition of phenological stages, act as significant bottlenecks determining yield and postharvest fruit quality (NASCIMENTO et al., 2020).

Irrigation requirements increase progressively until harvest due to the high metabolic demand for fruit formation and filling. Water demand doubles from the midpoint of the cycle onward, requiring approximately 150 L of water to produce 1 kg of melon. In the Brazilian semi-arid region, particularly within the major producing zones of Rio Grande do Norte and Ceará, irrigation water often contains elevated salinity levels, which directly impacts crop yield and soil microbial communities (DIAS et al., 2026). Simultaneously, nutrient demand increases throughout the melon life cycle. Among the most essential minerals, nitrogen (N), potassium (K^+), and calcium (Ca^{2+}) play critical roles in plant development and crop productivity (DONG et al., 2025). Their uptake accelerates sharply from the middle of the cycle, a phase where the plant's nutrient absorption curve increases exponentially in alignment with dry matter accumulation (DIAS et al., 2026).

From a global perspective, the combination of specific cultural practices establishes conditions that, when mismanaged, exacerbate adverse soil abiotic parameters, such as electrical conductivity (EC). Although EC primarily impacts plant growth and crop yield, it

also triggers negative secondary effects, such as imbalances in the rhizospheric microbiota, which chiefly affect plant-beneficial microorganisms (LIU et al., 2026).

Soil covering with polyethylene plastic film (mulching) is a common practice in melon cultivation, primarily adopted to suppress weed incidence and development throughout the cycle, improve water-use efficiency by conserving soil moisture for extended periods, and protect fruits from direct contact with the soil surface (ZHOU et al., 2023).

Consequently, the expansion of melon production, combined with the pressures of monoculture, minimal crop rotation, and the continuous use of plastic mulching, has altered soil microbial communities and favored the emergence of more aggressive soilborne phytopathogens under field conditions (ZHOU et al., 2023; LI et al., 2026). These biotic constraints represent a major barrier to productivity since several genera of soilborne pathogens are known to produce resistance structures that enable long-term survival in the soil. This is exemplified by the genus *Macrophomina* spp. Petr., which forms microsclerotia that can persist in the soil for up to 12 years (SALES JÚNIOR et al., 2020), and *Fusarium* spp. Link, which survives through the formation of chlamydospores (SILVA et al., 2023; SILVA et al., 2026).

In addition to these, genera such as *Didymella* spp. (Auersw.) Rehm, *Rhizoctonia* Kühn spp. (ALMEIDA et al., 2024), *Lasiodiplodia* spp. (Pat.) Griff. & Maubl. (SILVA et al., 2024), and *Monosporascus* spp. Pollack & Uecker (CAVALCANTE et al., 2020) are reported as the causal agents of the main diseases during the melon cycle, primarily attacking the root systems. Generally, these phytopathogens act simultaneously on the root system and can cause plant death at the onset of infection (damping-off), throughout cultivation, or up to the final weeks preceding harvest (ALMEIDA et al., 2024).

Notably, species of the genus *Fusarium* Link, as reported by Costa et al. (2026), such as *F. falciforme*, *F. sulawesiense*, *F. pseudensiforme*, and *F. silvicola*, possess the ability to colonize highly diverse environments. Consequently, they exhibit novel and distinct infection routes within the melon plant system, including organic sowing substrates, female flowers (due to high nectar content), plant roots, and the peduncle or openings on the fruit surface. This demonstrates that, contrary to conventional knowledge, pathogens such as *Fusarium* spp. employ dynamic infection strategies, frequently bypassing integrated pest management protocols and causing significant losses to the agricultural sector.

In traditional phytosanitary management, there are few active ingredients or formulations registered in Brazil for the control of phytopathogens in melon crops with soil-directed application; instead, management relies heavily on chemical formulations based on

imidazoles and strobilurins, which are the most conventional treatments for postharvest rots (AGROFIT, 2026). Although their application is permitted by the European market, their use is strictly constrained by the adoption of ultra-low maximum residue limits (EUROPEAN COMMISSION, 2025). Thus, the intersection of international restrictions on fungicide use and the decline in chemical efficacy over the years (MANIÇOBA et al., 2023) has driven the agricultural sector to search for alternative strategies to manage fungal diseases across various plant infection routes, such as the use of a biocontrol fungi belonging to the genus *Trichoderma*.

As a field-based biological strategy, the genus *Trichoderma* (Hypocreaceae) stands out as a cornerstone of biological control, comprising more than 300 cosmopolitan species. This genus exhibits multiple mechanisms of action, including mycoparasitism, antibiosis, competition, and the induction of plant resistance, alongside a proven potential for plant growth promotion (TANDON et al., 2020).

In a previous study, Almeida et al. (2024) observed that *Trichoderma* species, such as *T. asperellum* URM 5911, *T. harzianum* ESALQ 1306, and *T. longibrachiatum*, led to significant reductions (66% to 75%) in root rot severity in the 'Yellow' melon hybrid 'Natal' cultivated in soil naturally infested with soilborne pathogens. The authors verified the co-occurrence of diverse phytopathogenic genera causing root diseases in the crop, including *Macrophomina* spp., *Monosporascus* spp., *Fusarium* spp., and *Rhizoctonia* spp.

Consequently, the field efficacy of these biological control agents is directly dependent on their capacity for survival, persistence, and rhizospheric colonization throughout the crop cycle. In semi-arid regions, this biological performance faces severe constraints due to critical abiotic factors, such as salt-affected soils and high thermal amplitudes, where soil temperatures in the 10–20 cm surface layer frequently exceed 40 °C due to the use of polyethylene mulching combined with intense solar radiation (DIAS et al., 2026). These adverse conditions directly impair conidial germination, mycelial growth, and the viability of fungal resistance structures, thereby limiting the predictability of biocontrol agents under field conditions.

Continuing the melon production cycle, this diversity of routes demonstrates that *Fusarium* fruit rot is also a major limiting factor during the postharvest phase, a disease whose incidence in melon crops has been widely documented by several studies. For instance, Araújo et al. (2021) identified *F. falciforme*, *F. sulawesiense*, *F. pernambucanum*, and *F. kalimantanense* as the causal agents of this rot, noting that *F. falciforme* and *F. sulawesiense* exhibited the highest aggressiveness in 'Yellow' melons.

Subsequently, in a broader study, Nogueira et al. (2023) evaluated the pathogenicity of different *Fusarium* species across eight commercial melon hybrids belonging to the 'Yellow' ('Goldex' and 'Gold Mine'), 'Pele de Sapo' ('Grand Prix' and 'Flecha Verde'), 'Galia' ('McLaren' and 'DRG3228'), and 'Cantaloupe' ('SV1044MF' and 'Bonsai') groups. Their findings confirmed the pathogenicity of all evaluated species and highlighted *F. falciforme* and *F. sulawesiense* as the most aggressive pathogens, regardless of the commercial hybrid tested.

In this scenario, unmanaged pathogen infections lead to fungal rots, causing annual fresh fruit losses worldwide of approximately 20% to 40% (ULLAH et al., 2025), with the genus *Fusarium* alone capable of causing losses of up to 15% in total Brazilian fruit exports (OSTER et al., 2018). Thus, alternative strategies to traditional postharvest management that are efficient and residue-free are required, such as thermotherapy, 1-methylcyclopropene (1-MCP), and gibberellins.

Within alternative disease management, the use of thermotherapy has emerged as a valuable tool against postharvest *Fusarium* rot in melons, even though it is not yet commercially employed for this crop in Brazil. Defined as a physical method, it works by exposing fruits to high temperatures for short durations, inducing the reduction or eradication of the pathogen. This occurs through direct action leading to protein and cellular denaturation of spores and hyphae, thereby reducing the metabolic activity of phytopathogens such as *Fusarium* spp. Additionally, it acts indirectly by triggering the activation of metabolic defense mechanisms, including oxidative defense enzymes such as peroxidase, which reduce hydrogen peroxide (H_2O_2) levels in the plant tissue, consequently preserving the fruits and enhancing postharvest quality, as observed by Moura et al. (2024).

Other studies, such as that by Medeiros et al. (2024), corroborate these findings, demonstrating that copper-based treatments and cinnamon essential oil, when combined with thermotherapy in melons infected by *F. falciforme*, resulted in reduced fruit hydrogen peroxide content alongside a significant increase in pulp firmness.

The use of plant growth regulators such as 1-MCP and gibberellin has also been investigated as alternative management strategies. 1-MCP is classified as a synthetic growth regulator that acts as an ethylene antagonist, downregulating ACC-synthase and ACC-oxidase enzymes, which delays ripening and extends the shelf life of refrigerated fruits (TEDESCHI et al., 2023). Conversely, gibberellin is an endogenous phytohormone belonging to the diterpenoid class; its active form regulates plant growth and development, while also delaying fruit ripening and senescence, thereby indirectly restricting pathogen development (ZHANG et al., 2023).

Given the complexity of these combined biotic and abiotic stresses, it is evident that melon crop management requires a continuous and integrated phytosanitary approach capable of linking root system protection in the field with the maintenance of fruit metabolic integrity during the postharvest phase. Under this perspective, the present study investigates the phytopathological bottlenecks of the crop through two complementary research fronts. The first explores the survival dynamics, physiological plasticity, and resilience of commercial *Trichoderma* spp. isolates subjected to the thermal and osmotic fluctuations typical of the Brazilian semi-arid region during melon cultivation. The second front evaluates the defense mechanisms of fruits subjected to the combined management of thermotherapy and plant growth regulators to control postharvest rots caused by *F. falciforme* and *F. sulawesiense*.

REFERENCES

ABRAFRUTAS. Associação Brasileira dos Exportadores de Frutas e Derivados. **Export panels: Statistical data**. [S. l.], 2026. Disponível em: https://abrafrutas.org/dados-estatisticos/. Acesso em: 11 jul. 2026.

AGROFIT. Ministério da Agricultura, Pecuária e Abastecimento. Agrofit – Sistema de Agrotóxicos Fitossanitários. Brasília, DF: MAPA, 2026. Disponível em: https://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons. Acesso em: 11 jul. 2026.

ALMEIDA, B. H.; COSTA, M. H. D.; SILVA, J. L. S.; SILVA, D. J.; FRUTUOSO, R. M. S.; DIAS, A. M.; LOBO, J. T.; DA SILVA, W.; EVANGELISTA, L. F. B.; AMBRÓSIO, M. M. Q. Sustainable management of Fusarium rots in melons: A dual strategy combining thermotherapy and 1-MCP. **Crop Protection**, [S. l.], v. 202, 107522, 2026. Disponível em: https://doi.org/10.1016/j.cropro.2025.107522.

ALMEIDA, B. H.; NEGREIROS, A. M. P.; MELO, N. J. A.; AMBRÓSIO, M. M. Q.; ARMENGOL, J.; SILVA, W.; SALES JÚNIOR, R. Evaluation of fungicides and *Trichoderma* spp. for controlling soil-borne fungal pathogens in melon crops. **Revista Caatinga**, Mossoró, v. 37, e12462, 2024. Disponível em: http://dx.doi.org/10.1590/1983-21252024v3712462rc.

ARAÚJO, M. B. M.; MOREIRA, G. M.; NASCIMENTO, L. V.; NOGUEIRA, G. A.; NASCIMENTO, S. R. C.; PFENNING, L. H.; AMBRÓSIO, M. M. Q. Fusarium rot of melon is caused by several Fusarium species. **Plant Pathology**, [S. l.], v. 70, n. 3, p. 712-721, 2021. Disponível em: https://doi.org/10.1111/ppa.13328.

CAVALCANTE, A. L. A.; NEGREIROS, A. M. P.; TAVARES, M. B.; BARRETO, É. S.; ARMENGOL, J.; SALES JÚNIOR, R. Characterization of five new Monosporascus species: Adaptation to environmental factors, pathogenicity to cucurbits and sensitivity to fungicides. **Journal of Fungi**, [S. l.], v. 6, n. 3, 169, 2020. Disponível em: https://doi.org/10.3390/jof6030169.

COSTA, M. H. D.; SILVA, J. L. S.; SOUZA, J. J. F.; SILVA, I. V. P.; ALVES, T. R. C.; MOURA, A. P.; COSTA, T.; LIMA, J. S. S.; SILVA, W. L.; AMBROSIO, M. M. Q. Dynamics of Fusarium infection in melon (*Cucumis melo*): From roots to fruits. **Plant Pathology**, [S. l.], v. 75, e70140, 2026. Disponível em: https://doi.org/10.1111/ppa.70140.

DIAS, N. S.; MEDEIROS, J. F.; MORAIS, P. L. D.; ARRUDA, M. V. M.; DINIZ, A. A.; SALDANHA, E. C. M.; ARAGÃO, A. R. (ed.). **Cultivo do melão para exportação na bacia potiguar**. [S. l.]: LF Editorial, 2026.

DONG, Y.-H.; WANG, F.-G.; WANG, Y.-Q.; WANG, Z.; BAI, L.-F.; CHENG, Z.-P.; CAO, Y.-T.; SUN, L.; YU, J.-H.; XU, J.-Y.; WANG, Z.-G. Split application of NPK fertigation improve the economic benefits and fertilizer use efficiency in spring maize production. **Journal of Plant Nutrition and Fertilizers**, [S. l.], v. 31, n. 5, p. 926–942, 2025.

EUROPEAN COMMISSION. **Active substances, safeners and synergists**. [S. l.], 2025. Disponível em: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances. Acesso em: 11 jul. 2026.

FAO. Food and Agriculture Organization of the United Nations. **FAOSTAT: Countries by commodity: Cantaloupes and other melons**. Roma: FAO, 2024. Disponível em: https://www.fao.org/faostat/en/#rankings/countries_by_commodity. Acesso em: 11 jul. 2026.

HABIBAH, N.; NASRUDIN; RAMADHAN, R. A. M. Physical performance of *Cucumis melo* var. *inodorus* fruit under different storage conditions. **Journal Triton**, [S. l.], v. 17, p. 158–168, 2026. Disponível em: https://doi.org/10.47687/jt.v17i1.1690.

IBGE. Instituto Brasileiro de Geografia e Estatística. **Municipal agricultural production: Melon**. Rio de Janeiro: IBGE, 2024. Disponível em: https://www.ibge.gov.br/estatisticas/economicas/agricultura-e-pecuaria.html. Acesso em: 11 jul. 2026.

LI, X.; CAO, J.; LI, D.; JIN, K.; LIU, Y.; LI, W. Effects of different mulching practices on soil properties and soil microbial communities in tomato production. **Frontiers in Microbiology**, [S. l.], v. 16, 1734062, 2026. Disponível em: https://doi.org/10.3389/fmicb.2025.1734062.

LIU, Y.; SUN, X.; LAI, J.; WEI, S.; SHENG, Y.; ZHANG, Y.; ZHANG, Q.; YE, P.; HUANG, L.; ZENG, H. Research Progress on Rhizosphere Microbiota for Controlling Soil-Borne Diseases: Mechanisms, Applications, and Challenges. **Microorganisms**, [S. l.], v. 14, n. 4, 900, 2026. Disponível em: https://doi.org/10.3390/microorganisms14040900.

MANCHALI, S.; CHIDAMBARA MURTHY, K. N.; VISHNUVARDANA; PATIL, B. S. Nutritional composition and health benefits of various botanical types of melon (*Cucumis melo* L.). **Plants**, [S. l.], v. 10, n. 9, 1755, 2021. Disponível em: https://doi.org/10.3390/plants10091755.

MANIÇOBA, F. E.; NEGREIROS, A. M. P.; CAVALCANTE, A. L. A.; ALVES, C. P. S. S.; NASCIMENTO, M. T. A.; AMBRÓSIO, M. M. Q.; SALES JÚNIOR, R. Environmental factors and fungicide sensitivity of *Fusarium* spp. causing melon fruit rot. **Journal of Phytopathology**, [S. l.], v. 171, n. 11-12, p. 504–516, 2023. Disponível em: https://doi.org/10.1111/jph.13206.

MEDEIROS, A. S. de; ALVES, T. R. C.; SILVA, J. L. dos S.; MOURA, A. P. de; LIMA, J. S. S. de; SOUZA, J. J. F. de; FRANÇA, M. A. V. de; FERNANDES, J. da C.; EVANGELISTA, L. F. B.; AMBRÓSIO, M. M. Q. Thermotherapy combined with alternative products in the management of melon rot caused by *Fusarium falciforme*. **European Journal of Plant Pathology**, [S. l.], v. 169, n. 1, p. 195–207, 2024. Disponível em: https://doi.org/10.1007/s10658-023-02805-w.

MOURA, A. P. de; SOUZA, J. J. F. de; ALVES, T. R. C.; SILVA, J. L. dos S.; SILVA, I. V. P. da; NEGREIROS, A. M. P.; FIGUEIREDO, F. R. A.; EVANGELISTA, L. F. B.; AMBRÓSIO, M. M. de Q.; FREIRES, A. L. A. Thermotherapy in the management of galia melon rot caused by *Fusarium falciforme* and *Fusarium sulawesiense*. **European Journal of**

Plant Pathology, [S. l.], v. 169, n. 4, p. 1089–1099, 2024. Disponível em: https://doi.org/10.1007/s10658-024-02866-5.

NASCIMENTO, C. S.; NASCIMENTO, C. S.; CECÍLIO FILHO, A. B. N:K ratio for phenological growth stages of net melon cultivated in NFT hydroponic system. **Revista Caatinga**, Mossoró, v. 33, n. 1, p. 108-115, 2020. Disponível em: https://doi.org/10.1590/1983-21252020v33n112rc.

NOGUEIRA, G. A.; CONRADO, V. S. C.; FREIRES, A. L. A.; SOUZA, J. J. F.; FIGUEIREDO, F. R. A.; BARROSO, K. A.; ARAÚJO, M. B. M.; NASCIMENTO, L. V.; LIMA, J. S. S.; BEZERRA NETO, F.; SILVA, W. L.; AMBRÓSIO, M. M. Q. Aggressivity of different *Fusarium* species causing fruit rot in melons in Brazil. **Plant Disease**, [S. l.], v. 107, n. 3, p. 886–892, 2023. Disponível em: https://doi.org/10.1094/PDIS-04-22-0728-SR.

OSTER, A. H.; SILVA, E. de O.; BORDALLO, P. do N.; ZOCOLO, G. J.; SILVEIRA, M. R. S. da; OIRAM FILHO, F.; ARAÚJO, A. A. C. de. **Luz ultravioleta pulsada no controle de podridão pós-colheita e na qualidade de melão para exportação**. Fortaleza: Embrapa Agroindústria Tropical, 2018. (Boletim de Pesquisa e Desenvolvimento, 173). Disponível em: https://www.embrapa.br/agroindustria-tropical.

SALES JÚNIOR, R.; SILVA NETO, A. N.; NEGREIROS, A. M. P.; GOMES, T. R. R.; AMBRÓSIO, M. M. Q.; ARMENGOL, J. Pathogenicity of *Macrophomina* species collected from weeds in cowpea. **Revista Caatinga**, Mossoró, v. 33, n. 2, p. 395-401, 2020. Disponível em: https://doi.org/10.1590/1983-21252020v33n212rc.

SEBRAE. Serviço Brasileiro de Apoio às Micro e Pequenas Empresas. **Melon cultivation profile**. [S. l.]: SEBRAE, 2025. Disponível em: https://observatorio.sebrae.com.br/profile/industry/cultivo-de-melao. Acesso em: 11 jul. 2026.

SILVA, J. L. S.; BENTO, E. A.; MOURA, A. P.; ALVES, T. R. C.; SILVA, I. V. P.; SOUZA, V. M. G.; ÁVILA FILHA, V. M.; RIBEIRO, J. W. S.; SILVA, W. L.; AMBRÓSIO, M. M. Q. Characterization and aggressiveness of *Fusarium* spp. associated with root and stem rot in *Carica papaya* in Northeast Brazil. **Plant Disease**, [S. l.], v. 110, n. 3, 2026. Disponível em: https://doi.org/10.1094/PDIS-02-25-0400-RE.

SILVA, R. M.; AMBRÓSIO, M. M. Q.; SILVA NETO, J. A.; SILVA, J. L. S.; COSTA, T. E.; FIGUEIREDO, F. R. A.; BARROSO, K. A.; SILVA, W.; HOLANDA, I. S. A. First report of *Lasiodiplodia brasiliensis* causing root rot in melon plants in Northeastern Brazil. **Plant Disease**, [S. l.], v. 108, n. 1, p. 219, 2024. Disponível em: https://doi.org/10.1094/PDIS-07-23-1443-PDN.

SILVA, S. G. A.; COSTA, M. M.; CARDOSO, A. M. S.; NASCIMENTO, L. V.; BARROSO, K. A.; NUNES, G. H. S.; PFENNING, L. H.; AMBRÓSIO, M. M. Q. *Fusarium falciforme* and *Fusarium suttonianum* cause root rot of melon in Brazil. **Plant Pathology**, [S. l.], v. 72, n. 4, p. 721–730, 2023. Disponível em: https://doi.org/10.1111/ppa.13701.

TANDON, A.; FATIMA, T.; ANSHU, D.; SHUKLA, D.; TRIPATHI, P.; SRIVASTAVA, S.; SINGH, P. C. Phosphate solubilization by *Trichoderma koningiopsis* (NBRI-PR5) under abiotic stress conditions. **Journal of King Saud University – Science**, [S. l.], v. 32, p. 791–798, 2020. Disponível em: https://doi.org/10.1016/j.jksus.2019.02.001.

TEDESCHI, P.; MARZOCCHI, S.; MARCHETTI, N.; BARBA, F. J.; MAIETTI, A. Influence of post-harvest 1-methylcyclopropene (1-MCP) treatment and refrigeration on chemical composition, phenolic profile and antioxidant modifications during storage of Abate Fétel pears. **Antioxidants**, [S. l.], v. 12, n. 11, 1955, 2023. Disponível em: https://doi.org/10.3390/antiox12111955.

ULLAH, Q.; WAQAR, M.; SAJJAD, N.; AWLQADR, F. H.; ARSHAD, M. T.; MAQSOOD, S.; SAĞLAM, K.; MAQSOOD, N.; HOSSAIN, M. S.; IKRAM, A.; KHOMPHET, T.; GNEDEKA, K. T. Innovative strategies for postharvest disease management in fruits and vegetables: A comprehensive treatise. **Food Science & Nutrition**, [S. l.], v. 13, e70850, 2025. Disponível em: https://doi.org/10.1002/fsn3.70850.

WEN, M.; YANG, S.; HUO, L.; HE, P.; XU, X.; WANG, C.; ZHANG, Y.; ZHOU, W. Estimating Nutrient Uptake Requirements for Melon Based on the QUEFTS Model. **Agronomy**, [S. l.], v. 12, n. 1, 207, 2022. Disponível em: https://doi.org/10.3390/agronomy12010207.

ZHANG, J.; CAO, Y.; TANG, J.; HE, X.; LI, M.; LI, C.; REN, X.; DING, Y. Physiology and application of gibberellins in postharvest horticultural crops. **Horticulturae**, [S. l.], v. 9, n. 6, 625, 2023. Disponível em: https://doi.org/10.3390/horticulturae9060625.

ZHOU, W.; CHEN, J.; ZHAO, P. Temporal and habitat dynamics of soil fungal diversity in gravel-sand mulching watermelon fields in the semi-arid Loess Plateau of China. **Microbiology Spectrum**, [S. l.], v. 11, e0315022, 2023. Disponível em: https://doi.org/10.1128/spectrum.03150-22.

CHAPTER I

ADAPTIVE POTENTIAL AND SURVIVAL OF *Trichoderma* SPECIES UNDER MELON CULTIVATION IN SOILS OF THE BRAZILIAN SEMIARID REGION

ABSTRACT

Fungi of the genus *Trichoderma* are widely used in agriculture due to their ecological versatility and multiple mechanisms of action. However, controversies still exist regarding their use and their alleged inability to grow under adverse conditions. This study characterized the growth thresholds of eight commercial *Trichoderma* isolates under laboratory conditions (pH, salinity, and temperature) and validated their survival during a commercial field cycle of 'Pele de Sapo' melon. The studied *Trichoderma* isolates demonstrated broad physiological plasticity under contrasting abiotic conditions. Growth occurred over a wide pH range (2 to 10), at high salinity (up to 1000 mM NaCl), and across a broad temperature range (10°C to 35°C). Most *Trichoderma* isolates exhibited a survival rate above 74%, with the *T. asperellum* URM 5911 isolate standing out by achieving a 95% survival rate at the end of the experiment. The *Trichoderma* species stabilized and reduced the electrical conductivity under conditions of soil salinization cultivated with melon, with particular emphasis on the species *T. harzianum* IBLF006, *T. asperellum* URM 5911, *T. harzianum* IB19/17, *T. longibrachiatum*, *T. asperelloides* MMBF 94/17, and *T. asperellum* BV-10. In summary, this genus established itself as a resilient and functional bioinoculant capable of surviving the limitations of a semi-arid environment that utilizes polyethylene soil *mulching*; therefore, it is highly recommended for the sustainable management of this crop.

Keywords: Biological control, fungal resilience, soil conditions, chlamydospores, agricultural bioinoculant.

1. INTRODUCTION

Agricultural expansion, successive monoculture, and the recurrent use of susceptible cultivars have intensified the incidence and persistence of soilborne pathogens across diverse production systems (ALMEIDA et al., 2026; EVANGELISTA et al., 2026). Fungal genera, such as *Macrophomina*, *Fusarium*, and *Rhizoctonia* are widely recognized as some of the most challenging to manage and are frequently associated with substantial yield reductions in intensive melon cultivation (WANG, 2023; ALMEIDA et al., 2024). Their long-term survival in soil, even in the absence of host plants, is largely attributed to the formation of resistant structures (CAVALCANTE et al., 2020; NOGUEIRA et al., 2023).

Although chemical management remains widely adopted, its effectiveness has been increasingly constrained by reduced pathogen sensitivity, the development of resistance, and tightening regulatory restrictions on conventional active ingredients (MANIÇOBA et al., 2023; EUROPEAN COMMISSION, 2025). In Brazil, melon (*Cucumis melo* L.) production is characterized by up to three cultivation cycles per year, frequently without crop rotation and with the use of plastic mulching, conditions that contribute to an increase in the severity of soilborne pathogens. This scenario is exacerbated by the limited availability of chemical products for the control of the main soilborne pathogens (ALMEIDA et al., 2024; AGROFIT, 2026).

Furthermore, this low availability of efficient synthetic active ingredients, combined with the attack of aggressive phytopathogens such as *Fusarium* spp., causes severe losses across all stages of melon development, from seeding to post-harvest (COSTA et al., 2026). This directly impacts productivity and economic yield in the country, where this crop is responsible for more than 14,500 direct jobs and 80,000 indirect jobs (SEBRAE, 2025). In 2024, the Northeast region concentrated 98.35% of the national production (816,939 t), with emphasis on the states of Rio Grande do Norte (RN; 61.84%), Pernambuco (PE; 12%), Bahia (BA; 11%), and Ceará (CE; 8.68%) (IBGE, 2024).

This performance positions melon as the second most exported fruit by the country, with 113,753.16 t (US$ 95,537,443) in the first half of 2026 alone, with 94% of this total destined for the European market (Netherlands, Spain, and the United Kingdom) (ABRAFRUTAS, 2026).

Given the economic importance of this crop and the conventional phytosanitary limitations, alternative methods such as biological control have been adopted by agricultural producers, especially of melon, as a sustainable, residue-free, and efficient strategy for

managing soilborne pathogens. Based on this, the genus *Trichoderma* (Hypocreaceae) stands out due to its broad geographic distribution, rapid growth, and ability to colonize diverse soil environments. Comprising more than 300 species, notably, *T. harzianum*, *T. asperellum*, and *T. longibrachiatum* represent over 60% of commercially formulated biological products worldwide (MEYER et al., 2019). In Brazil, *Trichoderma*-based products represent a significant fraction of the biological control sector, accounting for approximately 67% of microbiological fungicides (AGROFIT, 2026).

The widespread adoption of these fungi is sustained by their varied mechanisms of action, such as mycoparasitism, antibiosis, competition for resources, enzyme secretion, and plant growth promotion (DRUZHININA et al., 2018; MARRA et al., 2019). This agronomic relevance is linked to the biological versatility of *Trichoderma* species (MEYER et al., 2019). However, this versatility is constantly challenged in regions of high environmental heterogeneity, such as Brazil, where edaphoclimatic variability imposes distinct constraints on microbial communities, influencing the biological performance of inoculants (SANTOS et al., 2018; IBGE, 2023).

Despite commercial formulations, the success of *Trichoderma* in the field remains inconsistent. The efficacy of the products under such conditions is dictated by ecological compatibility, genetic variability, and physiological resilience. Consequently, strains effective in one environment may exhibit reduced performance in other environments. The selection of appropriate isolates remains a limiting factor for the reliability of biological control, highlighting the need for studies that clarify the adaptive capacity, survival, and functional limits of the genus under variable environmental and soil conditions (MEYER et al., 2019).

In this context, the present study aimed to evaluate the physiological growth limits and stress tolerance of eight *Trichoderma* isolates under varying abiotic conditions in the laboratory, and to validate their survival and potential to modulate rhizospheric chemical attributes under the natural environmental fluctuations of a field-grown commercial melon production cycle.

2. MATERIALS AND METHODS

2.1 Fungal isolates

Eight isolates belonging to *Trichoderma* genus were evaluated, comprising five species (Table 1).

Table 1. The *Trichoderma* isolates used during the assays.

Tratamentos	**Produto**	**Empresa**
T. harzianum IBLF006	Ecotrich®	Ballagro
T. asperellum URM 5911	Quality®	Lallemand
T. harzianum IB19/17	Gratto Harz®	Ubyfol
T. endophyticum IBCB 56/12	Lalnix Resist®	Lallemand
T. harzianum ESALQ 1306	Trichodermil®	Koppert
T. longibrachiatum	TrichonemateMax®	Biofungi
T. asperelloides MMBF 94/17	Trichotrop®	Biotrop
T. asperellum BV-10	Tricho-Turbo®	Vittia

These isolates were obtained from commercial biological products and selected based on their cosmopolitan distribution, widespread use in scientific research, and application in biological control programs across different agricultural systems worldwide. The isolates were subcultured on potato dextrose agar (PDA) (Merck KGaA, Darmstadt, Germany) supplemented with tetracycline (0.05 g/L) and incubated in a BOD-type growth chamber at 28 °C under a 12-h light/12-h dark photoperiod for 7 days (YUSHU et al., 2017).

2.2 Laboratory assays

The assays (pH, salinity, and temperature) were conducted utilizing a completely randomized design (CRD) with eight treatments and five replicates per treatment, with each replicate consisting of a single Petri dish. The experiments were performed at the Plant Pathology and Microbiology laboratory of the Federal University of the Semi-Arid Region – UFERSA (5°12'05.44"S, 37°19'31.70"W), located in Mossoró, Rio Grande do Norte, Brazil.

Mycelial plugs (0.5 cm in diameter) excised from the margins of 7-day-old fungal colonies were transferred to the center of new PDA plates supplemented with tetracycline. The entire experimental series was performed in duplicate (two independent experimental runs).

2.3 pH and osmotic stress tolerance assays

For the pH assay, the PDA medium was adjusted to a range of 1 to 11 using 1.5 N hydrochloric acid (HCl) or 1.0 N sodium hydroxide (NaOH). For osmotic stress (salinity), the medium was supplemented with sodium chloride (NaCl) at concentrations of 0, 250, 500, 750, and 1000 mM. The electrical conductivity of the medium was measured using a benchtop conductivity meter (Bel W12D®). Plates were maintained in a BOD-type growth chamber at 28 ± 2 °C under a 12-h light/12-h dark photoperiod (CERVANTES-GARCÍA et al., 2003). Colony diameter was measured 7 days after inoculation in two perpendicular directions (average of the transverse and longitudinal axes) using a digital caliper (Lyman 7832218).

The collected data were used to calculate the mycelial growth rate (MGR – cm/d). This 7-day incubation period was established to evaluate the physiological plasticity of the isolates under prolonged exposure to adverse abiotic conditions, thereby allowing the observation of delayed responses and adaptive metabolic adjustments, which become evident only after sustained periods of environmental stress (MAURYA et al., 2024; JAISWAL et al., 2025).

2.4 Thermal stress tolerance assays

The assays were performed by incubating the fungal species at 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 °C in a BOD-type growth chamber under a 12 h light/12 h dark photoperiod for 7 days (CERVANTES-GARCÍA et al., 2003; HAO et al., 2022). Mycelial growth was determined as previously described. After the initial incubation, all plates were incubated again for an additional 7 days at 28 °C to evaluate physiological resilience and the ability to resume growth after periods of stress caused by unfavorable environmental conditions. The production of resistance structures was evaluated 7 days after transferring the fungi to 28 °C using light microscopy.

2.5 Field experimental design and area characterization

The field study comprised two identical and independent assays conducted from October to December 2025, during the commercial production cycle of the 'Pele de Sapo' melon cultivar ‘Waikiki’. The experiments were established at the Santa Júlia Farm - Agrícola Famosa, Mossoró, RN, Brazil (5°02'22.52"S; 37°23'03.13"W). The experimental design was a randomized complete block design (RCBD), arranged in a 9 × 5 factorial scheme (nine treatments × five sampling times), with five blocks. The nine treatments consisted of eight *Trichoderma* isolates and one control consisting of sterile substrate, and eight replicates, each consisting of a nylon bag filled with the prepared substrate.

The total land area occupied by each assay was 4,420 m², with a net experimental area of 2,700 m² per assay, where each treatment occupied a net plot of 300 m². Soil samples (classified as an Ultisol with a sandy texture) were collected for physical and chemical characterization following the analytical methodology of Teixeira et al. (2017). The soil attributes were as follows: 3.10 cmolc/dm³ of Ca^{2+}, 0.58 cmolc/dm³ of Mg^{2+}, 0.27 cmolc/dm³ of Na^{+}, 0.30 cmolc/dm³ of K^{+}, 4.25 cmolc/dm³ of cation exchange capacity (CEC at pH 7.0), 100% base saturation (V%), 6.60 g/kg of soil organic matter (SOM), 3.80 g/kg of organic carbon (OC), 75.50 ppm of available phosphorus (P), 5.50 ppm of iron, 22 ppm of manganese, 2.60 ppm of zinc, 0.80 ppm of boron, 0.60 ppm of copper, pH (1:2.5 v/v) of 7.20, and an EC of 1.39 dS/m.

2.6 Treatment preparation and experiment establishment

To produce the inoculum, the methodology proposed by Lefevre & Souza (1993) was followed. For this purpose, flasks containing moistened sand-organic substrate (weathered manure, washed sand, and oats [3:1:2% w/w, respectively]) were prepared, and subsequently sterilized at 120 °C for 60 min for three consecutive days at 24 h intervals. Active mycelial plugs of the isolates were inoculated into the flasks, which were incubated for 14 days at 28 ± 2 °C under daily agitation. Flasks containing the substrate without the fungus were maintained under the same conditions as a negative control. After incubation, synthetic fabric (nylon) bags were filled with 10 g of the substrate colonized by the fungi individually, which were buried at a depth of 10 cm, at a distance of 20 cm from the plant crown in each experimental plot.

2.7 Soil survival of *Trichoderma* spp.

Evaluations were performed from the transplanting of the melon plants until harvest, totaling five sampling time points (0, 17, 33, 50, and 65 days after transplanting). Sampling point 0 corresponds to the day of trial establishment, performed to confirm that the fungal isolates exhibited 100% survival. Flasks with inoculum of each fungal isolate were also maintained under laboratory conditions throughout all the assays, which were plated concurrently with the field survival evaluations to verify their survival under laboratory conditions. At each period, two nylon bags per treatment were collected from all blocks, totaling 90 bags per analysis and 450 per assay. Samples were removed through slits in the plastic mulch, which was subsequently sealed with transparent plastic tape to prevent potential interference with plant development. Subsequently, the samples were packaged and transported to the Plant Pathology and Microbiology laboratory of the Federal University of the Semi-Arid Region – UFERSA. There, they underwent surface disinfestation by immersion in 70% ethanol, followed by 2% sodium hypochlorite (60 s), and sterile distilled water, and then dried on flamed filter paper.

Plating was performed on *Trichoderma* semi-selective medium (TSM) according to the methodology of Elad et al. (1981), with modifications. The substrate from the two nylon bags was combined into a single representative sample for each treatment. Fifty portions were arranged equidistantly across five Petri dishes containing the TSM medium, totaling five replicates. The plates were incubated in a BOD-type growth chamber at 28 ± 2 °C under a 12-h light/12-h dark photoperiod for 4 days. After this period, the recovery frequency of the isolates (%) was determined, calculated as the ratio between the number of portions showing characteristic radial mycelial growth and the total number of plated portions, multiplied by 100.

2.8 Continuous agrometeorological monitoring

Micrometeorological variables in the area were continuously monitored throughout the crop production cycle. Data was recorded using an automatic weather station coupled to a CR1000 datalogger (Campbell Scientific, Logan, UT, USA), managed by PC400 v4.1 software. The system was powered by a deep-cycle battery (12 V; 7 Ah) connected to a photovoltaic solar panel (10 W), ensuring energy autonomy in the field. Soil temperature was monitored using Type T thermocouple sensors (copper-constantan) installed at a depth of 10 cm, adjacent to the nylon bags in all treatments. Additionally, air temperature and relative

humidity were recorded using an HMP45C thermohygrometer (Campbell Scientific, Logan, UT, USA). The system was programmed to take readings at 30-minute and 1-hour intervals, automatically storing hourly and daily averages.

2.9 Soil chemical analyses

Simultaneously with the survival evaluations, 100 g soil samples were collected around the nylon bags for chemical analyses, following the same sampling scheme. These analyses followed the protocols described by Teixeira et al. (2017). Soil from the two subsamples of each block was homogenized to form a representative sample for each treatment. Soil pH was determined using a pH meter (PHB-550, Incoterm, Brazil) in a soil-water suspension (1:2.5 v/v). Electrical conductivity (EC) was determined using a benchtop conductivity meter (Q405M, Quimis, Brazil) at a soil-to-water ratio of 1:1 (v/v).

2.10 Statistical analyses

In the results of the *in vitro* and field assays, the assumptions of normality and homoscedasticity of residuals were verified using the Shapiro–Wilk ($p \leq 0.05$) and Bartlett ($p \leq 0.05$) tests, respectively.

In the *in vitro assays,* preliminary ANOVA indicated no significant differences between the two independent experiments, allowing data pooling. MGR data were analyzed using regression, adopting specific models for each assay: a cubic polynomial model for pH, a four-parameter logistic (4PL) model for salinity, and a four-parameter Gaussian model for temperature. The optimum pH (O_{pH}) and optimum temperature (O_T) were determined from the maximum points of the fitted curves, while the EC_{50} (the saline concentration that reduces mycelial growth by 50%) was estimated from the inflection point of the logistic curve. Parameters were estimated using non-linear least squares, and the goodness-of-fit was evaluated using the coefficient of determination (R^2) and the standard error of the estimate. The statistical significance of the models and their coefficients was tested by ANOVA ($p \leq 0.05$). For multivariate analysis, MGR values for each isolate were standardized using Z-scores. A heatmap with hierarchical clustering (complete linkage, Euclidean distance) was constructed from the Z-score matrix.

For the field assays, population dynamics and physicochemical attributes (pH and electrical conductivity) data were analyzed in a 9 × 5 factorial scheme (treatments × sampling

times). Due to the nature of the variable, *Trichoderma* recovery frequency data were transformed to $y = \arcsen \sqrt{(x/100)}$ to meet the assumptions of normality (Shapiro-Wilk, $p \leq 0.05$) and homogeneity (Bartlett, $p \leq 0.05$), and are presented graphically with the original means. When a significant interaction was detected by the F-test ($p \leq 0.05$), factor slicing was performed, and means were compared using the Scott-Knott test ($p \leq 0.05$).

Soil temperature data (daily maximum, mean, and minimum) were submitted to descriptive statistical analysis to characterize the microclimate and thermal stress windows throughout the production cycle. Considering that the higher persistence of *Trichoderma* in the soil is potentially associated with the maintenance of its beneficial effects on soil properties, crop development, and, consequently, agricultural yield, the Pearson correlation analysis was performed using data from the longest fungal survival evaluation period, at 65 days after transplantation (DAT). For the temperature variable, we used the average of the minimum, maximum, and optimum temperatures recorded by the sensors installed in the experimental area throughout the entire evaluation period, as it comprehensively represents the thermal conditions to which the microorganism was subjected during its permanence in the soil. For the pH and electrical conductivity variables, the values obtained from the evaluation at 65 DAT were used to ensure temporal correspondence between the edaphic variables and the fungal survival data.

All analyses were performed using R software v. 4.4.0 (R CORE TEAM, 2024) within the RStudio interface (RSTUDIO TEAM, 2024), utilizing the packages *ExpDes.pt* (v. 1.2.2), *gplots*, *corrplot* v. 0.94, and *RColorBrewer*. Graphs for all sections were generated using SigmaPlot software v. 16.0 (SYSTAT SOFTWARE INC., 2021).

3. RESULTS

3.1 Laboratory assays

The mycelial growth rate (MGR) (cm/d) was described as a function of pH using a cubic polynomial model ($\hat{y} = y_0 + ax + bx^2 + cx^3$), with $R^2 > 0.71$ for all *Trichoderma* isolates (Fig. 1A and S1). The isolates exhibited growth across a wide pH range, from 2 to 10, with complete inhibition observed at extreme values (1 and 11). The O_{pH} ranged from 4.50 (*T. harzianum* ESALQ 1306) to 5.77 (*T. endophyticum* IBCB 56/12). Differences among the isolates were observed. Several isolates exhibited optimal growth (> 0.5 cm/d) across a broad pH range (4 to 7), including *T. harzianum* IBLF006, *T. asperellum* URM 5911, *T. longibrachiatum*, and *T. asperellum* BV-10.

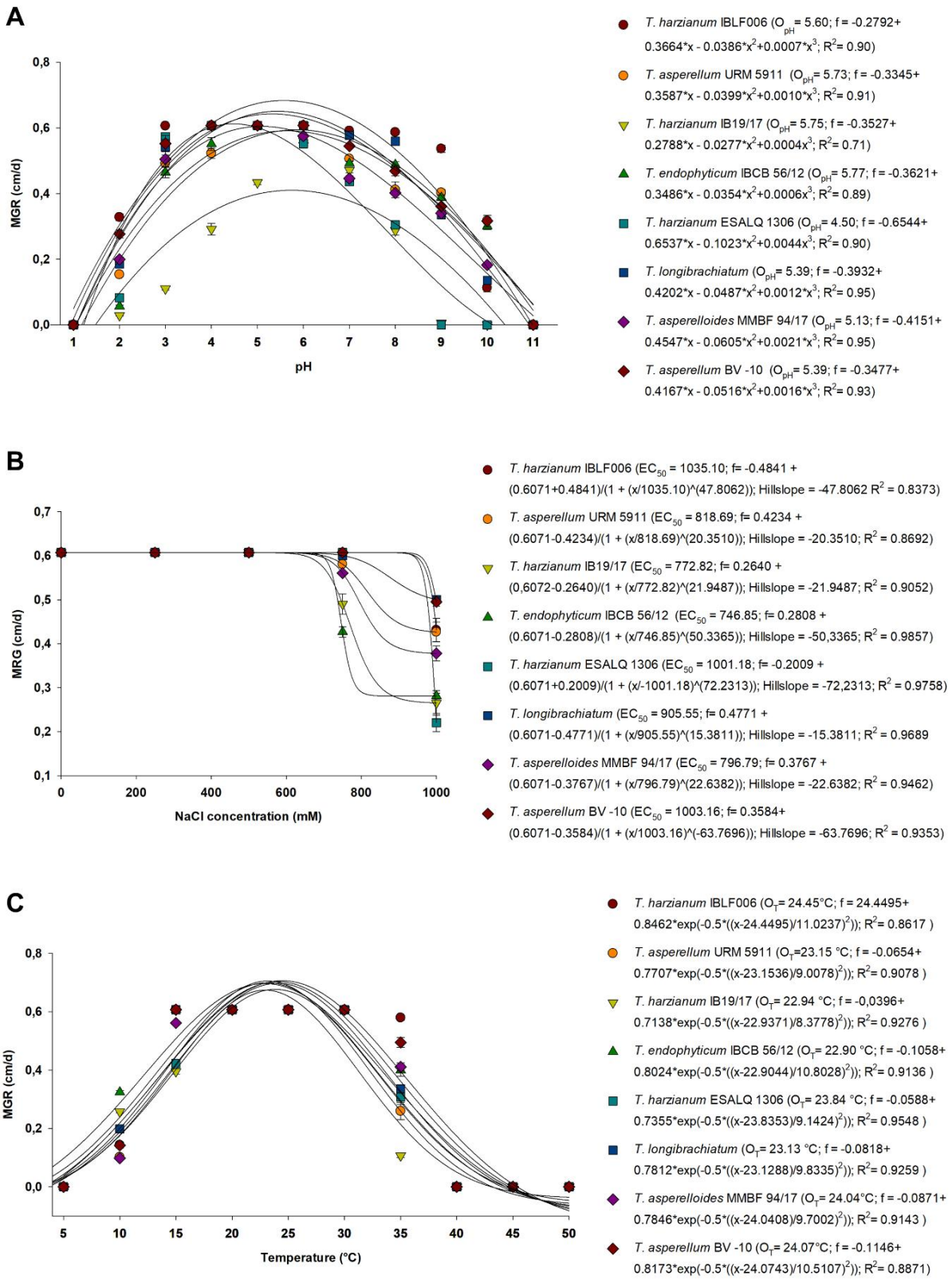

Figure 1. Mycelial growth rate (MGR – cm/d) of eight *Trichoderma* isolates under different pH values (A), NaCl concentrations (0 to 1000 mM) (B), and temperatures (5 to 50 °C) (C) after 7 days of incubation at 28 ± 2 °C. Data points represent the means of two independent

experiments ± standard error with five replicates per treatment. A = Lines correspond to cubic polynomial regression fits (f) adjusted to MGR values across pH 1 to 11; O_{pH} = optimum pH for mycelial growth of the isolates. B = Lines correspond to four-parameter logistic regression fits (f) adjusted to MGR values across different salt concentrations; EC_{50} (NaCl, mM) = saline concentration that reduces mycelial growth by 50%; Hillslope = slope coefficient of the curve. C = Lines correspond to four-parameter Gaussian regression fits (f) adjusted to MGR values across different temperatures; O_T (°C) corresponds to the maximum of the fitted curve, interpreted as the optimum temperature for mycelial growth of each isolate. R^2 = coefficient of determination.

The MGR (cm/d) was described as a function of salinity using a four-parameter logistic (4PL) model: f = min + (max − min) / [1 + (x / EC_{50})^(−Hillslope)], with $R^2 > 0.83$ for all isolates (Fig. 1B and S2). All *Trichoderma* isolates grew across all tested NaCl concentrations (0, 250, 500, 750, and 1000 mM), corresponding to electrical conductivity values of 2.07, 22.20, 30.45, 42.66, and 56.20 $dS \cdot m^{-1}$), respectively. A more pronounced reduction in growth was observed at concentrations above 750 mM (Fig. 1B). The EC_{50} ranged from 746.85 mM (*T. endophyticum* IBCB 56/12) to 1035.10 mM (*T. harzianum* IBLF006), evidencing differences in tolerance among the isolates. Despite the abrupt decline in growth near the critical threshold, none of the isolates exhibited complete inhibition at 1000 mM of NaCl.

The MGR (cm/d) was described as a function of temperature using a four-parameter Gaussian model: $\hat{y} = y_0 + a \cdot \exp[-0.5 \cdot ((x - x_0)/b)^2]$, with $R^2 > 0.86$, for all isolates (Fig. 1C and S3). During the first incubation period, the isolates exhibited growth between 10 °C and 35 °C. Within this range, maximum growth (> 0.6 cm/d) was observed from 20 °C to 30 °C. No mycelial growth occurred at 5 °C, 40 °C, 45 °C, or 50 °C (Figs. 1C and S3). The estimated O_T values ranged from 22.9 °C (*T. endophyticum* IBCB 56/12) to 24.45 °C (*T. harzianum* IBLF006). The isolates (*T. harzianum* [IBLF006, IB19/17, and ESALQ 1306], *T. longibrachiatum*, *T. asperelloides* MMBF 94/17, and *T. asperellum* BV-10) exposed to 5 and 40 °C resumed mycelial growth after re-incubation at 28 °C (Fig. S4). These same isolates also formed chlamydospores at these temperatures (Fig. 2). In contrast, temperatures above 45 °C were lethal to all tested fungi, with no isolate recovery.

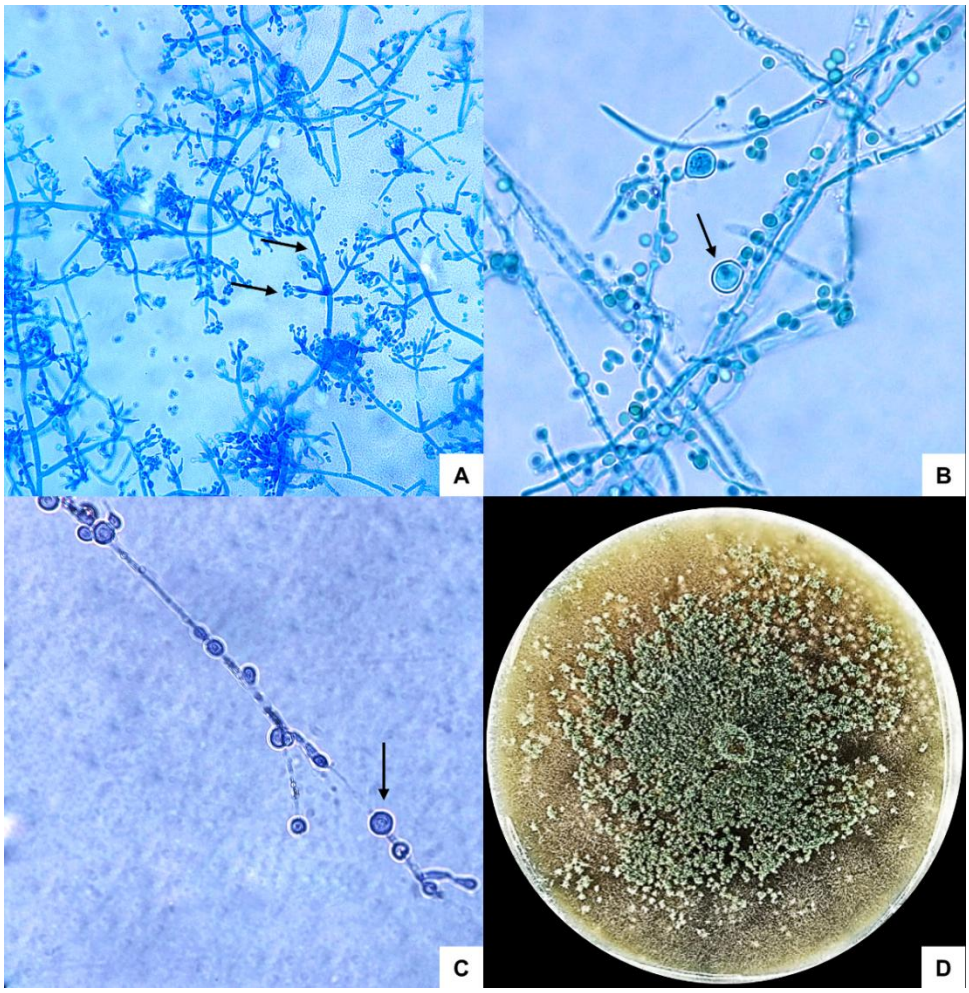

Figure 2. Morphological aspects of *Trichoderma* spp. on PDA medium, highlighting vegetative development and chlamydospore formation under thermal stress, were observed after incubation at 5 and 40 °C. (A) Branched conidiophores with phialides and abundant conidia, showing the typical sporulation pattern of the genus. (B) Hyphae with intercalary and terminal chlamydospores (black arrows), formed along the hyphae as resistance structures against stress. (C) Formation of chlamydospores along the hypha (black arrow), a thick-walled structure responsible for survival under unfavorable conditions. (D) Colony on PDA (28 ± 2 °C) showing dense mycelial growth and abundant sporulation, with the green coloration characteristic of the genus, 7 days after re-incubation. Light microscopy (A to C: 400×).

The heatmap (Fig. 3) defined the overall biological behavior of the isolates in response to abiotic stresses, clustering them into four groups. The first group (moderately alkalitolerant), composed solely of *T. endophyticum* IBCB 56/12, demonstrated tolerance to alkalinity but exhibited higher thermal and saline sensitivity. The second group (high physiological plasticity) clustered the largest number of isolates (*T. asperelloides* MMBF

94/17, *T. asperellum* URM 5911, *T. asperellum* BV-10, *T. harzianum* IBLF006, and *T. longibrachiatum*), which exhibited robust growth under optimal and intermediate conditions. The third group (low physiological plasticity) segregated *T. harzianum* IB19/17 due to its profile of low adaptation and high sensitivity to limiting salinity and temperature conditions. The fourth group (high acid tolerance) separated *T. harzianum* ESALQ 1306 into a distinct group characterized by high tolerance to acidic environments.

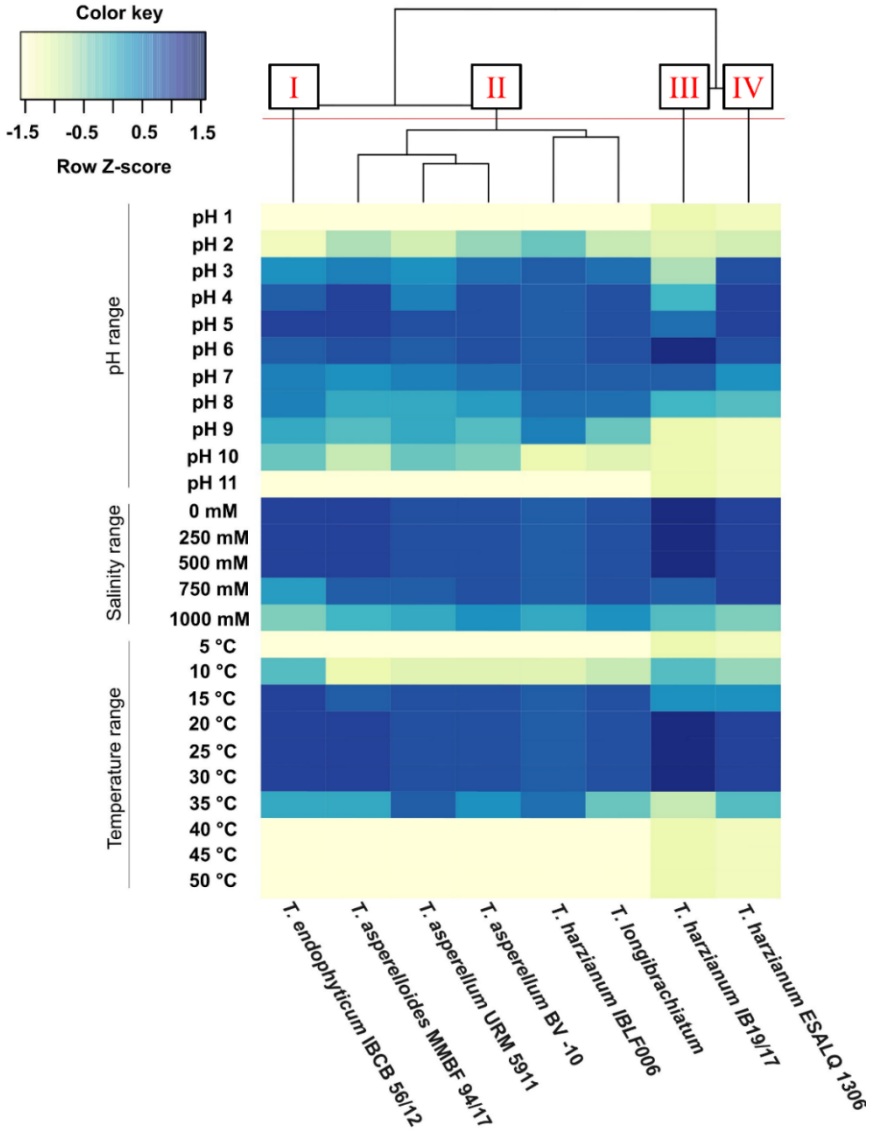

Figure 3. Heatmap representing the mycelial growth rate (MGR, cm/d) of eight *Trichoderma* isolates under different abiotic conditions of temperature (5 to 50 °C), salinity (0 to 1000 mM NaCl), and pH (1 to 11). Colors range from dark blue (higher relative growth, positive Z-scores) to light yellow (reduced or null growth, negative Z-scores). The top dendrogram shows hierarchical clustering of isolates based on their physiological response similarities.

3.2 Field assays

The survival (%) of *Trichoderma* species (Figs. 4 and 5) varied throughout the sampling period, with a more pronounced decline, for some isolates, at the 65-day sampling point ($p < 0.001$). On day 0 ($p < 0.001$), all isolates exhibited maximum survival (100%). At 17 days ($p < 0.001$), the isolates maintained high recovery rates, with *T. asperellum* URM 5911 and *T. asperelloides* MMBF 94/17 standing out at 100% survival during this period. At 33 days ($p < 0.001$), it was observed that the isolates *T. asperellum* URM 5911, *T. harzianum* IB19/17, *T. endophyticum* IBCB 56/12, and *T. asperellum* BV-10 showed 100% survival. At 50 days ($p < 0.001$), a more pronounced reduction in the survival of most species was observed. The isolates *T. harzianum* IBLF006, *T. asperellum* URM 5911, *T. harzianum* IB19/17, and *T. asperelloides* MMBF 94/17 demonstrated the highest survival rates of 99%, 100%, 100%, and 99%, respectively. In contrast, the isolates *T. longibrachiatum* and *T. asperellum* BV-10 began to exhibit a decline, reducing their survival to 68% and 72%, respectively.

At 65 days ($p < 0.001$), the soil survival of *Trichoderma* species was observed ranging from 51% for *T. longibrachiatum* to 95% for *T. asperellum* URM 5911. Regarding the horizontal slicing of the interaction across sampling times, the isolate *T. asperellum* URM 5911 stood out regarding survival throughout the 65 evaluated days, exhibiting a variation of only 5% throughout the experimental period. This isolate differed statistically from the other treatments within the same sampling time (65 days) (Fig. 4); furthermore, it did not present significant statistical differences when compared to the previous evaluations ($p = 0.5071$). In contrast, *T. longibrachiatum* exhibited the lowest survival rate at the end of the assays, showing a decrease of 49% compared to day 0.

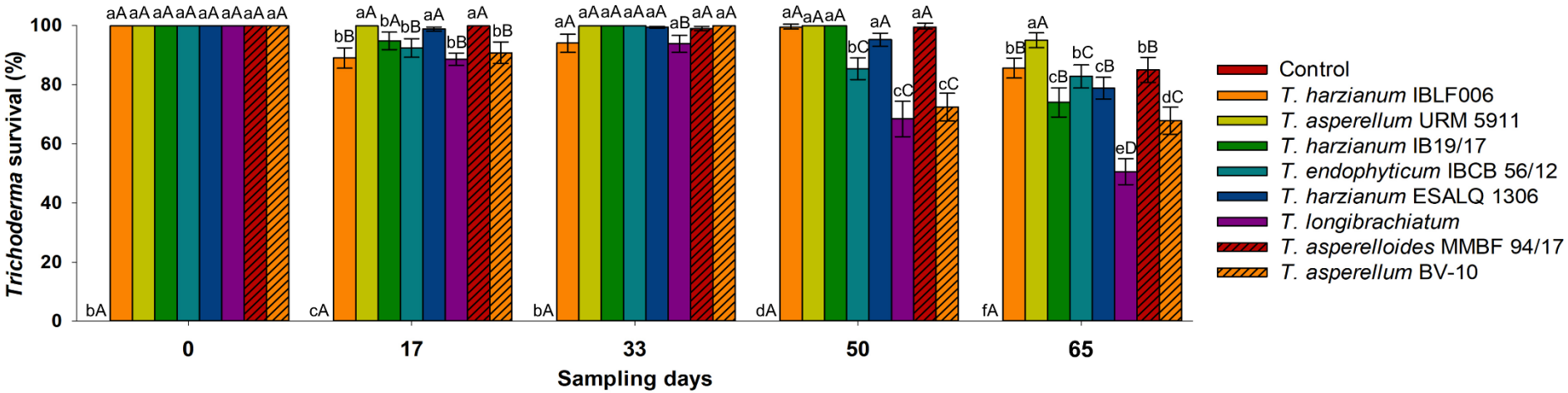

Figure 4. Survival of *Trichoderma* isolates and population dynamics during the commercial production cycle of the 'Pele de Sapo' melon 'Waikiki'. Bars represent the mean values of two independent experiments ± standard error. According to the slicing of the interaction, means followed by the same lowercase letters, within columns (among isolates) for each sampling time, or by the same uppercase letters across rows (among days) over time, do not differ statistically according to the Scott-Knott test ($p \leq 0.05$).

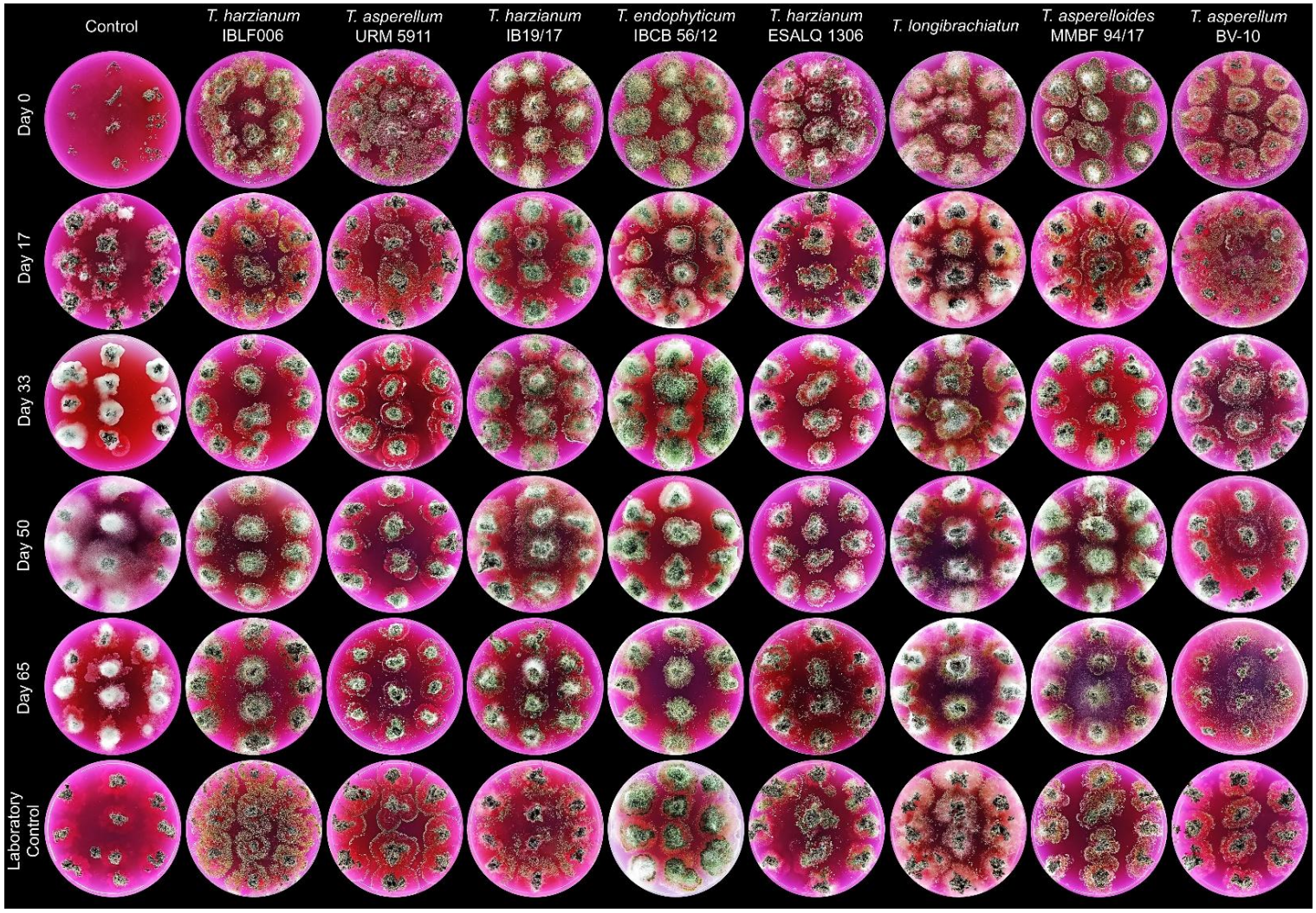

Figure 5. Macroscopic aspect of the survival of eight *Trichoderma* isolates and the control treatment on semi-selective medium (TSM) after 4 days of incubation at 28 ± 2 °C. Columns correspond to treatments, and rows represent the soil sampling times (0, 17, 33, 50, and 65 days after transplanting). The bottom row presents the growth pattern of the laboratory inoculum (positive control).

Continuous monitoring of soil temperature (Fig. 6) revealed that the maximum temperature (Fig. 6A) initially ranged from 35.6 ± 2.0 °C (day 0) to 37.7 ± 2.0 °C (day 13). From day 14 onward, a substantial increase in values was observed, resulting in a thermal peak exceeding 40 °C between days 15 and 18, with daily maximums recorded at 41.26 °C, 42.87 °C, 42.81 °C, and 41.31 °C, respectively. During this period, maximum temperatures occurred between 12:00 and 13:00 h, followed by a gradual decrease of ± 1 °C h^{-1} from 13:00 to 19:00 h, and of ± 0.5 °C h^{-1} from 19:00 to 00:00 h. From day 19 (daily mean of 38.7 ± 2.0 °C) to day 23 (daily mean of 35.4 ± 2.0 °C), a progressive thermal decline was observed, followed by the stabilization of values from day 24 to day 65. During this period, temperatures ranged between the extremes of 31.2 °C and 37.6 °C.

The mean soil temperature (Fig. 6B) and minimum soil temperature (Fig. 6C) showed a similar behavior, remaining stable during the first 14 days, followed by occasional fluctuations until day 24. From day 25 onwards, thermal stability was reestablished and remained until day 65, with minor variations. Regardless of the treatment, the daily thermal amplitude varied from 5.1 to 6.9 °C throughout the entire assay.

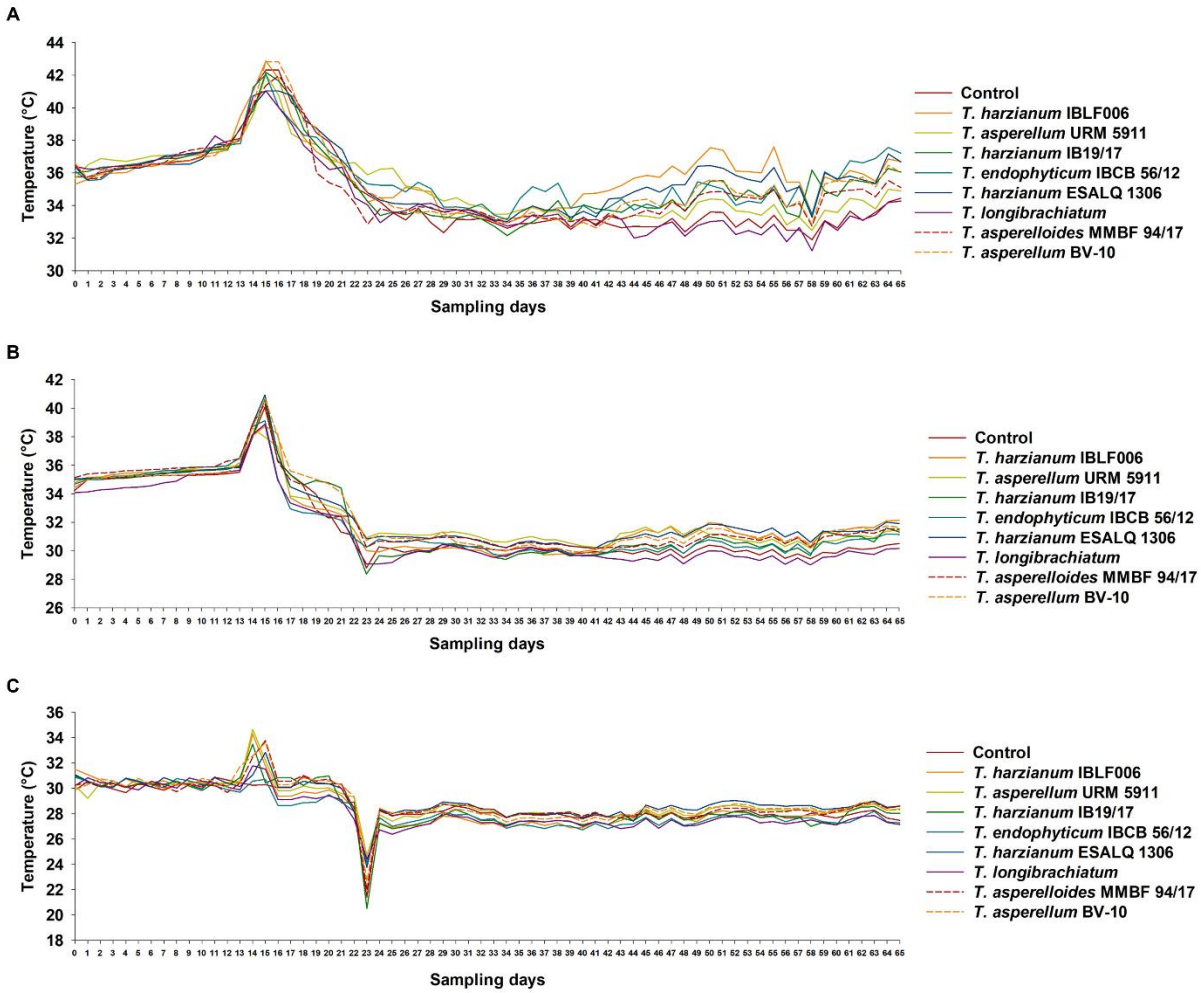

Figure 6. Soil thermal profile in plots treated with different *Trichoderma* isolates during the commercial production cycle of the 'Pele de Sapo' melon 'Waikiki'. (A) Daily maximum temperature (°C); (B) Daily mean temperature (°C); and (C) Daily minimum temperature (°C). Colored lines represent the evaluated *Trichoderma* isolates and the Control (absence of *Trichoderma*). Lines represent the daily values obtained from the continuous monitoring of each treatment.

Soil pH values (Fig. 7A) ranged from 6.19 to 7.22 ($p < 0.001$). The isolate *T. asperellum* BV-10 presented the highest absolute pH mean of the assay (7.22) at 50 days, while the isolate *T. longibrachiatum* presented the lowest pH value of the assay (6.19) at 33 days.

Soil electrical conductivity (EC) (Fig. 7B) was significantly influenced by the interaction between treatments and sampling periods ($p < 0.001$). At day 33, *T. endophyticum* IBCB56/12 maintained the lowest electrical conductivity and differed from the other treatments, reducing the EC by 46.07% compared to the Control. On day 50, a significant

increase in EC relative to day 0 was observed across all treatments, with cumulative variations ranging from 113.16% (*T. harzianum* IB19/17) to 318.64% (*T. harzianum* IBLF006). On day 65, the treatments varied from 102.63% (*T. harzianum* IB19/17) to 213.21% (*T. asperelloides* MMBF94/17), when compared to day 0. The control treatment demonstrated a constant increase throughout the entire assay, resulting in a 201.23% increment in soil EC, when comparing day 0 to day 65.

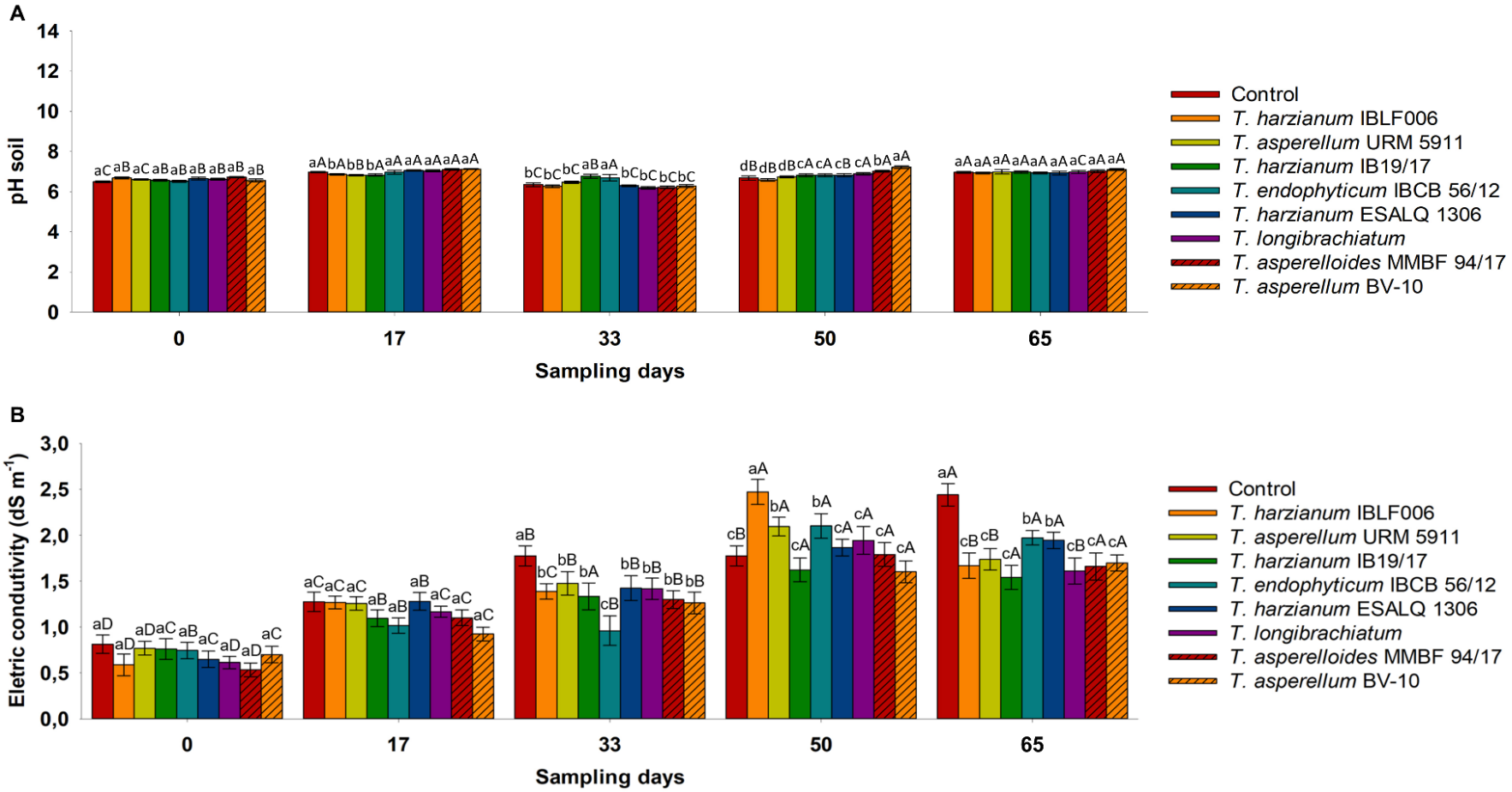

Figure 7. Soil pH (A) and electrical conductivity (B) monitoring under different treatments with *Trichoderma* spp. isolates and a Control treatment throughout the commercial production cycle of the 'Pele de Sapo' melon 'Waikiki'. Bars represent the mean values of two independent experiments ± standard error. According to the slicing of the interaction, means followed by the same lowercase letters within columns (among isolates) for each sampling time, or by the same uppercase letters across rows (among days) over time, do not differ statistically according to the Scott-Knott test ($p \leq 0.05$).

The Pearson correlation analysis (Fig. 8) revealed that at 65 days, survival exhibited a strong negative correlation with EC (r = -0.50). Soil pH exhibited positive coefficients with T_{max} (r = 0.36) and T_{med} (r = 0.32). T_{max} of soil exhibited positive coefficients with T_{med} (r = 0.84) and T_{min} (r = 0.52); and T_{med} with T_{min} (r = 0.85);

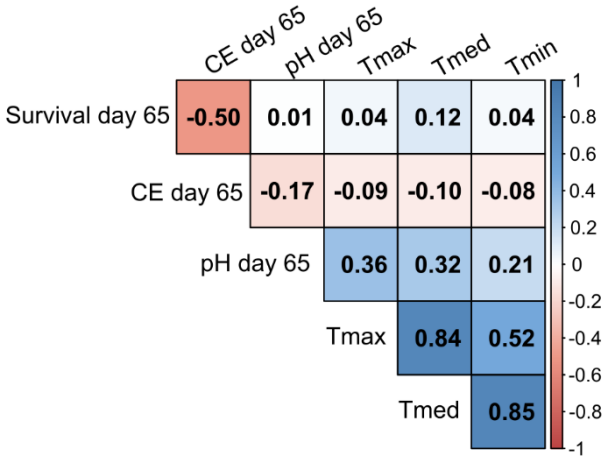

Figure 8. Pearson correlation matrix among *Trichoderma* spp. isolate survival, soil electrical conductivity (EC), and soil pH, at the final phase of the field assay (day 65), correlated with maximum (T_{max}), mean (T_{med}), and minimum (T_{min}) soil temperatures throughout the entire experimental period. The color scale and numbers indicate the Pearson correlation coefficient (r), ranging from -1 (red; perfect negative correlation) to +1 (blue; perfect positive correlation); bold values represent the strength of linear relationships.

4. DISCUSSION

Among the abiotic factors evaluated in laboratory, pH presented the widest growth range for the *Trichoderma* isolates (2 to 10) (Fig. 1A and S1), showcasing high physiological plasticity and classifying them as acidophilic to neutrophilic. All isolates maintained stable growth between pH 3 and 7, corroborating the findings of Cavalcante et al. (2025). This broad tolerance is linked to the genus's capacity to regulate microenvironment pH through the secretion of acidic and alkaline metabolites, thereby preserving cellular homeostasis and favoring growth under distinct conditions (Meyer et al., 2019). Furthermore, under more acidic conditions (pH 3 to 5), *Trichoderma* exhibits higher enzymatic activity associated with mycoparasitism and antagonism against phytopathogens, conferring greater competitiveness in tropical and semi-arid soils (NAIR et al., 2022).

Evaluation across a wide pH range allowed for a more accurate characterization of the isolates' physiological thresholds and adaptive capacity, simulating conditions frequently observed in agricultural applications, such as input tank mixtures and the *on-farm* production of microorganisms. These results demonstrate the high adaptation of *Trichoderma* to contrasting environments and provide solid ground for selecting more robust strains suited for adverse conditions (SANTOS et al., 2018; MEYER et al., 2019; DUTTA et al., 2022).

Among the treatments evaluated, high salinity levels reduced fungal growth, reflecting differences in tolerance associated with genetic variability among isolates (Fig. 1B and S2). The logistic model revealed the relationship between EC_{50} and the slope coefficient (Hillslope), indicating distinct physiological sensitivities to increasing saline concentration. The pronounced negative slope (Hillslope ≥ -50) indicates the transition phase between tolerance and inhibition under salt stress. This point corresponds to the critical threshold, estimated at around 750 mM, at which small changes in salt concentration cause abrupt reductions in mycelial growth rate (CHEN et al., 2025). Overall, isolates with higher EC_{50} and less negative slope, such as *T. longibrachiatum* and *T. asperellum* URM 5911, showed greater physiological stability under osmotic stress, indicating their suitability for cultivation systems under salinization stress.

In this study, fungal growth between 10 and 35 °C (Fig. 1C and S3) indicates the genetic versatility of the genus, reflecting its adaptive capacity across diverse environments. Notably, these data were obtained under controlled laboratory conditions with constant temperatures over a seven-day period. Such conditions differ substantially from those encountered in the field, where temperature fluctuations are frequent and stable thermal ranges generally persist for only short intervals.

The resumption of fungal growth observed in isolates previously exposed to 5 and 40 °C, following re-incubation at 28 °C, indicates the activation of adaptive and metabolic mechanisms in response to thermal stress (Fig. 2 and S4). This resilience is directly linked to the formation of chlamydospores and microsclerotia, demonstrating the capacity of the studied fungi to persist in the soil (JAISWAL et al., 2025). Such functional plasticity confers an ecological advantage to the genus, enabling its persistence and saprophytic activity in soils across diverse regions worldwide, thereby reinforcing its potential for application in varied agricultural systems (KUBIAK et al., 2023). However, specific management practices, such as the use of soil cover (polyethylene mulching) in high-temperature semi-arid regions, further elevate soil temperature, creating less favorable conditions for the development of antagonistic microorganisms.

We observed that *Trichoderma* soil survival (Figs. 4 and 5) demonstrated a dynamic behavior, being influenced not by a single environmental factor, but by the continuous interaction of temperature and electrical conductivity (Figs. 6 and 7B). As observed in Fig. 6A, with the increase in soil temperature above 40 °C during the cultivation of melon under plastic mulching, *Trichoderma* recovery (day 0 to 17) exhibited oscillations. This initial population instability is closely coupled with the soil temperature dynamics, which encompassed the period when the highest temperatures occurred along with the prolonged duration of these elevated thermal conditions. The populations recovered from day 17 to 33, coinciding with the stabilization of soil temperature to highly favorable conditions for the development of the genus (Fig. 1C). This recovery strongly corroborates the data obtained in the *in vitro* assays, where most *Trichoderma* species resumed growth after being transferred from unfavorable thermal conditions (40.0 ± 2.0 °C) back to favorable temperatures (28.0 ± 2.0 °C).

The mean soil temperature during most of the experiment remained constant around 29.0 ± 2.0 °C from day 25 to 65, remaining close to the optimal temperature for the growth of these fungi under laboratory conditions.

Our correlation data (Fig. 8) demonstrate that soil temperature during the experimental period did not negatively influence fungal survival (with correlation coefficients near zero, r = 0.04), even when subjected to a daily thermal amplitude of approximately 7 °C. Thus, we emphasize that the increase in temperature under productive conditions of the melon crop in the semi-arid region of Brazil, where polyethylene mulch is used, did not cause permanent negative impacts on *Trichoderma* populations, a factor associated with the development of resistance structures (chlamydospores and sclerotia), continuous irrigation, and the short period of exposure of the fungus to high temperatures; in which they remained exposed for only 1 h to a maximum temperature for the development of these fungi (40 °C), also as observed in the laboratory assays.

Under field conditions, we verified that from day 33 to 65 there was an increase in soil EC (Fig. 7B), causing an oscillation in the survival percentage of the isolates (Figs. 4 and 8). This occurrence is directly associated with the increased volume of fertilization and fertigation in the melon crop to support the initiation and full development of the reproductive phenological stages, ranging from flowering, fruiting, and fruit development (R1 to R2) until harvest (R3), with nitrogen and potassium (nutrients that most favor the increase in EC) inputs scaling weekly by up to 30% of the crop's nutritional requirements. Furthermore, the saline or brackish water used for irrigation in melon production also contributes to the

increase in EC (DIAS et al., 2026). However, at the 33-day sampling point, it is important to highlight that *T. endophyticum* IBCB 56/12 caused a significant reduction in electrical conductivity while maintaining 100% survival. This performance is associated with genetic and phenological factors, such as moderate tolerance to salt levels below the critical inhibitory threshold, characterized by its low Hillslope value (Fig. 1B; and Heatmap, Fig. 3).

Although an increase in soil EC was observed throughout the assays, in addition to a negative correlation between fungal survival and EC (Fig. 8), most *Trichoderma* isolates exhibited survival rates above 74%, with one specific isolate outstandingly reaching 95% survival at the end of the trials. This reflects the capacity of the genus to synthesize organic compounds that solubilize ions and regulate the expression of plant genes related to water transport and sodium balance (HU et al., 2025). This process facilitates nutrient uptake by the plant root system (WOO et al., 2023; BANDARA; KANG, 2024), consequently reducing the EC of the rhizospheric soil (TIRADO-MALAVER & TIRADO-LARA, 2025), as observed in our study, where we found reductions ranging from 19.26% (*T. endophyticum* IBCB56/12) to 36.89% (*T. harzianum* IB19/17) compared to the control, which increased uncontrollably until harvest, reaching its peak at day 65 (2.44 $dS \cdot m^{-1}$).

Thus, under high EC conditions, our findings reveal a specific modulation according to the species studied (Fig. 7B), which showed distinct reductions in EC according to the studied isolate, with *T. asperellum* and *T. harzianum* species being the most suitable for use in environments under salinization stress, aligning with our laboratory assays (Fig. 3), where differences in tolerance are associated with genetic variability among isolates (HU et al., 2025).

These field observations align with our laboratory assays (Fig. 1B and 3), where the logistic model and physiological screening identified distinct saline sensitivities among the isolates based on their EC_{50} and Hillslope values. This relationship between laboratory tolerance and field persistence was confirmed by *T. asperellum* URM 5911, which endured the high soil EC values and exhibited the highest reported survival rate (95%) at the end of the 65-day cycle, demonstrating its high capacity to withstand adverse salt-affected soil conditions and favoring the reduction of EC to optimal levels for crop development, as reported by Dias et al. (2026).

These findings demonstrate the pronounced physiological plasticity of *Trichoderma*, underscoring the genus's ecological versatility. Our field-based data validate this adaptive capacity under natural environmental fluctuations, proving that laboratory screening can accurately inform biological management strategies by selecting broadly tolerant strains.

Ultimately, this demonstrated adaptability highlights the strong potential of *Trichoderma* as an agricultural bioinoculant, particularly for sustainable crop management, such as melon cultivation in the semi-arid region of Brazil, where salt-affected soils with low water availability and the use of polyethylene mulch favor increased soil temperatures, thereby creating challenging environments for the development of microorganisms.

5. CONCLUSION

Based on the data obtained, the *Trichoderma* isolates studied demonstrated broad physiological plasticity under contrasting abiotic conditions. Growth occurred across a wide pH range of 2 to 10 and at high salinities of up to 1000 mM NaCl, with optimal development observed over a broad temperature range from 10°C to 35°C.

Most isolates of *Trichoderma* exhibited a survival rate above 74%, with the *T. asperellum* URM 5911 isolate standing out by achieving a 95% survival rate at the end of the assays. The *Trichoderma* species stabilized and reduced the electrical conductivity of melon-cultivated soils under salt stress, with particular emphasis on *T. harzianum* IBLF006, *T. asperellum* URM 5911, *T. harzianum* IB19/17, *T. longibrachiatum*, *T. asperelloides* MMBF 94/17, and *T. asperellum* BV-10. In summary, this genus consolidates itself as a resilient and functional bioinoculant capable of surviving the physicochemical limitations of a semi-arid environment that utilizes polyethylene soil *mulching*; therefore, it is highly recommended for the sustainable management of melon crops.

REFERENCES

ABRAFRUTAS. Associação Brasileira dos Exportadores de Frutas e Derivados. **Export panels: Statistical data**. [S. l.], 2026. Disponível em: https://abrafrutas.org/dados-estatisticos/. Acesso em: 11 jul. 2026.

AGROFIT. Ministério da Agricultura, Pecuária e Abastecimento. **Agrofit – Sistema de Agrotóxicos Fitossanitários**. Brasília, DF: MAPA, 2026. Disponível em: https://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons. Acesso em: 11 jul. 2026.

ALMEIDA, B. H.; COSTA, M. H. D.; EVANGELISTA, L. F. B.; FRUTUOSO, R. M. S.; DIAS, A. M.; SILVA, J. L. S.; SILVA, D. J.; LOBO, J. T.; SILVA, W. da; AMBRÓSIO, M. M. Q. Sustainable management of Fusarium rots in melons: A dual strategy combining thermotherapy and 1-MCP. **Crop Protection**, [S. l.], v. 202, 107522, 2026. Disponível em: https://doi.org/10.1016/j.cropro.2025.107522.

ALMEIDA, B. H.; NEGREIROS, A. M. P.; MELO, N. J. A.; AMBRÓSIO, M. M. Q.; ARMENGOL, J.; SILVA, W.; SALES JÚNIOR, R. Evaluation of fungicides and *Trichoderma* spp. for controlling soil-borne fungal pathogens in melon crops. **Revista Caatinga**, Mossoró, v. 37, e12462, 2024. Disponível em: http://dx.doi.org/10.1590/1983-21252024v3712462rc.

BANDARA, A. Y.; KANG, S. *Trichoderma* application methods differentially affect the tomato growth, rhizomicrobiome, and rhizosphere soil suppressiveness against *Fusarium oxysporum*. **Frontiers in Microbiology**, [S. l.], v. 15, 1366690, 2024. Disponível em: https://doi.org/10.3389/fmicb.2024.1366690.

CAVALCANTE, A. L. A.; NEGREIROS, A. M. P.; MELO, N. J. A.; SANTOS, F. J. Q.; SILVA, C. S. A. S.; PINTO, P. S. L.; KHAN, S.; SALES, I. M. M.; SALES JÚNIOR, R. Adaptability and sensitivity of *Trichoderma* spp. isolates to environmental factors and fungicides. **Microorganisms**, [S. l.], v. 13, n. 7, 1689, 2025. Disponível em: https://doi.org/10.3390/microorganisms13071689.

CAVALCANTE, A. L. A.; NEGREIROS, A. M. P.; TAVARES, M. B.; BARRETO, E. D. S.; ARMENGOL, J.; SALES JÚNIOR, R. Characterization of five new Monosporascus species: Adaptation to environmental factors, pathogenicity to cucurbits and sensitivity to fungicides. **Journal of Fungi**, [S. l.], v. 6, n. 3, p. 169–183, 2020. Disponível em: https://doi.org/10.3390/jof6030169.

CERVANTES-GARCÍA, D. C.; RAMIREZ, J. S. P.; SIMPSON, J.; MAYEK-PEREZ, J. Osmotic potential effects on in vitro growth, morphology, and pathogenicity of *Macrophomina phaseolina*. **Journal of Phytopathology**, [S. l.], v. 151, p. 456–462, 2003. Disponível em: https://doi.org/10.1046/j.1439-0434.2003.00751.x.

CHEN, X.; LU, Y.; LIU, X.; GU, Y.; LI, F. *Trichoderma*: Dual roles in biocontrol and plant growth promotion. **Microorganisms**, [S. l.], v. 13, n. 8, 1840, 2025. Disponível em: https://doi.org/10.3390/microorganisms13081840.

COSTA, M. H. D.; SILVA, J. L. S.; SOUZA, J. J. F.; SILVA, I. V. P.; ALVES, T. R. C.;

MOURA, A. P.; COSTA, T.; LIMA, J. S. S.; SILVA, W. L.; AMBROSIO, M. M. Q. Dynamics of Fusarium infection in melon (*Cucumis melo*): From roots to fruits. **Plant Pathology**, [S. l.], v. 75, e70140, 2026. Disponível em: https://doi.org/10.1111/ppa.70140.

DIAS, N. S.; MEDEIROS, J. F.; MORAIS, P. L. D.; ARRUDA, M. V. M.; DINIZ, A. A.; SALDANHA, E. C. M.; ARAGÃO, A. R. (ed.). **Cultivo do melão para exportação na bacia potiguar**. [S. l.]: LF Editorial, 2026.

DRUZHININA, I. S.; CHENTHAMARA, K.; ZHANG, J.; ATANASOVA, L.; YANG, D.; MIAO, Y.; RAHIMI, M. J.; GRUJIC, M.; CAI, F.; POURMEHDI, S.; SALIM, K. A.; PRETZER, C.; KOPCHINSKIY, A. G.; HENRISSAT, B.; KUO, A.; HUNDLEY, H.; WANG, M.; AERTS, A.; SALAMOV, A.; LIPZEN, A.; LABUTTI, K.; BARRY, K.; GRIGORIEV, I. V.; SHENG, Q.; KUBICEK, C. P. Massive lateral transfer of genes encoding plant cell wall-degrading enzymes to the mycoparasitic fungus *Trichoderma* from its plant-associated hosts. **PLoS Genetics**, [S. l.], v. 14, n. 4, e1007322, 2018. Disponível em: https://doi.org/10.1371/journal.pgen.1007322.

DUTTA, P.; DEB, L.; PANDEY, A. K. *Trichoderma* — from lab bench to field application. **Frontiers in Agronomy**, [S. l.], v. 4, 932839, 2022. Disponível em: https://doi.org/10.3389/fagro.2022.932839.

ELAD, Y.; CHET, I.; HENIS, Y. A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. **Phytoparasitica**, [S. l.], v. 9, n. 1, p. 59–67, 1981. Disponível em: https://doi.org/10.1007/BF03158330.

EUROPEAN COMMISSION. **Active substances, safeners and synergists**. [S. l.], 2025. Disponível em: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances. Acesso em: 11 jul. 2026.

EVANGELISTA, L. F. B.; ASSUNÇÃO, D. A.; BENTO, E. A.; SILVA, J. L. dos S.; ALMEIDA, B. de H.; ALVES, T. R. C.; MOURA, A. P. de; SILVA, I. V. P. da; SILVA, W. L. da; AMBRÓSIO, M. M. de Q. Comparative efficacy of soil-derived bacteria and commercial microbial formulations in controlling melon gray stem rot. **Journal of Plant Pathology**, [S. l.], 2026. Disponível em: https://doi.org/10.1007/s42161-026-02136-9.

HAO, D.; LANG, B.; WANG, Y.; WANG, X.; LIU, T.; CHEN, J. Designing synthetic consortia of *Trichoderma* strains that improve antagonistic activities against pathogens and cucumber seedling growth. **Microbial Cell Factories**, [S. l.], v. 21, 234, 2022. Disponível em: https://doi.org/10.1186/s12934-022-01959-2.

HU, G.; ZHAO, Z.; WEI, Y.; HU, J.; ZHOUI, Y.; LI, J.; YANG, H. *Trichoderma asperellum* 22043: Inoculation promotes salt tolerance of tomato seedlings through activating the antioxidant system and regulating stress-resistant genes. **Journal of Fungi**, [S. l.], v. 11, n. 4, 253, 2025. Disponível em: https://doi.org/10.3390/jof11040253.

IBGE. Instituto Brasileiro de Geografia e Estatística. **Biomas e sistema ambiental brasileiro**. Rio de Janeiro: IBGE, 2023. Disponível em: https://www.ibge.gov.br/geociencias/cartas-e-mapas/informacoes-ambientais/15842-biomas.html.

IBGE. Instituto Brasileiro de Geografia e Estatística. **Municipal agricultural production:**

Melon. Rio de Janeiro: IBGE, 2024. Disponível em: https://www.ibge.gov.br/estatisticas/economicas/agricultura-e-pecuaria.html.

JAISWAL, P.; KHADKA, R. B.; HUSSAIN BHAT, A.; BAIDYA, S.; KESHARI, A. K. Morphological and molecular characterization of *Trichoderma* isolates from vegetable crop rhizospheres in Nepal. **F1000Research**, [S. l.], v. 13, 1088, 2025. Disponível em: https://doi.org/10.12688/f1000research.153701.3.

KUBIAK, A.; WOLNA-MARUWKA, A.; PILARSKA, A. A.; NIEWIADOMSKA, A. Fungi of the *Trichoderma* genus: Future perspectives of benefits in sustainable agriculture. **Applied Sciences**, [S. l.], v. 13, 6434, 2023. Disponível em: https://doi.org/10.3390/app13116434.

LEFEVRE, A. F.; SOUZA, N. L. Determinação da temperatura letal para *Rhizoctonia solani* e *Sclerotium rolfsii* e efeito da solarização sobre a temperatura do solo. **Summa Phytopathologica**, Botucatu, v. 19, n. 2, p. 107–112, 1993.

MANIÇOBA, F. E.; NEGREIROS, A. M. P.; CAVALCANTE, A. L. A.; ALVES, C. P. S. S.; NASCIMENTO, M. T. A.; AMBRÓSIO, M. M. Q.; SALES JÚNIOR, R. Environmental factors and fungicide sensitivity of *Fusarium* spp. causing melon fruit rot. **Journal of Phytopathology**, [S. l.], v. 171, p. 504–516, 2023. Disponível em: https://doi.org/10.1111/jph.13206. Acesso em: 11 jul. 2026.

MARRA, R.; LOMBARDI, N.; D'ERRICO, G.; TROISI, J.; SCALA, G.; VINALE, F.; WOO, S. L.; BONANOMI, G.; LORITO, M. Application of *Trichoderma* strains and metabolites enhances soybean productivity and nutrient content. **Journal of Agricultural and Food Chemistry**, [S. l.], v. 67, n. 7, p. 1814–1822, 2019. Disponível em: https://doi.org/10.1021/acs.jafc.8b06503.

MAURYA, M. K.; SRIVASTAVA, M.; HARSHITA. Effect of various pH levels on the growth and sporulation of *Trichoderma viride* isolates and assessing their antagonistic activity against soil-borne pathogens. **Journal of Pure and Applied Microbiology**, [S. l.], v. 18, n. 4, p. 2516–2527, 2024. Disponível em: https://doi.org/10.22207/JPAM.18.4.23.

MEYER, M. C.; MAZARO, S. M.; SILVA, J. C. (ed.). **Trichoderma: uso na agricultura**. Brasília, DF: Embrapa, 2019.

NAIR, A.; SIBLE, G. V.; CYRIAC, A.; THARA, S. S.; JOHNSON, J. M.; RADHIKA, N. S.; SONI, K. B. Characterization of novel strains of *Trichoderma* spp. and their utilization in management of damping off disease in tomato. **Journal of Biological Control**, [S. l.], v. 36, n. 1, p. 31–46, 2022. Disponível em: https://doi.org/10.18311/jbc/2022/30015.

NOGUEIRA, G. A.; CONRADO, V. S. C.; FREIRES, A. L. A.; SOUZA, J. J. F.; FIGUEIREDO, F. R. A.; BARROSO, K. A.; ARAÚJO, M. B. M.; NASCIMENTO, L. V.; LIMA, J. S. S.; BEZERRA NETO, F.; SILVA, W. L.; AMBRÓSIO, M. M. Q. Aggressivity of different *Fusarium* species causing fruit rot in melons in Brazil. **Plant Disease**, [S. l.], v. 107, n. 3, p. 886–892, 2023. Disponível em: https://doi.org/10.1094/PDIS-04-22-0728-SR.

R CORE TEAM. R: A language and environment for statistical computing (Version 4.4.0). Viena: R Foundation for Statistical Computing, 2024. Disponível em: https://www.R-project.org.

RSTUDIO TEAM. **RStudio**: Integrated Development for R. Boston: RStudio, PBC, 2024. Disponível em: http://www.rstudio.com/.

SANTOS, H. G.; JACOMINE, P. K. T.; ANJOS, L. H. C.; OLIVEIRA, V. A.; LUMBRERAS, J. F.; COELHO, M. R.; ALMEIDA, J. A.; ARAÚJO FILHO, J. C.; OLIVEIRA, J. B.; CUNHA, T. J. F. **Sistema Brasileiro de Classificação de Solos**. 5. ed. Brasília, DF: Embrapa Solos, 2018.

SEBRAE. Serviço Brasileiro de Apoio às Micro e Pequenas Empresas. **Melon cultivation profile**. [S. l.]: SEBRAE, 2025. Disponível em: https://datampe.sebrae.com.br/profile/industry/cultivo-de-melao. Acesso em: 11 jul. 2026.

SYSTAT SOFTWARE INC. **SigmaPlot for Windows** (Version 16.0). San Jose: Systat Software Inc., 2021.

TEIXEIRA, P. C.; DONAGEMMA, G. K.; FONTANA, A.; TEIXEIRA, W. G. (ed.). **Manual de métodos de análise de solo**. 3. ed. Brasília, DF: Embrapa, 2017.

TIRADO-MALAVER, R. H.; TIRADO-LARA, R. Combined use of humic acids and *Trichoderma harzianum* as sustainable alternatives to alliviate salt stress in bell pepper. **Brazilian Journal of Biology**, São Carlos, v. 85, e289492, 2025. Disponível em: https://doi.org/10.1590/1519-6984.289492.

WANG, H. Epidemiology and control of fungal diseases in crop plants. **Agronomy**, [S. l.], v. 13, 2327, 2023. Disponível em: https://doi.org/10.3390/agronomy13092327.

WOO, S. L.; HERMOSA, R.; LORITO, M.; MONTE, E. *Trichoderma*: A multipurpose, plant-beneficial microorganism for eco-sustainable agriculture. **Nature Reviews Microbiology**, [S. l.], v. 21, n. 5, p. 312–326, 2023. Disponível em: https://doi.org/10.1038/s41579-022-00819-5.

YUSHU, Z.; GUO, Q.; YANAN, Z.; YUTING, L.; XUEZHENG, W. Controlling *Fusarium* wilt in melon using tillered onion bulb extract. **Pakistan Journal of Botany**, Karachi, v. 49, p. 1615–1623, 2017.

SUPPLEMENTARY MATERIAL

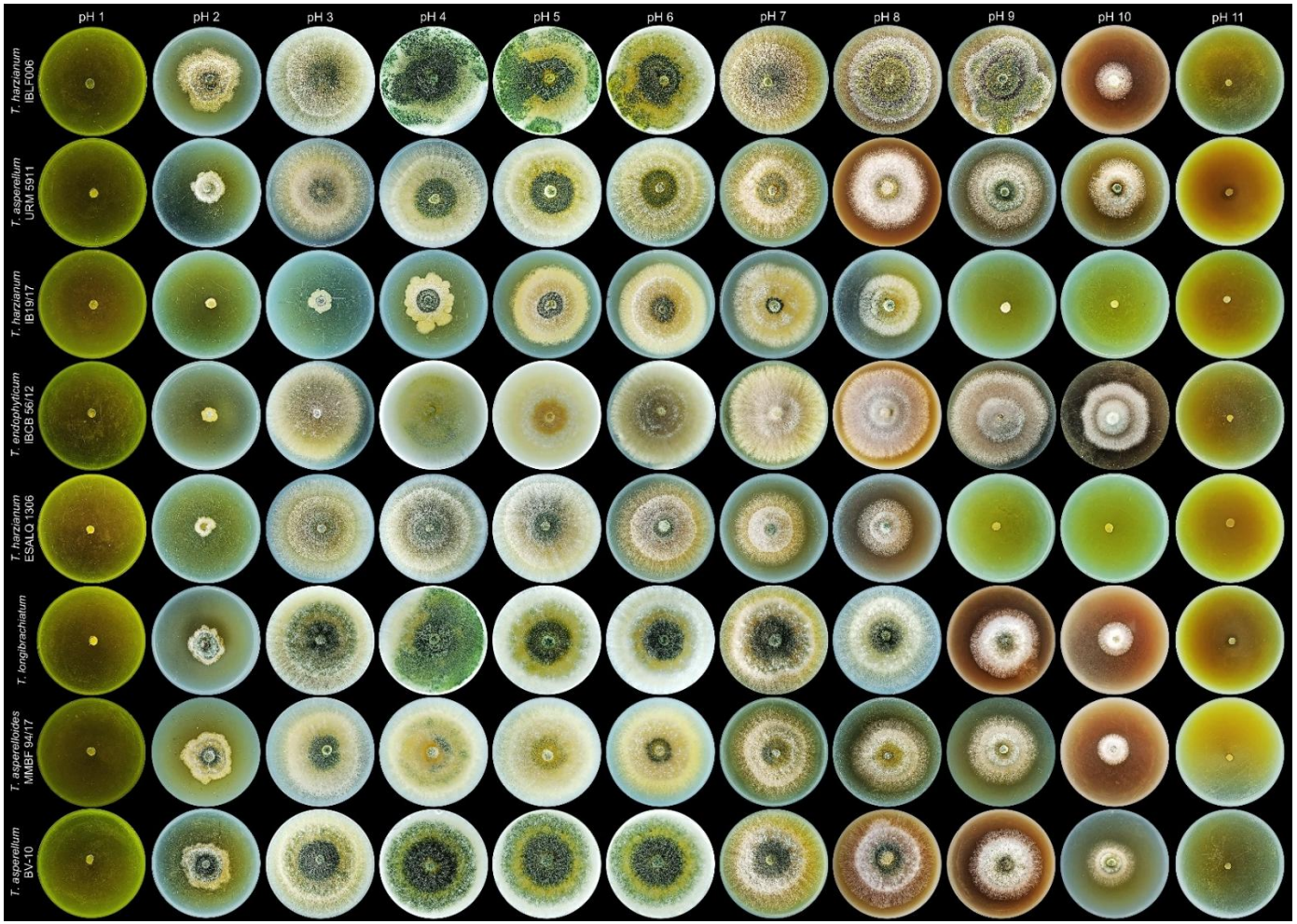

Figure S1. Mycelial growth of eight *Trichoderma* isolates after 7 days of incubation on PDA medium at different pH values (1 to 11), incubated at 28 ± 2 °C.

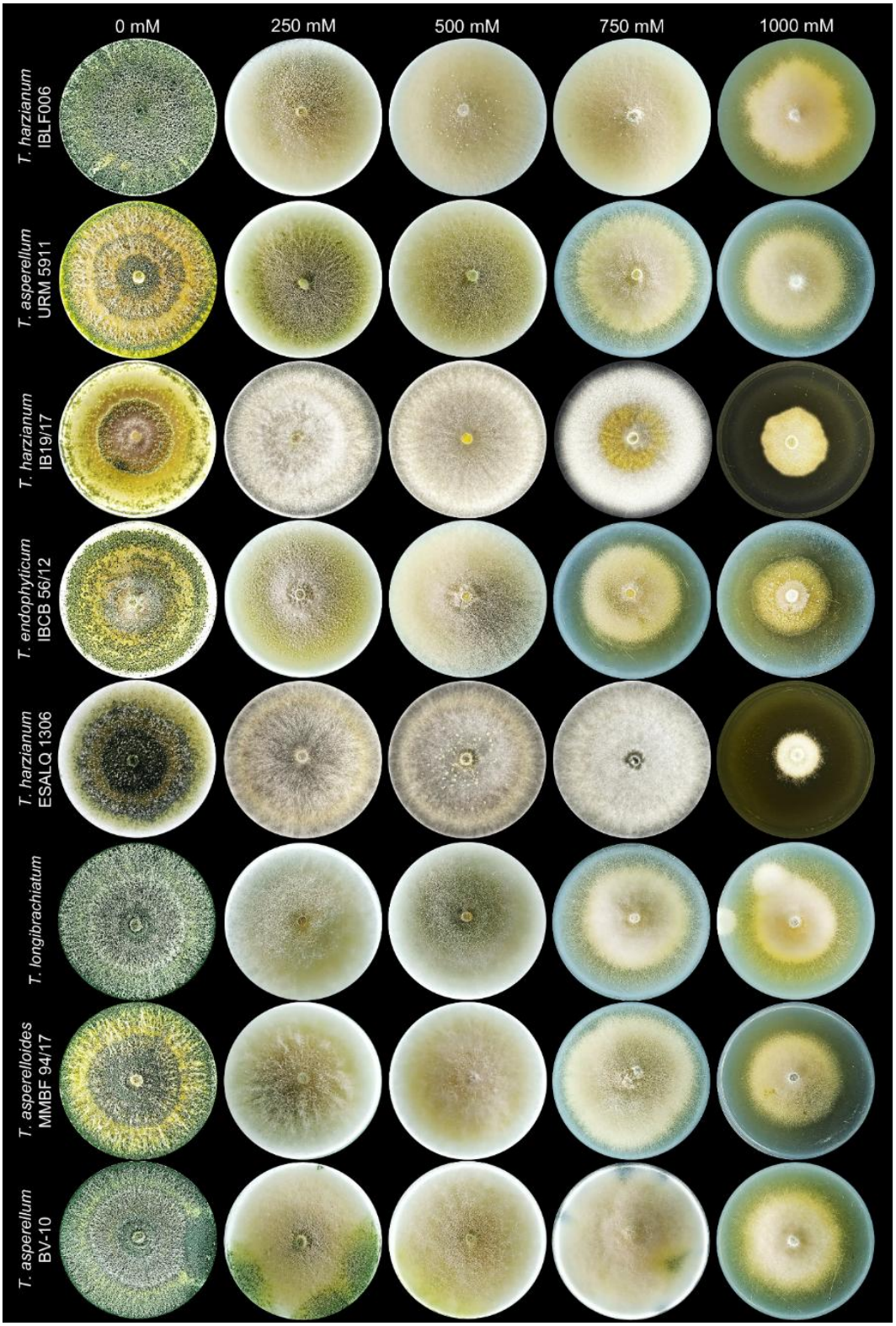

Figure S2. Mycelial growth of eight *Trichoderma* isolates on PDA medium supplemented with different NaCl concentrations (0 to 1000 mM) after 7 days of incubation at 28 ± 2 °C.

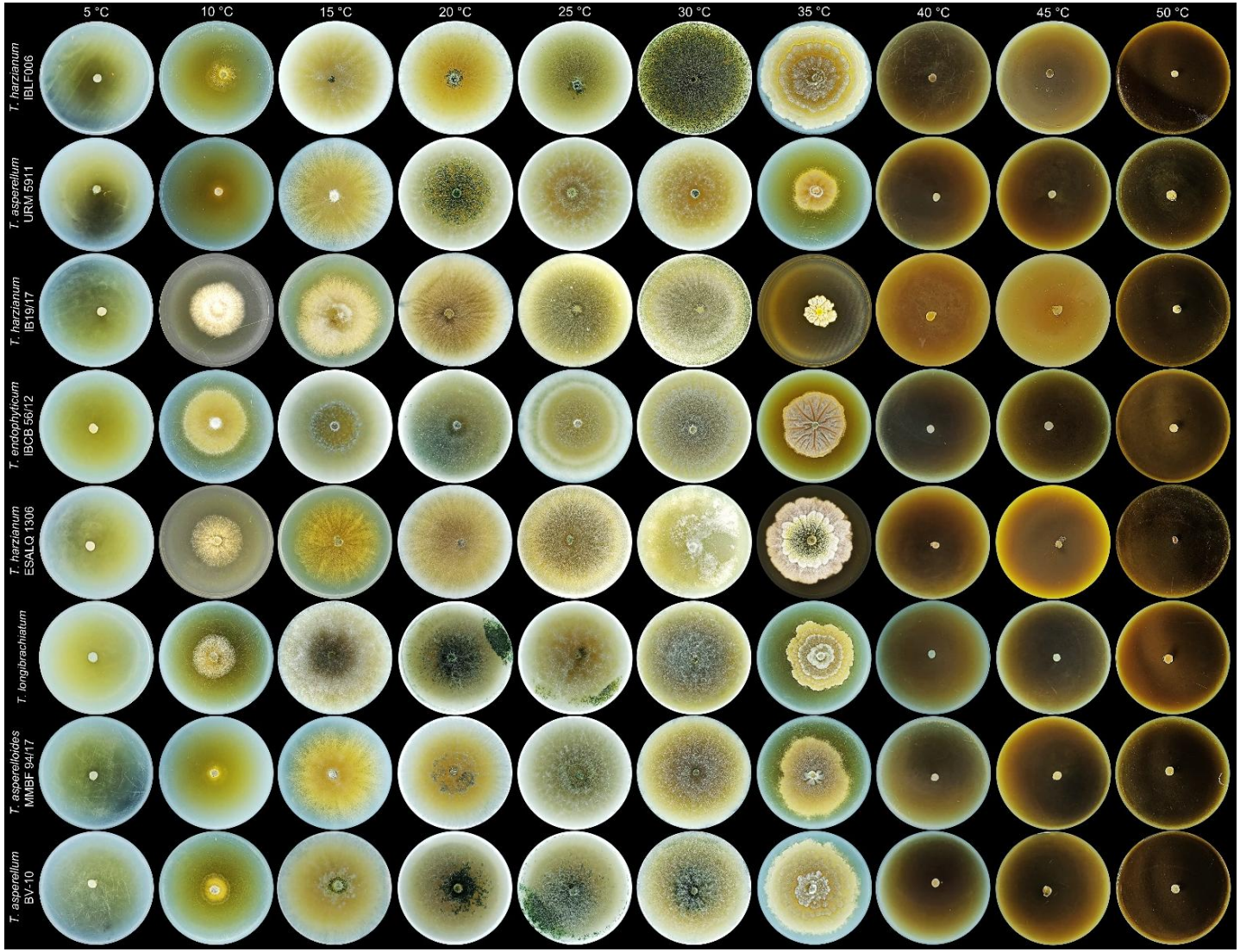

Figure S3. Mycelial growth of eight *Trichoderma* isolates at different temperatures (5 to 50 °C) after 7 days of incubation on PDA medium.

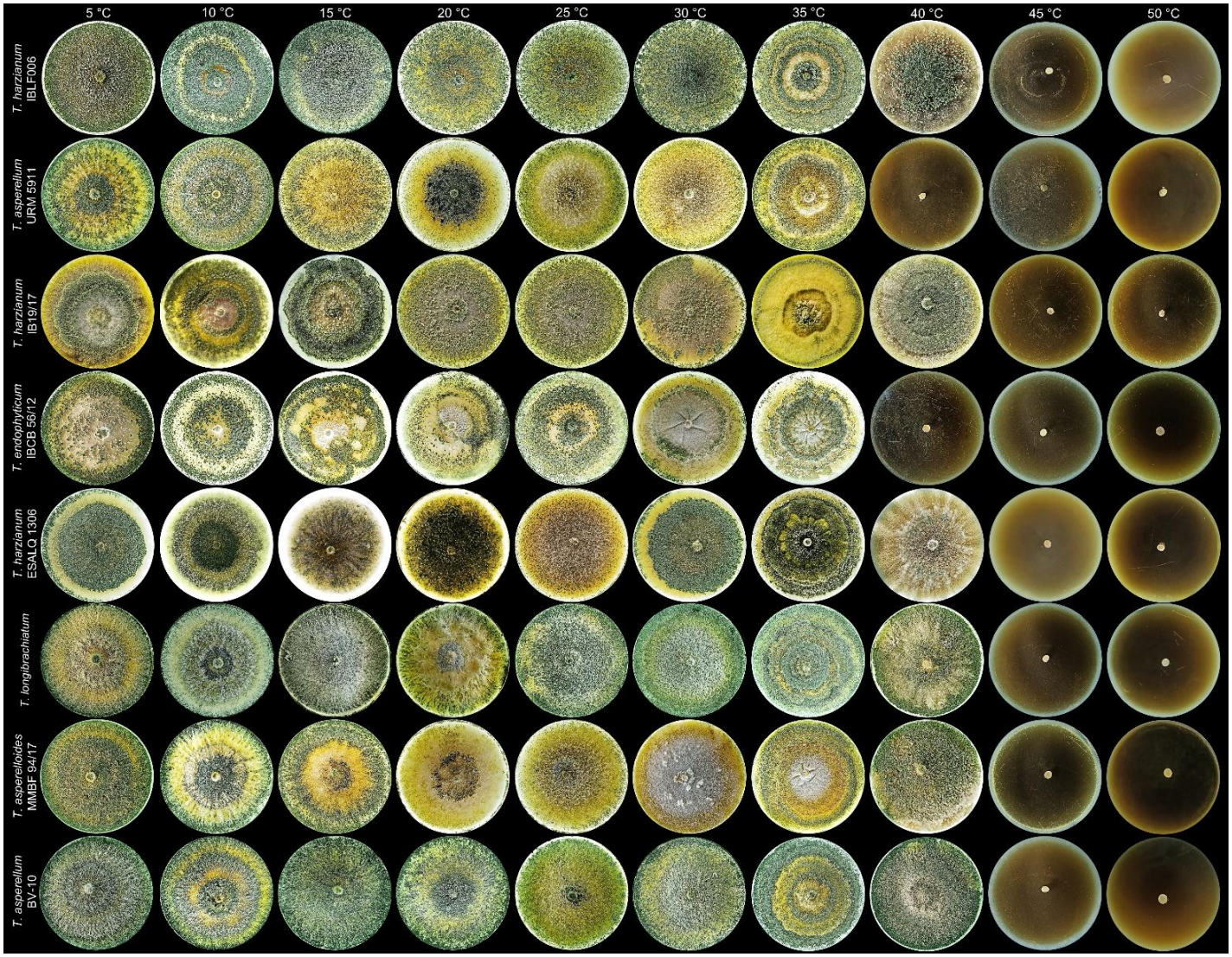

Figure S4. Mycelial growth of *Trichoderma* isolates after initial incubation (7 days, 5 to 50 °C) and subsequent recovery for 7 days at 28 °C on PDA medium.

CHAPTER II
SUSTAINABLE MANAGEMENT OF FUSARIUM ROTS IN MELONS: A DUAL STRATEGY COMBINING THERMOTHERAPY AND 1-MCP

ABSTRACT

Brazil is a leading producer and exporter of Galia melon (*Cucumis melo* L.), a variety renowned for its exceptional sensory qualities. However, postharvest diseases caused by *Fusarium* spp. lead to significant economic losses, estimated at up to US$22 million annually. The current reliance on fungicides, such as Imazalil, is unsustainable due to regulatory restrictions and the increasing fungal resistance to current chemistry, which calls for the development of alternative control strategies. In this study, we investigated the effectiveness of thermotherapy combined with ripening phytoregulators for managing rots caused by *F. falciforme* and *F. sulawesiense* in Galia melons. Inoculated fruits underwent eight different treatments [Mock (control), HWT (hot water treatment), 1-MCP (1-methylcyclopropene), HWT + 1-MCP, GA (gibberellin), HWT + GA, GRAD (Graduate A+®), and HWT + GRAD] and were stored for 20 days at 10 ± 2°C. We evaluated disease severity, melon fruit physicochemical parameters, and the activity of plant defense enzymes. Most treatments significantly reduced rot severity compared to the control. Notably, Graduate A+® achieved up to a 72.9% reduction, and its efficacy was enhanced when combined with thermotherapy. Furthermore, 1- methylcyclopropene (1-MCP) treatment, alone and with thermotherapy, preserved postharvest quality and enhanced defense-related enzyme activity. These findings suggest that the combination of thermotherapy and 1-MCP represents a promising and sustainable approach to reducing Fusarium rot in Galia melons destined for export, with no associated regulatory risks.

Keywords: Postharvest, Phytopathogens, Phytoregulators, Antioxidant defenses.

1. INTRODUCTION

Melon (*Cucumis melo* L.) is one of the most widely consumed fresh fruits globally, with Brazil ranking among the leading producers and the second-largest exporter (MAPA, 2024). In the 2023/2024 season, Brazil's melon exports reached 138,000 tons, generating USD 118 million in revenue (HF BRASIL, 2025). The Northeast region accounts for over 98% of national production, with approximately 73% of this output destined for the European Union market (HF BRASIL, 2025). In 2023, Brazil's melon production totaled 862,387 tons, with about 88% concentrated in the states of Rio Grande do Norte (RN), Bahia (BA), and Ceará (CE) (IBGE, 2023). Melon cultivation in Brazil has a significant socioeconomic impact, creating around 12,000 direct jobs across 190 active companies. This sector not only fosters regional development but also substantially contributes to national income (SEBRAE, 2025). Among the cultivated varieties, Galia melon (*C. melo* L. var. *reticulatus*) is prominent in both national and international markets due to its medium size, high nutritional value, and excellent aroma and flavor (MOURA et al., 2024). However, this variety is characterized by a short shelf life, largely due to its susceptibility to physiological deterioration and phytopathogen attacks (TERÃO et al., 2021).

The expansion of agricultural areas, coupled with intensive cultivation practices lacking crop rotation, has enabled up to three production cycles per year in Brazil's Northeast (NOGUEIRA et al., 2023). While these strategies increase melon productivity, they also promote the proliferation of phytopathogens (CAVALCANTE et al., 2020), particularly those that cause rots such as *Fusarium* spp. (NOGUEIRA et al., 2023), which compromises fruit quality and reduces marketable yield (OSTER et al., 2018). Disease development frequently occurs during transportation to importing countries (OSTER et al., 2018). In this context, melon exportation via refrigerated shipping containers poses a logistical challenge, as transit time from Brazil to Europe can range from 9 to 20 days, directly influencing fruit quality (ABRAFRUTAS, 2025). It is therefore crucial to consider transport duration and conditions to minimize the impact of these infections and ensure product integrity. This is particularly worrisome, given that approximately 15% of melon exports are lost during shipping due to rots, resulting in losses exceeding USD 22 million (OSTER et al., 2018). To make matters worse, more than 20 *Fusarium* species have been reported to cause rots in melons (ARAÚJO et al., 2021; SILVA, 2023). Recently, five *Fusarium* species were identified as causal agents of rot in eight melon hybrids, with *F. falciforme* and *F. sulawesiense* standing out as the most aggressive on the evaluated fruits (NOGUEIRA et al., 2023).

Chemical formulations such as imidazoles (Imazalil), strobilurins (Azoxystrobin), and phenylpyrroles (Fludioxonil) are among the most used fungicides for postharvest disease management (MANIÇOBA et al., 2023). Although these products are permitted in the European Union, Brazil's main melon export market, their use is limited by stringent maximum residue level (MRL) regulations for fruits (EUROPEAN COMMISSION, 2025). There is also an increasing push for close to zero traces of fungicides and other chemicals in food supplies, triggering policy changes around the world. Just recently, the European Commission published a proposal for a new regulation on the current use of pesticides, which plans to reduce the use and risk of chemical pesticides by 50% by 2030 (EUROPEAN COMMISSION, 2022), as outlined in the European Green Deal (EUROPEAN COMMISSION, 2019). These restrictions and regulations, combined with a decline in fungal control efficacy by those fungicides over time, present significant challenges for the melon industry (MANIÇOBA et al., 2023). In fact, Terão et al. (2021) observed that Galia melons with peduncular rot caused by *F. pallidoroseum* developed symptoms 15 days after treatment with Imazalil and storage in cold chambers (10 ± 2°C). The growing consumer demand for residue-free products, along with the pursuit of healthier lifestyles and reduced environmental impact, is pressuring the agricultural sector to adopt alternative methods for effective pathogen control (TERÃO et al., 2021). Thus, independent methods and mechanisms may be integrated into functional strategies to manage Fusarium rot in melons, such as the application of thermotherapy and metabolic modulators like gibberellin and 1-methylcyclopropene (1-MCP) on fruits (TERÃO et al., 2009; ZHANG et al., 2023).

Thermotherapy is a physical method involving the exposure of fruits to high temperatures for a short duration. This promotes protein denaturation and reduces cellular activity in pathogens, thereby decreasing disease symptoms, preserving the fruits, and even enhancing postharvest quality (KOLTZ et al., 2020; TAN et al., 2022). In Brazil, this technique is not yet commonly adopted by melon growers; however, studies suggest that it is a promising method for postharvest disease management in fruits (TERÃO et al., 2021; MEDEIROS et al., 2023; MOURA et al., 2024). An alternative to chemical control also includes the use of 1-methylcyclopropene (1-MCP), an exogenous metabolic modulator from the cyclopropene class. It acts as a competitive antagonist of ethylene by binding to its receptors in plant cells, inhibiting cellular signaling and suppressing the metabolic activation associated with fruit ripening and senescence (DIAS et al., 2021), as well as temporarily reducing ethylene production (ASREY et al., 2023). Its application contributes to maintaining the quality of many fruits (HAN et al., 2015). Similarly, gibberellin (GA), a diterpenoid class

phytohormone, when applied postharvest, competes for the ethylene-binding site, regulating protein synthesis, delaying ripening and senescence, and increasing resistance to oxidative stress caused by phytopathogens (SALAZAR-CEREZO et al., 2018; ZHANG et al., 2023). Taken all together, the present study aimed to evaluate the effect of thermotherapy associated with ripening phytoregulators on the management of rot caused by *Fusarium falciforme* and *Fusarium sulawesien*se, the most aggressive rot pathogens in Galia melon. Unlike previous reports, this study provides the first integrated evaluation of thermotherapy combined with 1-MCP and GA under postharvest conditions in Galia melons, highlighting a sustainable approach to reducing Fusarium rot and extending fruit shelf life.

2. MATERIALS AND METHODS

2.1 Fruit collection and study location

A total of 224 Galia melons (*Cucumis melo* var. *reticulatus* 'Gália Citrino') at 65 days after transplanting were collected in August 2024 from Agrícola Famosa farm (4°51'25"S, 37°20'58"W) in Icapuí, Ceará, Brazil. The fruits were then transported to the Food Technology Laboratory at the Universidade Federal Rural do Semi-Árido (UFERSA) (5°12'17"S, 37°19'21" W), located in Mossoró, Rio Grande do Norte, Brazil, where they were washed with running water and selected for uniformity, ripeness, and absence of physical or physiological damage. The experiment was conducted from August to September 2024.

2.2 Inoculation with *Fusarium falciforme* and *Fusarium sulawesiense*

The selection of *F. falciforme* and *F. sulawesiense* for this study was based on their documented prevalence and high pathogenic potential in melons produced in Northeastern Brazil, where they have been identified as the main causal agents of postharvest rot (ARAÚJO et al., 2021; NOGUEIRA et al., 2023; MOURA et al., 2024). Two *Fusarium* species (*F. falciforme* strain CFM 17C and *F. sulawesiense* strain CFM 03A), isolated from melons grown in the production region of Rio Grande do Norte in Brazil, were used in the experiments (ARAÚJO et al., 2021). These strains, known for their high aggressiveness toward various melon types (NOGUEIRA et al., 2023), are deposited in the Lavras Mycological Collection (LMC) at the Universidade Federal de Lavras (UFLA), and were identified based on EF-1α and RPB2 gene sequences (GenBank: MT476611/MT461687 for *F. falciforme* and MT476613/MT461677 for *F. sulawesiense*). Their pathogenicity to melon

was confirmed before the experiments. Isolates were cultured on Potato Dextrose Agar (PDA; Merck KGaA, Darmstadt, Germany) supplemented with tetracycline (0.05 g/L) and incubated in a BOD-type growth chamber at 28 ± 2 °C, under a 12:12 h light:dark photoperiod, for 10 days. Before inoculation, spore suspensions were prepared at a concentration of 1×10^6 conidia/mL using a Neubauer counting chamber (YUSHU et al., 2017).

2.3 Experimental design

Two independent experiments were conducted for each *Fusarium* species, using a completely randomized design (CRD) with eight treatments and seven replicates, each represented by one melon. The treatments were: MOCK (control), HWT (hot water treatment), 1-MCP (1-methylcyclopropene), HWT + 1-MCP, GA (gibberellin), HWT + GA, GRAD (Graduate A+®), and HWT + GRAD. For each *Fusarium* species, 56 fruits were used per experiment. The experiments with both fungi were repeated, totaling 224 melons.

2.4 Treatment preparation and application

Selected fruits were disinfected by spraying with 70% ethanol, followed by immersion in 0.1% sodium hypochlorite for 60 s, rinsed twice with sterile distilled water, and naturally dried at 23 ± 2 °C. The experiments were conducted in 2024. For each *Fusarium* species and in each independent experiment, 12 wounds (3 mm deep) were made at the peduncle base using sterile needles, followed by inoculation with 20 µL of the spore suspension (1×10^6 conidia/mL) (YUSHU et al., 2017). After inoculation, fruits were kept for 12 h at 23 ± 2 °C and 66% relative humidity to allow pathogen establishment before treatment application. The incubation conditions were defined based on previous studies that used similar methodologies for the establishment of *Fusarium* spp. in melon fruits (YUSHU et al., 2017; ARAÚJO et al., 2021; MEDEIROS et al., 2023; MOURA et al., 2024; SILVA, 2024). The heat treatment involved immersion in hot water at 58 °C for 150 s, followed by immersion in cold water (20 ± 2 °C) for 2 min and natural drying (25 ± 2 °C) (Moura et al., 2024). The 1-MCP was applied via fumigation (600 nL/L) for 12 h in a modified atmosphere chamber at 28 ± 2 °C. The 1-MCP treatment (600 nL/L for 12 h) followed the concentration and application method routinely used in commercial melon packing facilities in northeastern Brazil, according to standardized operational practices adopted by the production sector. Thus, previously optimized and widely applied parameters were employed to ensure the reproducibility and

practical applicability of the results obtained. Gibberellin (GA, 2.5 g/10 L) was applied directly to the peduncle using a sponge soaked in the solution, maintained in contact with the site for 30 s. The fungicide Graduate A+® (GRAD, 3 mL/L) was applied directly to the peduncle according to the manufacturer's instructions. The control (MOCK) consisted only of *Fusarium* inoculation without any treatment. After treatment, fruits were stored for 20 days in cold chambers (9 ± 2 °C) at UFERSA, simulating the approximate maximum shipping duration to the European Union markets (TERÃO et al., 2021; ABRAFRUTAS, 2025).

2.5 Epidemiological component assessment

Disease severity (SEV) was evaluated 20 days after cold storage by measuring both transverse and longitudinal lesion lengths on the peduncle using a digital caliper (Lyman 7832218), with results expressed in millimeters.

2.6 Postharvest evaluations

Total soluble solids (°Brix) were determined from pulp concentrate using a portable digital refractometer (MA871 Milwaukee). Pulp firmness was measured with a penetrometer (PTR-300), considering the average of two measurements at opposite points on the longitudinally cut pulp. Pulp pH was measured using a digital pH meter (PHB-550).

2.7 Biochemical and enzymatic analyses

Peel samples (5 g) were collected and flash-frozen in liquid nitrogen (-80 °C) for subsequent analysis. Subsamples (0.5 g) were weighed, homogenized with 25 mg polyvinylpyrrolidone (PVP) and liquid nitrogen, and extracted using 2.5 mL of acetate buffer (0.1 M, pH 5.0) and 0.5 mL EDTA (1 mM). After centrifugation (10.000 g, 4 °C, 10 min), the supernatant (protein extract) was stored at -80 °C (BEZERRA NETO; BARRETOS, 2011). Catalase (CAT) activity was determined using 100 µL of the extract, 2.75 mL potassium phosphate buffer (100 mM, pH 7.5), and 120 µL hydrogen peroxide, with absorbance readings at 240 nm at 0 and 60 seconds (AZEVEDO et al., 1998). Peroxidase (POX) activity was measured using 25 µL guaiacol (0.2 M), 250 µL hydrogen peroxide (0.38 M), 1 mL sodium phosphate buffer (0.2 M, pH 6.0), and 25 µL extract, with absorbance readings at 470 nm every 10 seconds for 1 minute (BEZERRA NETO & BARRETOS, 2011). Polyphenol

oxidase (PPO) activity was evaluated using 1.8 mL potassium phosphate buffer (0.05 M, pH 6.0), 50 µL extract, 50 µL catechol (0.1 M), and 100 µL perchloric acid (1.4%), with readings at 395 nm (CAMPOS et al., 2004). Phenylalanine ammonia-lyase (PAL) activity was measured using 300 µL extract, 1.2 mL Tris-HCl buffer (25 mM, pH 8.8), and 1.5 mL L-phenylalanine (50 mM), with readings at 290 nm (UMESHA, 2006). Hydrogen peroxide concentration (H_2O_2) was quantified using 200 µL extract, 200 µL potassium phosphate buffer (100 mM, pH 7.5), and 800 µL potassium iodide (1 M), with readings at 390 nm (ALEXIEVA et al., 2001). Distilled water was used as a blank in all analyses.

2.8 Statistical analyses

Preliminary ANOVA indicated no significant differences between experiments, allowing them to be combined. Variables with homogeneous distribution (Scott-Knott, $p \leq 0.05$, using the ExpDes.pt v.1.2.2 statistical package) included °Brix, pH, firmness, POX, PPO, and PAL in *F. falciforme*; °Brix, firmness, CAT, POX, PPO, PAL, and H_2O_2 in *F. sulawesiense*. Variables with non-homogeneous distribution (Kruskal-Wallis, $p \leq 0.05$, using the agricolae v. 1.3-7 statistical package) included disease severity for both pathogens; pH, CAT, and H_2O_2 in *F. falciforme*; and pH in *F. sulawesiense*. Correlation analyses were performed using the Pearson method, and the results were plotted using the corrplot v. 0.94 statistical package. All analyses were performed using R software (R CORE TEAM, 2020) in the RStudio interface (RSTUDIO TEAM, 2024).

3. RESULTS

Both *Fusarium* species caused rot in the Galia melons used in our experiments (Fig. 1 and Fig. 2). Analysis of variance revealed that disease severity differed significantly among treatments within each *Fusarium* species (*F. falciforme*: $p = 5.55 \times 10^{-16}$; *F. sulawesiense*: $p = 3.33 \times 10^{-16}$).

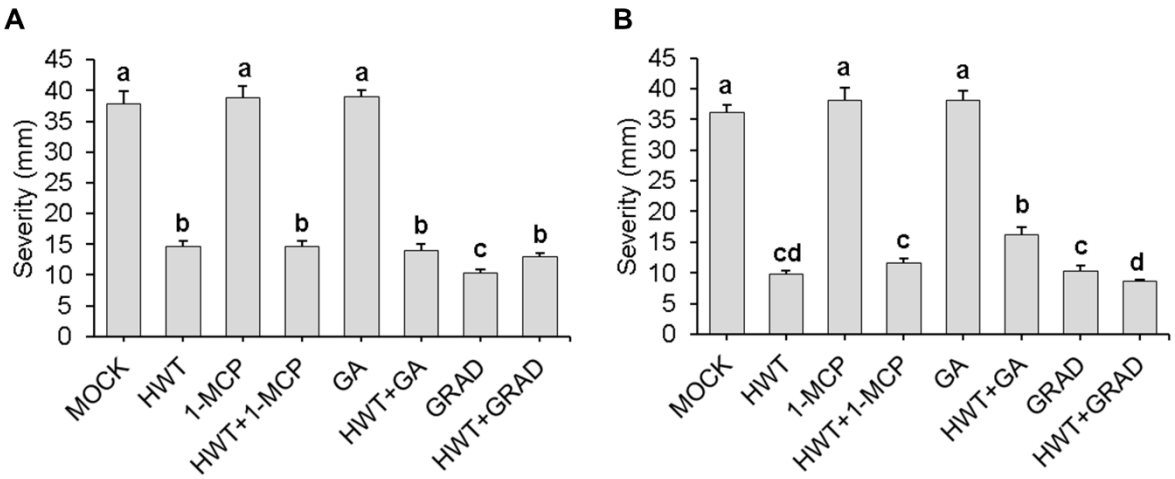

Figure 1. Severity of rot caused by *F. falciforme* (A) and *F. sulawesiense* (B) in Galia melons. MOCK = Control; HWT = Hot Water Treatment; 1-MCP = 1-Methylcyclopropene; GA = Gibberellin; GRAD = Graduate A+®. Bars followed by the same letter are not statistically different according to the Kruskal-Wallis test at 5% significance. Data represent means of two experiments per fungus ± standard error, with seven replicates (melons) per treatment in each experiment.

Figure 2. Effect of treatments on rot severity in Galia melons caused by *F. falciforme* (A–H) and *F. sulawesiense* (I–P), respectively. A and I = Control (MOCK); B and J = Hot Water Treatment (HWT); C and K = 1-Methylcyclopropene (1-MCP); D and L = HWT + 1-MCP; E and M = Gibberellin (GA); F and N = HWT + GA; G and O = Graduate A+® (GRAD); H and P = HWT + GRAD.

In fruits inoculated with *F. falciforme*, the lowest disease severity (Fig. 1A) was observed in the GRAD treatment, which was 72.9% lower than the control. Other treatments (HWT, HWT+1-MCP, HWT+GA, and HWT+GRAD) also showed significant reductions of 61.4%, 61.4%, 63.2%, and 65.7%, respectively, compared to the control (Fig. 1A). For fruits inoculated with *F. sulawesiense*, the treatments HWT and HWT+GRAD showed the lowest lesion severity (Fig. 1B), with reductions of 72.7% and 76.2%, respectively, compared to MOCK. Additionally, treatments HWT+1-MCP and GRAD reduced lesion size by 11.59 mm

and 10.36 mm, corresponding to 67.9% and 71.3% reductions, respectively, compared to MOCK.

Regardless of the *Fusarium* species inoculated, no statistical difference was observed in °Brix content among treatments (Table 1). For fruits inoculated with *F. falciforme*, MOCK, GA, HWT+GA, and HWT+GRAD treatments had higher pH values and were statistically different from the others. Notably, treatments with 1-MCP and HWT+1-MCP showed the highest firmness, with increases of 98.1% and 131.1%, respectively, compared to MOCK. For fruits inoculated with *F. sulawesiense*, pH was highest in fruits treated with GA, which differed statistically from HWT+GA and HWT+GRAD. Firmness was significantly higher in fruits treated with 1-MCP, HWT+1-MCP, and GRAD, with increases of 110.9%, 163.7%, and 124.4%, respectively, compared to MOCK.

Table 1. Brix, pH, and firmness (N) in Galia melons inoculated with *F. falciforme* and *F. sulawesiense* and subjected to different treatments.

Treatments	*F. falciforme*		
	°Brix [1]	pH[1]	Firmness (N)[1]
MOCK	10.65 ± 00.37 a	05.93 ± 00.06 a	08.79 ± 00.80 c
HWT	09.73 ± 00.36 a	05.70 ± 00.05 b	11.68 ± 02.51 bc
1-MCP	10.72 ± 00.65 a	05.74 ± 00.04 b	17.41 ± 00.36 ab
HWT +1-MCP	10.32 ± 00.42 a	05.77 ± 00.05 b	20.31 ± 01.55 a
GA	10.00 ± 00.78 a	05.84 ± 00.05 a	09.60 ± 00.92 c
HWT + GA	10.27 ± 00.55 a	05.85 ± 00.06 a	11.02 ± 02.07 bc
GRAD	10.73 ± 00.59 a	05.73 ± 00.05 b	10.54 ± 00.63 c
HWT +GRAD	10.62 ± 00.41 a	05.93 ± 00.04 a	08.95 ± 01.01 c
CV (%)[2]	12.62	2.27	28.24
Treatments	*F. sulawesiense*		
	°Brix [1]	pH[1,4]	Firmness (N)[1]
MOCK	10.30 ± 00.55 a	05.83 (30.16) ± 00.07 ab	08.45 ± 00.40 b
HWT	09.82 ± 00.59 a	05.73 (23.58) ± 00.06 ab	11.74 ± 00.80 b
1-MCP	09.68 ± 00.42 a	05.72 (22.08) ± 00.04 ab	17.82 ± 00.97 a
HWT +1-MCP	10.52 ± 00.57 a	05.74 (23.16) ± 00.06 ab	22.28 ± 01.01 a
GA	10.68 ± 00.29 a	05.87 (37.33) ± 00.40 a	12.89 ± 01.29 b
HWT + GA	09.78 ± 00.43 a	05.80 (21.16) ± 00.14 b	12.61 ± 00.89 b

GRAD	10.22 ± 00.67 a	05.73 (23.33) ± 00.03 ab	18.96 ± 01.56 a
HWT +GRAD	09.23 ± 00.64 a	05.63 (15.16) ± 00.06 b	10.53 ± 00.68 b
CV (%)[2]	13.04	3.05 [3]	22.26

[1]Values followed by the same letter within columns are not statistically different. Data represent means of two experiments ± standard error, with seven replicates (melons) per treatment.[2]CV (%) = coefficient of variation significance for parametric analysis (Scott-Knott test, $p \leq 0.05$).[3]CV (%) = coefficient of variation significance for non-parametric analysis (Kruskal-Wallis test, $p \leq 0.05$).[4]Data are mean ranks ± standard error. MOCK = Control; HWT = Hot Water Treatment; 1-MCP = 1-Methylcyclopropene; GA = Gibberellin; GRAD = Graduate A+®.

In melons inoculated with *F. falciforme*, the highest CAT activity (Fig. 3A) was observed in HWT and 1-MCP treatments, with increases of 539.3% and 397.4%, respectively, compared to MOCK. POX activity (Fig. 3B) was similar between MOCK and HWT but significantly different from the other treatments. PPO activity (Fig. 3C) was highest in MOCK, which was statistically different from the others, 426.4% higher than the 1-MCP treatment, which had the lowest activity. PAL activity (Fig. 3D) increased by 511.3%, 491.6%, and 509.1% in the HWT+1-MCP, GRAD, and HWT+GRAD treatments, respectively, compared to MOCK. MOCK also showed the highest accumulation of hydrogen peroxide (H_2O_2), significantly different from other treatments, while GRAD reduced H_2O_2 concentration by 52% compared to MOCK (Fig. 3E).

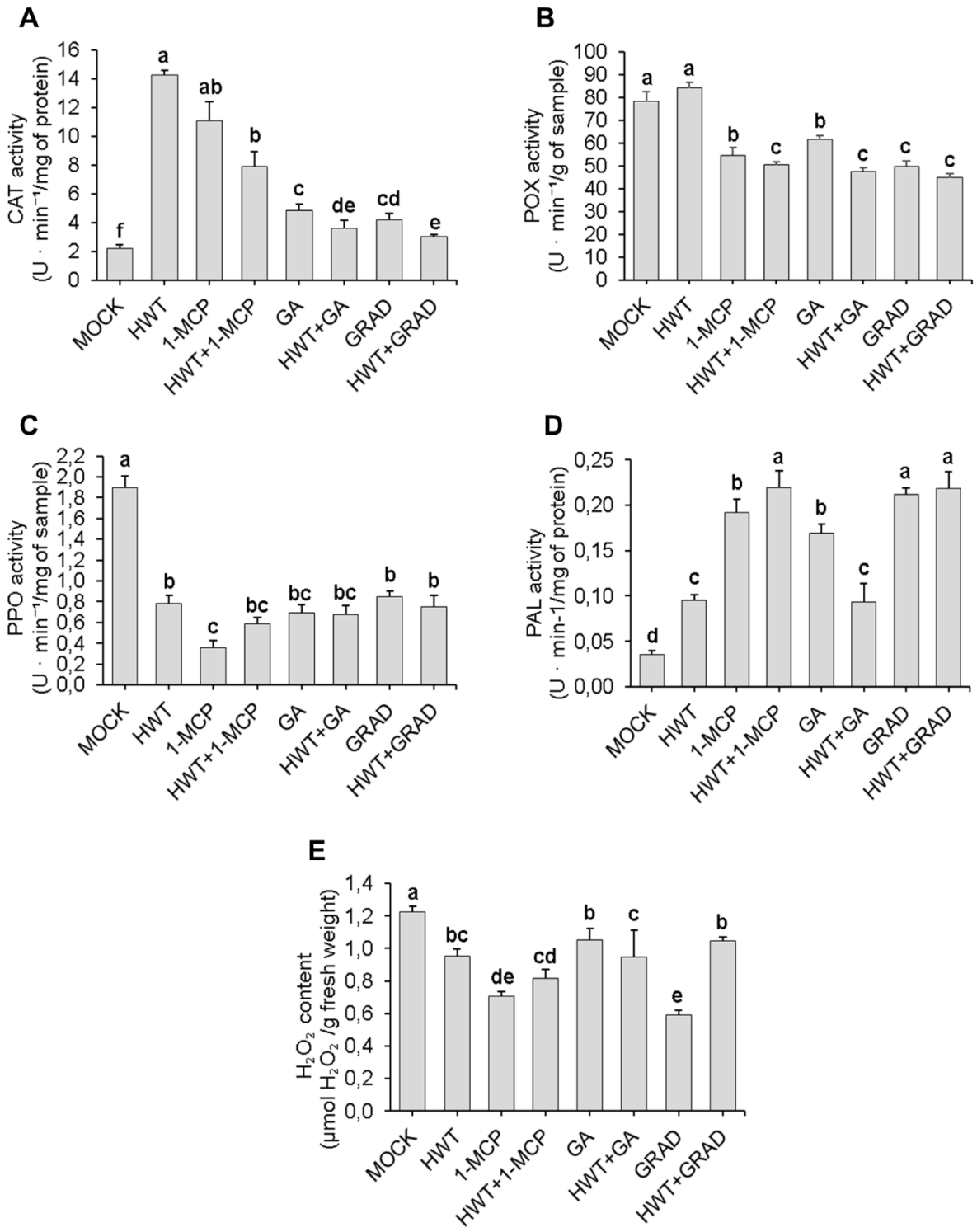

Figure 3. Mean values of Catalase (A), Peroxidase (B), Polyphenol oxidase (C), Phenylalanine ammonia-lyase (D), and Hydrogen peroxide quantification (E) in Galia melons inoculated with *F. falciforme*, under isolated and combined thermotherapy treatments. MOCK = Control; HWT = Hot Water Treatment; 1-MCP = 1-Methylcyclopropene; GA = Gibberellin; GRAD = Graduate A+®. Bars followed by the same letter in the same graph are not statistically different according to the Kruskal-Wallis test ($p \leq 0.05$) for Figures A and E,

and the Scott-Knott test ($p \leq 0.05$) for Figures B, C, and D. Data represent means of two experiments ± standard error, with seven replicates (melons) per treatment in each experiment.

In melons inoculated with *F. sulawesiense*, CAT activity (Fig. 4A) was highest in the HWT+1-MCP treatment, which was not statistically different from MOCK, GRAD, and HWT+GRAD, but significantly higher than the respective isolated treatments (HWT and 1-MCP). For POX activity (Fig. 4B), 1-MCP and GA treatments resulted in the highest levels, significantly different from other treatments. PPO activity (Fig. 4C) peaked in MOCK, HWT, GRAD, and HWT+GRAD treatments, with these treatments showing statistically similar PPO activity levels, all significantly higher than 1-MCP, the treatment with the lowest activity. PAL activity (Fig. 4D) was highest in GA, significantly different from the other treatments, with a 136.9% increase compared to MOCK, which had the lowest activity. MOCK treatment showed the highest H_2O_2 accumulation in fruits inoculated with *F. sulawesiense*, while HWT+1-MCP reduced H_2O_2 levels by 66.5% compared to MOCK (Fig. 4E).

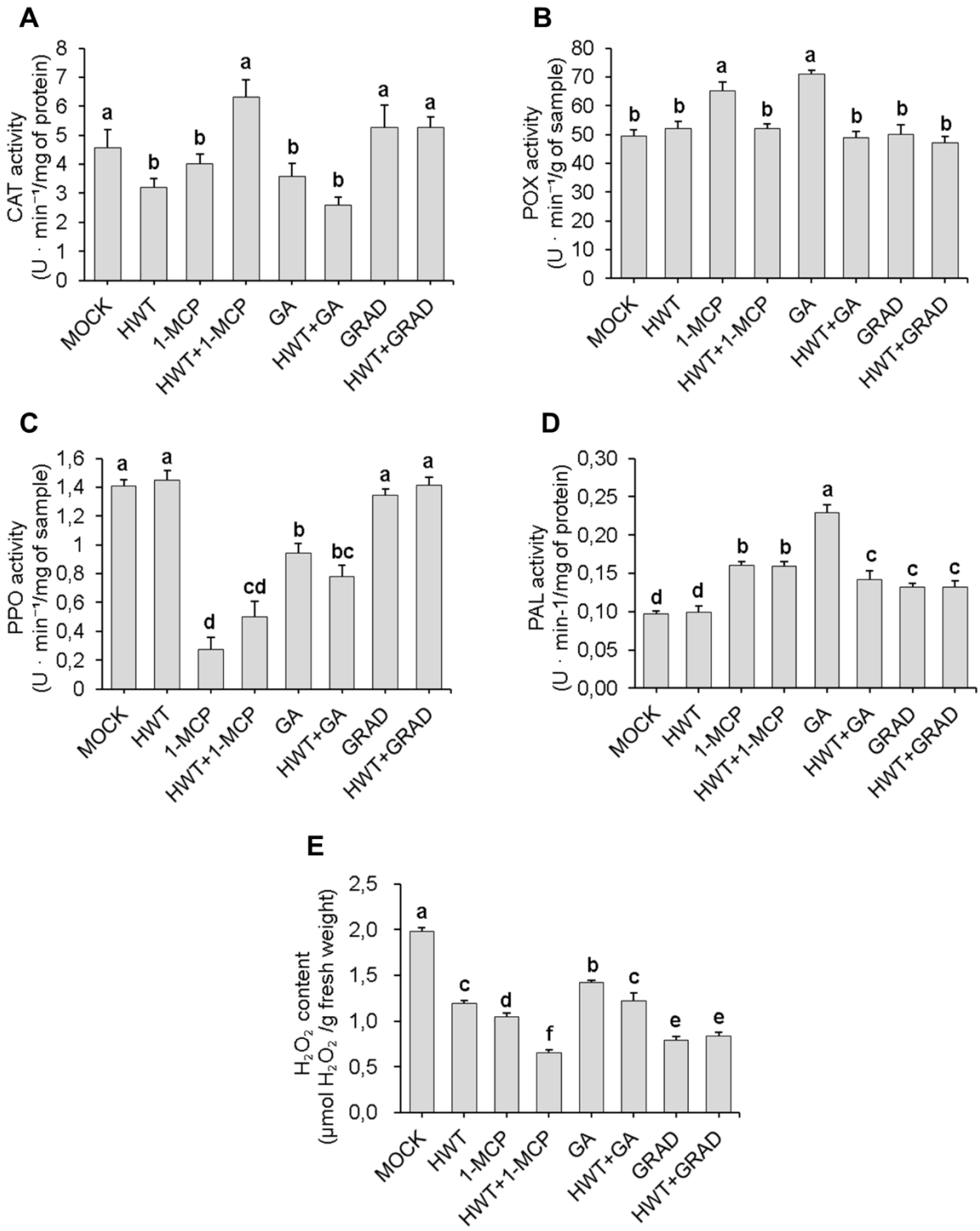

Figure 4. Mean values of Catalase (A), Peroxidase (B), Polyphenol oxidase (C), Phenylalanine ammonia-lyase (D), and Hydrogen peroxide quantification (E) in Galia melons inoculated with *F. sulawesiense*, under different treatment conditions. MOCK = Control; HWT = Hot Water Treatment; 1-MCP = 1-Methylcyclopropene; GA = Gibberellin; GRAD = Graduate A+®. Bars followed by the same letter in the same graph are not statistically different according to the Scott-Knott test ($p \leq 0.05$). Data represent means of two

experiments ± standard error, with seven replicates (melons) per treatment in each experiment.

In melons inoculated with both fungal species, positive correlations were observed between disease severity and enzymatic activities: for *F. falciforme*, POX (0.35), PPO (0.22), and H_2O_2 (0.25); and for *F. sulawesiense*, POX (0.63), PAL (0.38), and H_2O_2 (0.62). Additionally, for fruits inoculated with *F. falciforme*, negative correlations were found between disease severity and firmness (–0.02) and PAL activity (–0.25). For fruits inoculated with *F. sulawesiense*, severity showed negative correlations with firmness (–0.16), CAT (–0.21), and PPO (–0.30). The strongest negative correlation was between firmness and H_2O_2 (–0.62) (Fig. 5).

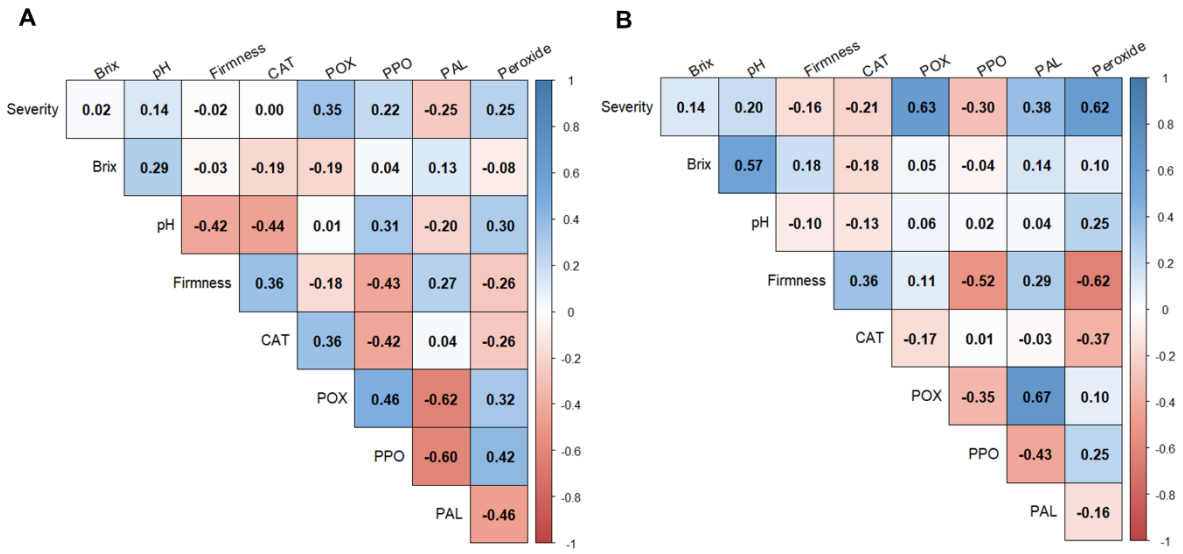

Figure 5. Pearson correlation matrix for disease severity, °Brix, pH, Firmness, Catalase (CAT), Peroxidase (POX), Polyphenol oxidase (PPO), Phenylalanine ammonia-lyase (PAL), and Hydrogen peroxide quantification (H_2O_2) in Galia melons inoculated with *F. falciforme* (A) and *F. sulawesiense* (B), subjected to isolated and combined thermotherapy treatments.

4. DISCUSSION

In this study, both *Fusarium* species caused rots in Galia melons, presenting characteristic symptoms of pathogenesis, such as the development of dark, water-soaked spots, progressing to cracking at the inoculation site and evident mycelial growth (Fig. 2), as previously reported by Araújo et al. (2021). Similar results were found by Nogueira et al.

(2023), who evaluated the aggressiveness of different *Fusarium* species in eight melon hybrids in Brazil, including Galia melon. In that study, *F. falciforme* also caused greater disease severity.

Based on the severity data (Fig. 1), although Graduate A+® resulted in the most significant reduction of peduncular lesions among treatments, it did not achieve complete disease control. According to the literature, its efficacy appears to be decreasing (MANIÇOBA et al., 2023). This reduction may reflect decreased sensitivity of phytopathogens to chemical actives, likely due to genetic and epigenetic mutations, variability mechanisms, or even the fungicide's mode of action itself (AZEVEDO, 2007; PARREIRA et al., 2009).

The findings of this study demonstrate that hot water treatments (HWT) were highly effective in disease control throughout storage. Similar results were reported by Moura et al. (2024), where hot water treatment at 58 °C for 150 s significantly reduced disease severity caused by *F. falciforme* and *F. sulawesiense* by more than 35%. Hot water can eradicate or weaken phytopathogens (Brito et al., 2023), as many *Fusarium* species have lethal thermal thresholds above 42 °C (MANIÇOBA et al., 2023). Moreover, fruit stress induced by high temperature may stimulate the synthesis of fungitoxic compounds such as chitinase, β-1,3-glucanase, and phytoalexins (MOURA et al., 2024), which contribute to disease suppression (SIDDIQUI, 2018).

Among the treatments, 1-MCP alone was ineffective against both *Fusarium* species; however, when combined with HWT, it led to a reduction in peduncular lesions caused by both fungi. Nguyen et al. (2020), also observed low effectiveness of 1-MCP at 5, 10, and 20 °C in naturally infected Cantaloupe melons. Similarly, Terão et al. (2009) evaluated the influence of 1-MCP (29 ± 1 °C) on the control of *F. pallidoroseum* in Orange melons and observed disease symptoms 15 days after storage at 10 ± 2 °C. Nevertheless, in our study, 1-MCP and its combined treatments maintained greater fruit firmness, regardless of the *Fusarium* species. This is attributed to its action in inhibiting ethylene perception, as 1-MCP binds to ethylene receptors and prevents activation of ripening processes such as pulp softening (NGUYEN et al., 2020).

The combination of HWT and the application of 1-MCP represents a distinctive strategy for managing Fusarium rot in fruits. HWT acts directly on pathogen structures, reducing spore germination and tissue colonization, in addition to inducing host defense responses (MOURA et al., 2024). In contrast, 1-MCP acts indirectly by inhibiting ethylene action and delaying fruit ripening and senescence, thereby maintaining tissue integrity,

limiting infection, and extending shelf life (DIAS et al., 2021). This complementary action gives the combination of these techniques an additive effect, unlike other physical or biological methods that act individually on either the pathogen or the host. Moreover, it is a safe technology, free of residues, and approved by international regulations (EUROPEAN COMMISSION, 2025), making it suitable for fruits intended for export. Therefore, the integration of these two techniques represents a sustainable, technically viable approach with commercial application potential, combining *Fusarium* control in melons with the maintenance of postharvest quality.

Another strategy tested was the use of gibberellin. While the hormone alone was ineffective in controlling rots caused by both *Fusarium* species, it significantly reduced disease severity when combined with HWT. Similar results were found by Zhang et al. (2023), who showed that although gibberellin does not directly inhibit *Fusarium* growth, its combination with azoxystrobin resulted in superior disease control compared to individual treatments. These results suggest that GA may enhance host resistance to pathogen penetration and delay postharvest senescence, despite not having direct antifungal activity.

HWT and 1-MCP increased CAT activity in melons inoculated with both fungal species (Figs. 3A and 4A). Biotic stress induced by *Fusarium* infection and abiotic stress from heat treatment produce the overproduction of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), as observed in this study (MOURA et al., 2024). The accumulation of ROS in plant tissues acts as a chemical signal that triggers antioxidant defense pathways, including gene expression of CAT and POX. These enzymes catalyze the breakdown of H_2O_2 into H_2O and O_2, preventing toxic accumulation and oxidative damage to cellular structures (DING et al., 2024). CAT plays a central role in antioxidant defense, as H_2O_2 metabolism is crucial for regulating senescence and maintaining cellular integrity in stored fruit (WANG et al., 2023a; WANG et al., 2023b). Whereas, 1-MCP enhances antioxidant responses by suppressing ethylene action, prolonging enzyme activity, and contributing to firmness retention and disease resistance during storage. Thus, the combination of thermal and hormonal stress triggers defense mechanisms, enhancing antioxidant responses and limiting the deleterious effects of *Fusarium* infection (NGUYEN et al., 2020). Herein, the negative correlation between CAT activity and H_2O_2 levels, as well as the positive correlation between CAT activity and fruit firmness (Fig. 3A, 4A and 5), indicates that mitigation of oxidative stress is associated with the preservation of cell wall integrity, which in turn enhances shelf life.

The increased POX activity and reduced H_2O_2 levels in melons inoculated with both *Fusarium* species, except in the MOCK treatment (Fig. 3B and 4B), indicate an adaptive response to early ROS accumulation. These results suggest that the combination of biotic stress from fungal infection and physiological responses to HWT and 1-MCP promotes ROS production. Consequently, POX is activated to catalyze H_2O_2 decomposition and phenolic compound oxidation (GUO et al., 2025), as also observed by Véras et al. (2019), Terão et al. (2021), Medeiros et al. (2023), and Moura et al. (2024).

The lack of statistical difference between MOCK and HWT may be attributed to the timing of enzymatic induction. Previous studies show that the peak of POX activity occurs up to 72 h post-treatment, followed by a gradual decline (TERÃO et al., 2021). Additionally, 1-MCP has been shown to stimulate the expression of genes such as *GbPOD1* in *Gynura bicolor* DC associated with POX production during early stress phases. This helps reduce H_2O_2 accumulation during storage and prevents excessive phenolic oxidation (GUO et al., 2025), preserving fruit quality. Similarly, exogenous GA applications have been shown to increase POX activity and reduce H_2O_2 levels, contributing to postharvest quality (ZHANG et al., 2023).

Stress induced by *Fusarium* inoculation, along with 1-MCP and GA treatments, activated phenylpropanoid pathway defenses (PPO and PAL), as previously observed by Zhang et al. (2021) and Moura et al. (2024). Our study revealed differential activation of these enzymes, which play complementary roles in antioxidant defense. PAL catalyzes the first step in phenolic biosynthesis, including flavonoids and anthocyanins, which function as non-enzymatic antioxidants (DING et al., 2024; GUO et al., 2025), while PPO oxidizes phenols into reactive quinone compounds with strong fungicidal potential (LI et al., 2022).

Low PPO activity, particularly in 1-MCP and GA treatments, may have limited the phenol-to-quinone conversion, reducing infection containment (Figs. 1 and 2). This imbalance, characterized by the accumulation of non-oxidized phenols, suggests a partial or uncoordinated defense response, possibly caused by regulator interference in PPO gene expression and the depletion of non-enzymatic antioxidants (ZHANG et al., 2021; GUO et al., 2025). Previous studies showed that while GA and 1-MCP may induce enzymes like POX, APX (ascorbate peroxidase), and SOD (superoxide dismutase), they may inhibit PPO, reflecting selectivity in signaling pathways (Zhang et al., 2023). Therefore, postharvest antioxidant efficacy depends not only on the synthesis of phenolic compounds but also on coordinated activation of PAL and PPO enzymes to ensure an effective defense against pathogenesis.

5. CONCLUSION

Based on the data obtained, HWT at 58 °C for 150 s, both alone and in combination with 1-MCP, proved to be effective alternatives to conventional fungicide-based management for controlling rots in Galia melons caused by *F. falciforme* and *F. sulawesiense*. These treatments also resulted in increased fruit firmness, reduced H_2O_2 levels, and influenced the activity of the enzymes CAT, POX, PPO, and PAL, contributing to a significant reduction in disease severity. Therefore, HWT, whether applied alone or in combination with 1-MCP, not only improves the postharvest quality of the fruit but also triggers physiological defense mechanisms.

REFERENCES

ABRAFRUTAS. Brazilian Association of Fruit Producers and Exporters. **From trucks to ships: fruit exports face infrastructure bottlenecks in Ceará**. [S. l.], 2025. Disponível em: https://abrafrutas.org/2022/08/de-caminhoes-e-navios-exportacao-de-frutas-enfrenta-gargalos-de-infraestrutura-no-ceara/?utm_source=chatgpt.com. Acesso em: 12 jan. 2025.

ALEXIEVA, V.; SERGIEV, I.; MAPELLI, S.; KARANOV, E. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. **Environmental and Experimental Botany**, [S. l.], v. 45, p. 1337–1344, 2001. Disponível em: https://doi.org/10.1046/j.1365-3040.2001.00778.x.

ARAÚJO, M. B. M.; MOREIRA, G. M.; NASCIMENTO, G. A. N.; NASCIMENTO, S. R. C.; PFENNING, L. H.; AMBRÓSIO, M. M. Q. Fusarium rot of melon is caused by several Fusarium species. **Plant Pathology**, [S. l.], v. 70, p. 712–721, 2021. Disponível em: https://doi.org/10.1111/ppa.13328.

ASREY, R.; SHARMA, S.; BARMAN, K.; PRAJAPATI, U.; NEGI, N.; MEENA, N. K. Biological and postharvest interventions to manage the ethylene in fruit: a review. **Sustainable Food Technology**, [S. l.], v. 1, p. 803–826, 2023. Disponível em: https://doi.org/10.1039/d3fb00037k.

AZEVEDO, L. A. S. **Fungicidas sistêmicos: teoria e prática**. Campinas: EMOPI, 2007.
AZEVEDO, R. A.; ALAS, R. M.; SMITH, R. J.; LEA, P. J. Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation in barley. **Physiologia Plantarum**, [S. l.], v. 104, p. 280–292, 1998. Disponível em: https://doi.org/10.1034/j.1399-3054.1998.1040217.x.

BEZERRA NETO, E.; BARRETO, L. P. **Análises químicas e bioquímicas em plantas**. Mossoró: UFERSA, 2011.

BRITO, C. H.; COSTA, N. P.; BATISTA, J. C.; NASCIMENTO, L. C.; OLIVEIRA, H. D.; BARRETO, E. S. Thermotherapy for the control of postharvest pathogens in cajazeira fruits. **Acta Scientiarum. Agronomy**, Maringá, v. 30, p. 19–23, 2023. Disponível em: https://doi.org/10.4025/actasciagron.v30i1.1117.

CAMPOS, A. D.; FERREIRA, A. G.; HAMPE, M. M. V.; ANTUNES, I. F.; BRANCÃO, N.; SILVEIRA, E. P.; OSÓRIO, V. A.; AUGUSTIN, E. Peroxidase and polyphenol oxidase activity in bean resistance to anthracnose. **Pesquisa Agropecuária Brasileira**, Brasília, DF, v. 39, p. 637–643, 2004. Disponível em: https://doi.org/10.1590/S0100-204X2004000700004.

CAVALCANTE, A. L. A.; NEGREIROS, A. M. P.; TAVARES, M. B.; BARRETO, E. D. S.; ARMENGOL, J.; SALES JÚNIOR, R. Characterization of five new Monosporascus species: adaptation, pathogenicity and fungicide sensitivity. **Journal of Fungi**, [S. l.], v. 6, p. 169–183, 2020. Disponível em: https://doi.org/10.3390/jof6030169.

DIAS, C.; RIBEIRO, T.; RODRIGUES, A. C.; FERRANTE, A.; VASCONCELOS, M. W.; PINTADO, M. Improving the ripening process after 1-MCP application: implications and strategies. **Trends in Food Science & Technology**, [S. l.], v. 113, p. 382–396, 2021. Disponível em: https://doi.org/10.1016/j.tifs.2021.05.012.

DING, X.; MA, J.; LIU, S.; DONG, X.; PAN, X.; DONG, B. Acid electrolytic water treatment improves the quality of fresh-cut red pitaya fruit by regulating ROS metabolism. **Postharvest Biology and Technology**, [S. l.], v. 207, 112636, 2024. Disponível em: https://doi.org/10.1016/j.postharvbio.2023.112636.

EUROPEAN COMMISSION. **A European Green Deal**. New Circular Economy Action Plan, p. 1–4, 2019. Disponível em: https://environment.ec.europa.eu/strategy/circular-economy-action-plan_en. Acesso em: 27 ago. 2025.

EUROPEAN COMMISSION. **Active substances, safeners and synergists**. [S. l.], 2025. Disponível em: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances. Acesso em: 2 fev. 2025.

EUROPEAN COMMISSION. **Approval of active substances**. [S. l.], 2022. Disponível em: https://food.ec.europa.eu/plants/pesticides/approval-active-substances-safeners-and-synergists. Acesso em: 27 ago. 2025.

GUO, F.; HAN, J.; JIN, M.; XIE, Y.; JIANG, L. Effects of sucrose and 1-MCP on antioxidants in postharvest Gynura bicolor DC. **Plant Physiology and Biochemistry**, [S. l.], v. 219, 109319, 2025. Disponível em: https://doi.org/10.1016/j.plaphy.2024.109319.

HAN, G.; ZUO, J.; WANG, Q.; XU, L.; WANG, Z.; DONG, H.; GAO, L. Effects of 1-MCP on postharvest physiology and quality of bitter melon. **Scientia Horticulturae**, [S. l.], v. 182, p. 86–91, 2015. Disponível em: https://doi.org/10.1016/j.scienta.2014.07.024.

HF BRASIL. **Melon/CEPEA: on the rise! Exports from the 2023/24 harvest are higher**. Piracicaba: CEPEA, 2024. Disponível em: https://www.hfbrasil.org.br/br/melao-cepea-em-alta-exportacoes-da-safra-2023-24-estao-maiores.aspx. Acesso em: 19 jan. 2025.

IBGE. Instituto Brasileiro de Geografia e Estatística. **Melon production**. Rio de Janeiro: IBGE, 2023. Disponível em: https://www.ibge.gov.br/explica/producao-agropecuaria/melao/rn. Acesso em: 2 fev. 2025.

KOLTZ, E. A.; SANTOS, I.; DALLEMOLE-GIARETTA, R.; PAZOLINI, K.; LEITE, C.; STEILMANN, P. Combining Brassica sachets and thermotherapy against green mold of orange. **Scientia Horticulturae**, [S. l.], v. 268, 109389, 2020. Disponível em: https://doi.org/10.1016/j.scienta.2020.109389.

LI, Z.; LI, B.; LI, M.; FU, X.; ZHAO, X.; MIN, D.; LI, F.; LI, X.; ZHANG, X. Hot air pretreatment alleviates browning of fresh-cut pitaya by regulating phenylpropanoid pathway. **Postharvest Biology and Technology**, [S. l.], v. 190, 111954, 2022. Disponível em: https://doi.org/10.1016/j.postharvbio.2022.111954.

MANIÇOBA, F. E.; NEGREIROS, A. M. P.; CAVALCANTE, A. L. A.; ALVES, C. P. S. S.; NASCIMENTOS, M. T. A.; AMBRÓSIO, M. M. Q.; SALES JÚNIOR, R. Environmental factors and fungicide sensitivity of *Fusarium* spp. causing melon fruit rot. **Journal of Phytopathology**, [S. l.], v. 171, p. 504–516, 2023. Disponível em: https://doi.org/10.1111/jph.13206.

MAPA. Ministry of Agriculture and Livestock. **The fruit sector stands out in Brazilian exports**. Brasília, DF: MAPA, 2024. Disponível em: https://www.gov.br/agricultura/pt-br/assuntos/noticias/setor-de-fruticultura-se-destaca-nas-exportacoes-brasileiras. Acesso em: 2 fev. 2025.

MEDEIROS, A. S.; ALVES, T. R. C.; SILVA, J. L. S.; MOURA, A. P.; LIMA, J. S. S.; SOUZA, J. J. F.; FRANÇA, M. A. V.; FERNANDES, J. C.; EVANGELISTA, L. F. B.; AMBRÓSIO, M. M. Q. Thermotherapy with alternative products in melon rot management. **European Journal of Plant Pathology**, [S. l.], v. 70, n. 3, p. 712-721, 2023. Disponível em: https://doi.org/10.1111/ppa.13328.

MOURA, A. P.; ALVES, T. R.; SILVA, J. L. S.; SILVA, I. V. P.; SOUZA, J. J. F.; NEGREIROS, A. M. P.; EVANGELISTA, L. F. B.; FREIRES, A. L. A.; FIGUEIREDO, F. R. A.; AMBRÓSIO, M. M. Thermotherapy in the management of galia melon rot caused by *Fusarium falciforme* and *Fusarium sulawesiense*. **European Journal of Plant Pathology**, [S. l.], v. 169, p. 715–725, 2024. Disponível em: https://doi.org/10.1007/s10658-024-02866-5.

NGUYEN, L. P. L.; ZSOM, T.; DAM, M. S.; BARANYAI, L.; HITKA, G. Comparison of 1-MCP treatment on melon cultivars using different temperatures. **Journal of Applied Botany and Food Quality**, [S. l.], v. 93, p. 122–129, 2020. Disponível em: http://dx.doi.org/10.5073/JABFQ.2020.093.016.

NOGUEIRA, G. A.; CONRADO, V. S. C.; FREIRES, A. L. A.; SOUZA, J. J. F.; FIGUEIREDO, F. R. A.; BARROSO, K. A.; ARAÚJO, M. B. M.; NASCIMENTO, L. V.; LIMA, J. S. S.; BEZERRA NETO, F.; SILVA, W. L.; AMBRÓSIO, M. M. Q. Aggressivity of *Fusarium* spp. causing melon fruit rot in Brazil. **Plant Disease**, [S. l.], v. 107, p. 886–892, 2023. Disponível em: https://doi.org/10.1094/PDIS-04-22-0728-SR.

OSTER, A. H.; SILVA, E. O.; BORDALLO, P. N.; ZOCOLO, G. H.; SILVEIRA, M. R. S.; OIRAM FILHO, F.; ARAÚJO, A. A. C. Pulsed ultraviolet light in the control of melon rot. **Boletim de Pesquisa e Desenvolvimento**, Fortaleza, n. 173, 2018. (Embrapa Agroindústria Tropical).

PARREIRA, D. F.; NEVES, W. S.; ZAMBOLIM, L. Resistance of fungi to quinone-inhibiting fungicides. **Revista Trópica: Ciências Agrárias e Biológicas**, Chapadinha, v. 3, n. 2, p. 24–30, 2009. Disponível em: https://doi.org/10.0000/rtcab.v3i2.36.

R CORE TEAM. **R**: A language and environment for statistical computing. Viena: R Foundation for Statistical Computing, 2024. Disponível em: https://www.R-project.org. Acesso em: 9 fev. 2025.

RSTUDIO TEAM. **RStudio**: Integrated Development for R. Boston: RStudio, PBC, 2024. Disponível em: http://www.rstudio.com/. Acesso em: 25 ago. 2025.

SALAZAR-CEREZO, S.; MARTÍNEZ-MONTIEL, N.; GARCÍA-SÁNCHEZ, J.; PÉREZ-Y-TERRÓN, R.; MARTÍNEZ-CONTRERAS, R. D. Gibberellin biosynthesis and metabolism in plants, fungi, and bacteria. **Microbiological Research**, [S. l.], v. 208, p. 85–98, 2018. Disponível em: https://doi.org/10.1016/j.micres.2018.01.010.

SEBRAE. **Melon cultivation**. [S. l.]: SEBRAE, 2025. Disponível em: https://datampe.sebrae.com.br/profile/industry/cultivo-de-melao. Acesso em: 27 mar. 2025.
SIDDIQUI, M. W. **Postharvest disinfection of fruits and vegetables**. Londres: Academic Press, 2018.

SILVA, D. E. M. **Identification and genetic diversity of Fusarium isolates associated with melon rot in northeastern Brazil**. 2023. Dissertação (Mestrado em Fitotecnia) – Universidade Federal Rural do Semi-Árido, Mossoró, 2023.

TAN, G. H.; ALI, A.; SIDDIQUI, Y. Current strategies and challenges in control of papaya postharvest diseases. **Scientia Horticulturae**, [S. l.], v. 301, 111139, 2022. Disponível em: https://doi.org/10.1016/j.scienta.2022.111139.

TERÃO, D.; NECHET, K. L.; FRIGHETTO, R. T.; ANJOS, V. D.; MAIA, A. H.; HALFELD-VIEIRA, B. A. Control of Fusarium rot in Galia melon with UV-C radiation and hot water. **Tropical Plant Pathology**, [S. l.], v. 46, p. 350–359, 2021. Disponível em: https://doi.org/10.1007/s40858-021-00432-6.

TERÃO, D.; OLIVEIRA, S. M. A.; VIANA, F. M. P.; SÁ, C. R. L.; MOURA, R. D.; GONDIM, D. M. F. Management of melon rot using 1-MCP under two storage conditions. **Summa Phytopathologica**, Botucatu, v. 35, n. 2, p. 110–115, 2009. Disponível em: https://doi.org/10.1590/S0100-54052009000200005.

UMESHA, S. Phenylalanine ammonia lyase activity in tomato seedlings and its relation to bacterial canker resistance. **Phytoparasitica**, [S. l.], v. 34, p. 68–71, 2006. Disponível em: http://dx.doi.org/10.1007/BF02981341.

VÉRAS, M. L. M.; ARAÚJO, F. F.; GOMES JUNIOR, J.; FINGER, F. L. Enzymatic activity and quality of 'Galia' melon under storage temperatures and atmosphere. **Revista Brasileira de Ciências Agrárias**, Recife, v. 14, e5617, 2019. Disponível em: http://dx.doi.org/10.5039/agraria.v14i1a5617.

WANG, C.; WU, J.; TANG, Y.; MIN, Y.; WANG, D.; MA, X.; LI, H.; LI, J.; CHEN, Y.; CHEN, S.; LIU, Z. Changes of phenylpropanoid metabolism and lignin accumulation in cassava root during storage. **Scientia Horticulturae**, [S. l.], v. 310, 111765, 2023b. Disponível em: https://doi.org/10.1016/j.scienta.2022.111765.

WANG, Q.; HAN, Y.; YANG, R.; ZHANG, X.; ZHU, Y.; OYOM, W.; LI, Y.; PRUSKY, D.; BI, Y. Melatonin facilitates wound suberin and lignin deposition in potato tubers. **Postharvest Biology and Technology**, [S. l.], v. 198, 112270, 2023a. Disponível em: https://doi.org/10.1016/j.postharvbio.2023.112270.

YUSHU, Z.; GUO, Q.; YANAN, Z.; YUTING, L.; XUEZHENG, W. Controlling Fusarium wilt in melon using tillered onion bulb extract. **Pakistan Journal of Botany**, Karachi, v. 49, p. 1615–1623, 2017.

ZHANG, J.; CAO, Y.; TANG, J.; HE, X.; LI, M.; LI, C.; REN, X.; DING, Y. Physiology and application of gibberellins in postharvest horticultural crops. **Horticulturae**, [S. l.], v. 9, n. 6, p. 625, 2023. Disponível em: https://doi.org/10.3390/horticulturae9060625.

ZHANG, W.; JIAMG, H.; CAO, J.; JIANG, W. Biochemical mechanisms and control technologies for chilling injury in postharvest fruits. **Trends in Food Science & Technology**, [S. l.], v. 113, p. 355–365, 2021. Disponível em: https://doi.org/10.1016/j.tifs.2021.05.009.