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Original article

Effect of *Trichoderma harzianum* and *Pseudomonas fluorescens* on the control of anthracnose in mango fruit (*Mangifera indica* L.) during post-harvest

Efecto de *Trichoderma harzianum* y *Pseudomonas fluorescens* en el control de Antracnosis en frutos de mango (*Mangifera indica* L.) en pos cosecha

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ABSTRACT: The objective of the study was to evaluate the effect of *Trichoderma harzianum* and *Pseudomonas fluorescens* on the control of anthracnose in post-harvest mango fruit. An experimental study was conducted on Tommy Atkins mangoes using beneficial microorganisms at different concentrations as treatments. A completely randomized design was established with six treatments and ten replicates; the microorganisms were applied by spraying for four months. The incidence, severity, and total soluble solids (°Brix) were evaluated, and the data were analyzed using ANOVA and Tukey ($\alpha = 0.05$). Molecular identification confirmed the high purity of *T. harzianum* (KF624792), *P. fluorescens* (MG977684), and the pathogen *C. gloeosporioides* (MH700455). The results showed that all treatments with beneficial microorganisms achieved 0% incidence, while the control treatment had a 10 % incidence with visible necrotic lesions. In addition, the consortium of both microorganisms (T5) recorded the highest concentration of soluble solids (15 °Brix), showing an improvement in fruit quality. The use of biological control microorganisms, both individually and in consortium, strengthened the fruit's defense mechanisms against *C. gloeosporioides*, providing post-harvest protection, increasing °Brix, and extending the shelf life of mangoes.

Key words: Fruit quality, fungal disease, fruit, fungi, beneficial microorganisms, rot, soluble solids (°Brix).

RESUMEN: El objetivo del estudio fue evaluar el efecto de *Trichoderma harzianum* y *Pseudomonas fluorescens* en el control de la antracnosis en frutos de mango en postcosecha. Se efectuó un estudio experimental en mangos de la variedad Tommy Atkins usando microorganismos benéficos a diferentes concentraciones de microorganismos benéficos como tratamientos. Se estableció un diseño completamente al azar con seis tratamientos y diez repeticiones; los microorganismos se aplicaron por aspersión durante cuatro meses. Se evaluó la incidencia, severidad y los sólidos solubles totales (°Brix) y los datos fueron analizados mediante ANOVA y Tukey ($\alpha = 0,05$) La identificación molecular confirmó la alta pureza de *T. harzianum* (KF624792), *P. fluorescens* (MG977684) y el patógeno *C. gloeosporioides* (MH700455). Los resultados mostraron que todos los tratamientos con microorganismos benéficos lograron 0%, de incidencia, mientras que el tratamiento control presentó 10 % de incidencia con lesiones necróticas visibles. Además, el consorcio de ambos microorganismos (T5) registró la mayor concentración de sólidos solubles (15 °Brix), evidenciando una mejora en la calidad del fruto. El empleo de los microorganismos de control biológico tanto de forma individual como en consorcio, fortaleció los mecanismos de defensa del fruto frente a *C. gloeosporioides*, brindando protección en postcosecha, incrementando los °Brix y prolongando la vida útil del de mango.

Palabras clave: Calidad de fruto, enfermedad fúngica, fruta, hongos, microorganismos benéficos, podredumbre, sólidos solubles (°Brix).

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INTRODUCTION

Mango (*Mangifera indica* L.) is considered one of the most important tropical fruits worldwide, not only for its characteristic flavor and wide variety of culinary uses, but also for its high nutritional value (1). It is an abundant source of vitamins A, C, and E, as well as antioxidant compounds and fiber, which has driven a sustained increase in its global demand (2). However, as a climacteric fruit, its rapid ripening generates significant losses, representing a challenge for both producers and marketers seeking to maintain fruit quality from harvest to the final consumer (3).

Among the main problems affecting mango commercialization are fungal diseases, with anthracnose being the most relevant. This disease, caused mainly by phytopathogenic fungi, begins with small dark spots that expand rapidly as the fruit ripens, affecting both the external appearance and the internal pulp (4,5). The associated economic losses are considerable, especially during the postharvest period, when factors such as humidity and temperature favor pathogen proliferation (6).

Historically, the control of anthracnose has depended almost exclusively on synthetic fungicides (7). Although effective in the short term, their intensive application has led to the emergence of resistant strains, negative impacts on the environment, and the presence of chemical residues in the fruits (8). Currently, international food safety regulations are becoming increasingly strict, limiting access to premium markets for products exceeding maximum residue limits (9).

In response to this situation, biological control has emerged as an efficient and sustainable alternative. This strategy uses beneficial microorganisms capable of reducing pathogen populations and protecting the fruit without affecting the ecosystem (10). Among the most studied microorganisms for biocontrol is *Trichoderma harzianum*, which acts through mycoparasitism, competition for nutrients and space, and the production of hydrolytic enzymes such as chitinases and glucanases that degrade the cell wall of phytopathogenic fungi (11). Its ability to adapt to different environments and grow rapidly makes it a good candidate for the control of anthracnose in fruits (12).

On the other hand, bacteria of the genus *Pseudomonas*, especially *Pseudomonas fluorescens* (a Gram-negative bacillus belonging to the Pseudomonadaceae family), have proven to be excellent bioprotection agents. These bacteria are well known for their ability to coexist within plant tissues and produce secondary metabolites with antifungal function, such as siderophores and antibiotics (13). Furthermore, *P. fluorescens* can generate systemic defense in the fruit, strengthening its natural defenses against pathogen attack, which greatly aids the action of other biological agents (14). The study is based on evaluating the effect of *Trichoderma harzianum* and *Pseudomonas fluorescens* on the control of anthracnose in postharvest mango fruits.

MATERIALS AND METHODS

Study area

For the study, mango fruits were considered from the experimental farm of the Agrarian University of Ecuador, Palestina extension, in Guayas province, Ecuador, located within the coordinates 1°37'03.1"S 79°58'31.6"W, at an altitude of 10 meters above sea level. This site is characterized by a very warm and humid tropical climate with average temperatures between 23 and 25 °C, clayey soils, and flat topography. The mango trees are approximately 5 years old, under organic crop management.

Genetic material

The study used mango fruits of the 'Tommy Atkins' cultivar, selected for their commercial relevance and quality characteristics. The selected fruits had an average of 7.5 °Brix.

Obtaining microorganisms and pathogen

The beneficial microorganisms were acquired from the Ecuadorian laboratory specialized in agricultural microbiology (Microbiolab) in liquid formulation, activated with metabolites, spores, and chlamydospores. For the identification of the anthracnose species, a fruit with disease symptoms was sent to the molecular identification laboratory IDgen in Quito for its respective isolation and molecular identification of the species.

Experimental design

A completely randomized design (CRD) was used for the experiment, with six treatments and ten replicates. Each experimental unit consisted of one mango tree (Table 1).

Table 1. Treatments evaluated, microorganism concentration, and biological control objective for anthracnose in *Mangifera indica*

Symbol	Treatment	Concentration (CFU/mL)
T0	Without microorganisms	Control
T1	*P. fluorescens*	1×10^{7}
T2	*P. fluorescens*	1×10^{8}
T3	*T. harzianum*	1×10^{7}
T4	*T. harzianum*	1×10^{8}
T5	*P. fluorescens* + *T. harzianum*	1×10^{7}

CFU: Colony Forming Units

Application of treatments

Treatments were applied by spraying using a motorized fumigation pump, starting in the flowering season and continuing until the fruits began to ripen (approximately 4 months), with an application frequency of every 20 days.

Selection and storage of fruits

From each treatment replicate, four mango fruits were selected. These were stored under controlled temperature and relative humidity conditions (10 °C; 85 %), kept in darkness for 24 hours; for a total of 240 fruits evaluated.

Evaluation of incidence and severity

Observations were made four days after storage, evaluating the percentage of fruits with visible symptoms of anthracnose. The external appearance of the mango was assessed visually. Incidence was calculated by dividing the number of affected fruits by the total evaluated and multiplying by 100.

$$\text{Incidence}(\%) = \frac{\text{Number of fruits with symptoms}}{\text{total number of fruits evaluated}} \quad x100$$

Severity of anthracnose was determined by cutting slices of the fruit using a visual scale, based on the percentage of the fruit flesh area affected by the disease.

Fruit quality measurement

Brix degrees were measured with a manual refractometer, placing a few drops of crushed and filtered mango juice on the prism of the equipment.

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) and Tukey's mean comparison test (α = 0.05) using SAS® statistical software version 9.4 (2015).

RESULTS AND DISCUSSION

The molecular analysis results (Table 2) accurately confirmed the identity of the biocontrol agents *T. harzianum* and *P. fluorescens*, along with the pathogen *C. gloeosporioides.*

Molecular characterization accurately confirmed the identity of the beneficial microorganism species for *P. fluorescens* and *T. harzianum*, and with 98 % certainty for the pathogen *C. gloeosporioides*. This accuracy is key because using gene fragments such as the 16S gene in bacteria and ITS in fungi is a way to compare the genetic similarity among these microorganism species. According to some authors, correct species identification allows for greater certainty in predicting the effectiveness of beneficial microorganisms against specific pathogens, ensuring the validity of biocontrol assays (15).

During postharvest evaluation, the external appearance of the fruits at harvest time and the changes observed during the first four days were recorded, as well as the internal condition of the pulp. These analyses allowed for the identification of anthracnose symptoms and the assessment of tissue integrity under the different applied treatments. The appearance of the fruits under the different experimental conditions is shown in Figure 1.

Only the control treatment (T0, without microorganisms) showed anthracnose damage during postharvest. Several replicates of this treatment (R1, R2, R3, and R7) exhibited brown and black necrotic lesions extending from the skin into the pulp, typical symptoms of *C. gloeosporioides,* which could compromise fruit commercial quality and shelf life. Figure 2 illustrates these internal damages.

The incidence analysis showed that only fruits from the T0 treatment (Control, without microorganisms) presented anthracnose symptoms, reaching 10 % incidence. In contrast, fruits from the other treatments with beneficial microorganisms showed no signs of the disease (Table 3). These results confirm the efficacy of *T. harzianum* and *P. fluorescens* for the control of phytopathogenic fungi in mango during postharvest.

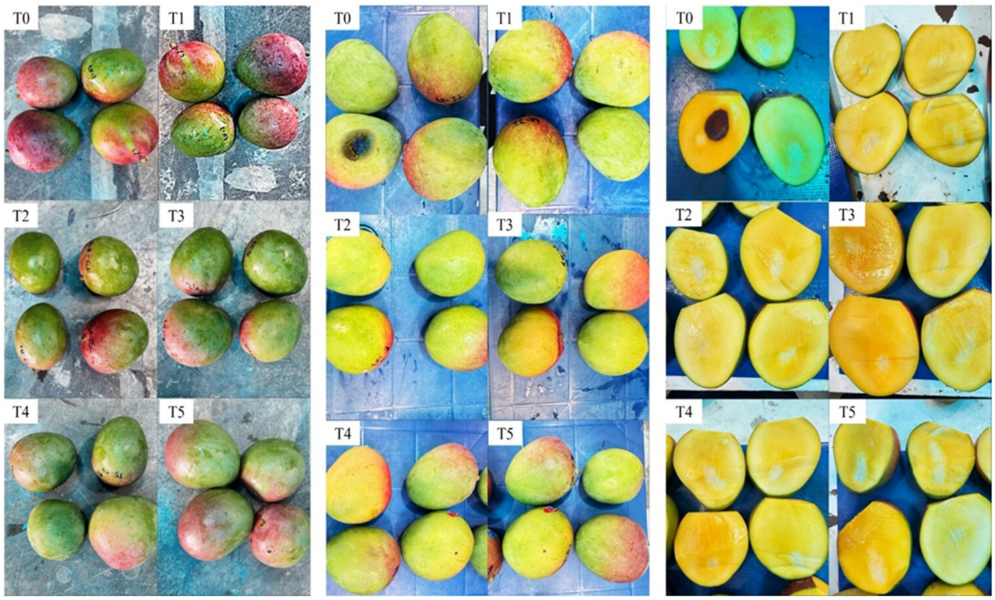
In contrast, fruits from the control treatment (T0) showed typical necrotic lesions of *C. gloeosporioides*, extending from the epidermis into the pulp, reflecting the action of the pathogen, which remains latent in the tissues until fruit ripening. According to some authors (16), the filamentous fungus *Trichoderma* forms a protective layer on the fruit surface and produces antifungal metabolites, acting as a biological barrier that inhibits the reproduction of pathogen spores, which explains the absence of symptoms in the treated fruits.

On the other hand, the treatment with *P. fluorescens* showed a notable reduction in external and internal severity, acting through nutrient competition and siderophore production. Even at a concentration of 1×10^7 CFU/mL, the bacterium was able to limit fungal progression. As stated by some researchers, *Pseudomonas* bacteria can induce systemic resistance in the fruit, strengthening cell walls and preventing the spread of pathogen hyphae into the pulp (17), which explains why treatments T1 and T2 maintained good internal quality of the mango during the four days postharvest.

Table 2. Summary of results of the molecular identification of microorganisms

Organism	Gene	Length	Identity	Accession	Coverage	E-value
Trichoderma harzianum	ITS	350 bp	100 %	KF624792	100 %	8e-162
Pseudomona fluorescens	16S	1.198 bp	100 %	MG977684	100 %	0.0
Colletotrichum gloeosporioides	ITS	560 bp	98 %	MH700455	97 %	0.0

ITS (Internal Transcribed Spacer), 16S (16S ribosomal RNA gene), and bp (base pairs). The accession numbers correspond to the species showing the highest similarity following comparison with the GenBank database using the BLAST tool



T0 (Control, without microorganisms), T1 (*P. fluorescens* 1×10⁷ CFU/mL), T2 (*P. fluorescens* 1×10⁸ CFU/mL), T3 (*T. harzianum* 1×10⁷ CFU/mL), T4 (*T. harzianum* 1×10⁸ CFU/mL), T5 (*P. fluorescens* + *T. harzianum* 1×10⁷ CFU/mL)

Figure 1. Effect of different inoculation treatments on ripening and anthracnose symptom development in mango fruits after four days of storage

T0 (Control, without microorganisms), R1 - R2 - R3 - R7 (Replicates)

Figure 2. Types of internal damage in mango fruit under the control treatment

Table 3. Anthracnose incidence in postharvest mango fruits under different beneficial microorganism treatments

Treatment	Total number of fruits	Fruits with symptoms	Incidence (%)
T0	40	4	10
T1	40	0	0
T2	40	0	0
T3	40	0	0
T4	40	0	0
T5	40	0	0

T0 (Control, without microorganisms), T1 (*P. fluorescens* 1×10⁷ CFU/mL), T2 (*P. fluorescens* 1×10⁸ CFU/mL), T3 (*T. harzianum* 1×10⁷ CFU/mL), T4 (*T. harzianum* 1×10⁸ CFU/mL), T5 (*P. fluorescens* + *T. harzianum* 1×10⁷ CFU/mL)

Additionally, the concentration of soluble solids (Brix degrees) in the pulp was evaluated, showing that the consortium of *P. fluorescens* + *T. harzianum* (T5) reached the highest value (15 °Brix), while treatments with individual microbial strains also showed increases compared to the control (10 °Brix) (Table 4).

Table 4. Soluble solids content (Brix degrees) of mango pulp

Treatment	Brix degrees
T0	10^{d}
T1	14^{ab}
T2	13^{b}
T3	13^{b}
T4	12^{c}
T5	15^{a}
S.E.	0.84
S^2	4.27

T0 (Control, without microorganisms), T1 (*P. fluorescens* 1×10^{7} CFU/mL), T2 (*P. fluorescens* 1×10^{8} CFU/mL), T3 (*T. harzianum* 1×10^{7} CFU/mL), T4 (*T. harzianum* 1×10^{8} CFU/mL), T5 (*P. fluorescens* + *T. harzianum* 1×10^{7} CFU/mL). S.E. (Standard error), S^2 (Variance)

This increase in soluble solids suggests that the microbial interaction not only protects the fruit from anthracnose but also enhances organoleptic quality and carbohydrate metabolism, promoting starch-to-sugar conversion during ripening. According to some researchers (18), microbial consortia can activate enzymatic processes that accelerate this conversion, potentially improving mango's commercial profile and market acceptance.

CONCLUSIONS

The use of the biological control microorganisms *T. harzianum* and *P. fluorescens*, whether as individual pure strains or as a consortium, ensures the defense mechanisms of the mango tree against anthracnose caused by *C. gloeosporioides*, providing protection to the fruit even after harvest, unlike the untreated control plantations.

The use of the microbial consortium (T5) *T. harzianum* + *P. fluorescens* not only supports the health status of the plants through the fruit but also improves fruit quality for commercialization by increasing soluble solids concentration (15 °Brix), thereby extending the shelf life of both the mango fruit and the crop.

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