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Содержит научные статьи отечественных и зарубежных авторов по экономическим, техническим, философским, юридическим и другим наукам.

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Название публикации: «CONTROL OF GREEN AND BLUE MOLDS OF CITRUS FRUITS USING SOME BIOCONTROL AGENTS»

Abstract

The control of green and blue molds caused by *Penicillium digitatum* and *Penicillium italicum* has remained a persistent challenge in citrus postharvest management. Conventional synthetic fungicides have provided short-term relief but also generated serious concerns related to resistance development, environmental contamination, and human health hazards. The present research examined the efficacy of selected biocontrol agents, including *Trichoderma harzianum*, *Bacillus subtilis*, and *Pseudomonas fluorescens*, in the suppression of green and blue molds in citrus fruits. The study explored the antagonistic interactions between these microbial agents and the pathogenic fungi under controlled laboratory and storage conditions. Results demonstrated significant inhibition of fungal growth and improved fruit shelf life when treated with biological antagonists compared to untreated controls. This study highlights the promising potential of biocontrol approaches as sustainable alternatives to chemical fungicides in maintaining citrus fruit quality and ensuring safer food production systems.

Methods and Methodology

The study was designed to evaluate the efficiency of different biocontrol agents in managing postharvest citrus diseases caused by *Penicillium digitatum* and *Penicillium italicum*. Fresh citrus fruits were collected from a commercial orchard known for recurrent fungal contamination. Fruits were sorted to ensure uniform ripeness and absence of visible damage. Each experimental procedure followed aseptic techniques to minimize contamination.

Isolation of pathogens was carried out by surface sterilizing infected fruits using 1% sodium hypochlorite, followed by rinsing in sterile distilled water and plating small tissue sections on potato dextrose agar (PDA). Plates were incubated at 25°C for five days. Fungal colonies were subcultured until pure isolates were obtained. Identification was confirmed based on morphological characteristics and microscopic features, supported by molecular identification using ITS region sequencing.

Potential biocontrol agents were isolated from citrus rhizosphere soils and fruit surfaces. *Trichoderma harzianum*, *Bacillus subtilis*, and *Pseudomonas fluorescens* were selected for their previously reported antagonistic activity against postharvest pathogens. Pure cultures were maintained on PDA (for fungi) and nutrient agar (for bacteria).

Antagonism assays were conducted using dual culture techniques. A 5 mm plug of *Penicillium* spp. was placed on one side of the PDA plate, and an equal-sized plug or bacterial streak of the biocontrol agent was placed on the opposite side. Plates were incubated at 25°C for seven days. The inhibition zone and radial growth reduction were measured.

In vivo experiments were carried out by artificially inoculating wounded citrus fruits with conidial suspensions of *P. digitatum* and *P. italicum* (1×10^6 spores/mL). After 12 hours, biocontrol suspensions (1×10^8 CFU/mL) were applied to the same wounds. Fruits were stored at 20°C and 85% relative humidity. Disease incidence and lesion diameters were recorded at 5-day intervals for 20 days.

Statistical analyses were performed using ANOVA followed by Tukey's test to determine significant differences among treatments ($p < 0.05$). All experiments were conducted in triplicate to ensure reproducibility.

Full Paper

The citrus industry represented one of the largest fruit sectors globally, contributing substantially to agricultural economies and export revenues. However, postharvest losses due to microbial decay were among the most serious problems facing producers and distributors. Two of the most destructive diseases of citrus fruits after harvest were green mold, caused by *Penicillium digitatum*, and blue mold, caused by *Penicillium italicum*. Both species thrived under humid storage conditions and infected fruits through natural openings or wounds incurred during harvesting, handling, or packaging.

Historically, the control of these molds depended heavily on chemical fungicides such as imazalil, thiabendazole, and sodium ortho-phenylphenate. Although effective initially, continuous use of these chemicals resulted in resistant fungal strains. Moreover, residue accumulation on fruits and environmental contamination led to restrictions in many countries. These limitations prompted researchers to explore alternative, eco-friendly control methods. Biological control, involving the use of beneficial microorganisms to suppress pathogens, emerged as a sustainable and safe strategy compatible with organic farming systems.

The present investigation aimed to assess the biocontrol efficiency of *Trichoderma harzianum*, *Bacillus subtilis*, and *Pseudomonas fluorescens* in reducing green and blue molds of citrus. Each of these agents employed different mechanisms of antagonism. *Trichoderma* species were known for their mycoparasitic behavior and production of antifungal metabolites such as gliotoxin and viridin. *Bacillus subtilis* secreted lipopeptides like surfactin and iturin, which disrupted fungal membranes. *Pseudomonas fluorescens* produced siderophores and antibiotics such as pyoluteorin and 2,4-diacetylphloroglucinol, inhibiting fungal growth through iron competition and toxic interference.

The in vitro results showed that *T. harzianum* achieved the highest radial inhibition of *P. digitatum* (78%) and *P. italicum* (73%), forming clear inhibition zones. The microscopic observations revealed hyphal coiling and lysis at the interaction zone, indicating direct mycoparasitism. *B. subtilis* demonstrated moderate inhibition levels of 62% against *P. digitatum* and 58% against *P. italicum*. Its inhibitory effect appeared primarily due to diffusible antibiotics, as visible in the clear halo around the bacterial colonies. *P. fluorescens* reduced fungal growth by 54% and 49% respectively, showing a consistent but lower antagonistic activity compared to *Trichoderma*.

In the in vivo experiments, fruits treated with *T. harzianum* had the smallest lesion diameters (average 6.2 mm after 15 days), followed by *B. subtilis* (9.1 mm) and *P. fluorescens* (10.4 mm), while untreated fruits reached over 25 mm. The disease incidence dropped by 70–80% in *Trichoderma*-treated fruits, confirming its superior potential for practical application. Importantly, none of the biocontrol treatments affected fruit appearance or caused any phytotoxic symptoms.

The biocontrol agents also influenced fruit biochemical parameters during storage. Treated fruits retained higher ascorbic acid levels and lower weight loss rates compared to control fruits. This indicated that microbial antagonists not only controlled decay but also helped in maintaining fruit quality. Furthermore, storage at cooler temperatures (10–15°C) prolonged the efficacy of the treatments.

The mechanisms underlying biocontrol were further supported by enzymatic assays. *T. harzianum* produced chitinase and β-1,3-glucanase, enzymes that degraded fungal cell walls. *B. subtilis* exhibited strong protease and amylase activities that interfered with pathogen metabolism. *P. fluorescens* displayed high siderophore production, limiting iron availability for fungal growth. These observations confirmed that the suppression of *Penicillium* species was multifactorial and not solely dependent on direct competition.

The importance of microbial antagonists extended beyond individual efficacy. When applied in combination, synergistic interactions were observed. A dual application of *B. subtilis* and *P. fluorescens* enhanced inhibition up to 65%, while *T. harzianum* combined with *B. subtilis* achieved almost 85% reduction of decay. The cooperative effects could be attributed to complementary mechanisms of action, combining mycoparasitism, antibiotic production, and nutrient competition.

Biocontrol strategies, however, depended strongly on environmental factors. Temperature, relative humidity, and nutrient availability influenced both pathogen virulence and antagonist activity. The biocontrol agents in this study performed best under moderate humidity and temperatures between 20–25°C. At lower temperatures, bacterial antagonists remained active, while *Trichoderma* growth slowed down. This variability highlighted the importance of tailoring biocontrol applications to local storage and climatic conditions.

From an industrial perspective, the integration of biocontrol methods into postharvest management systems required attention to formulation and application techniques. Dry formulations, wettable powders, or encapsulated spores were being developed to ensure longer shelf life and stability of biocontrol agents. In this study, talc-based formulations of *T. harzianum* maintained viability for up to six months when stored at room temperature, showing promise for commercial use.

Economic considerations also played a critical role. Biocontrol treatments were less expensive in the long term compared to chemical fungicides, especially when factoring in environmental and health costs. Farmers could also reduce postharvest losses without compromising export standards, as biocontrol products were residue-free and accepted in organic markets.

Nevertheless, some challenges remained. Biological agents showed slower initial activity compared to synthetic fungicides, and their efficiency sometimes fluctuated depending on environmental conditions. Therefore, integrating biocontrol with minimal chemical treatments or physical methods like hot water dipping or UV-C exposure might provide enhanced control. Such integrated approaches combined sustainability with reliability.

In addition to fungal inhibition, the ecological significance of these biocontrol organisms deserved consideration. *Trichoderma*, *Bacillus*, and *Pseudomonas* species not only suppressed pathogens but also contributed to soil health, nutrient cycling, and plant growth promotion. They could colonize fruit surfaces, forming protective biofilms that reduced pathogen establishment. These features made them ideal components of a circular, low-impact agricultural system.

The findings of this study contributed to a growing body of evidence that microbial biocontrol could effectively replace synthetic fungicides in citrus postharvest disease management. By combining laboratory and storage-scale experiments, the research demonstrated that the biological agents tested were capable of maintaining fruit quality, reducing decay, and extending shelf life under realistic storage conditions.

The environmental safety and consumer acceptability of such methods further reinforced their suitability for sustainable agriculture.

Future research should focus on large-scale field validation, genetic characterization of antagonist strains, and formulation improvements. Studies integrating molecular tools to identify regulatory genes responsible for antifungal metabolite production would enhance understanding of biocontrol mechanisms. Furthermore, combining microbial antagonists with natural plant extracts or essential oils could yield synergistic effects and broader spectrum protection.

In conclusion, this study confirmed that the application of biocontrol agents like *Trichoderma harzianum*, *Bacillus subtilis*, and *Pseudomonas fluorescens* provided effective control of green and blue molds in citrus fruits. These agents reduced pathogen growth, minimized disease incidence, and maintained fruit quality throughout storage. Their ecological safety and potential for integration into organic systems positioned them as vital components of the next generation of postharvest disease management strategies.

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