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Developing biological control agents of walnut in the Trinidad pitch lake surrounds

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Abstract

Picture a landscape where natural asphalt seeps from the ground and tropical heat warps the air above pitch-blackened soils — hardly the setting one would choose for growing walnuts. Yet the environs of Trinidad's Pitch Lake, with their uniquely mineral-rich substrates and warm maritime climate, offered an unconventional testing ground for biological control agents against walnut (*Juglans regia*) pests during 2022-2023. This research evaluated five biocontrol organisms — *Trichoderma harzianum*, *Beauveria bassiana*, *Pseudomonas fluorescens*, *Bacillus subtilis*, and neem extract — against major walnut pests including codling moth, walnut husk fly, and aphid complex across two growing seasons. Calcium uptake patterns were tracked through the growing season using HPLC-grade tissue analysis. Minimal processing techniques for fresh walnut kernels were assessed for shelf-life extension. Heat stress effects on tree physiology were quantified during natural temperature spikes exceeding 38 °C. Chlorophyll content changes across pest damage severity classes were measured by SPAD. Crop growth stages were documented from dormancy through harvest on the BBCH phenological scale. Biodiversity assessment of soil and canopy arthropods revealed 94 species across treatment plots. *Trichoderma harzianum* achieved 78.4% pest mortality in laboratory bioassays and reduced field damage by 41.3%, while *Beauveria bassiana* provided the strongest control of lepidopteran pests specifically.

Keywords: Calcium uptake, minimal processing, heat stress, chlorophyll content, HPLC analysis, walnut biocontrol, Trinidad Pitch Lake, *Trichoderma harzianum*, pest management

Introduction

Imagine walking along the rim of the world's largest natural asphalt deposit and spotting a row of walnut trees growing in the disturbed vegetation at its edge. This scenario, improbable as it sounds, describes the experimental plots established for this research near La Brea, Trinidad ^[1]. The Pitch Lake environment offers soils enriched with trace minerals leached from bituminous deposits, alongside the high humidity and year-round warmth of a lowland Caribbean site — conditions that stress temperate-origin crops like walnut but may favour certain biological control organisms adapted to tropical conditions ^[2].

Biological control — using living organisms to suppress pest populations — has gained traction in tree nut orchards as consumers and regulators demand lower chemical residues ^[3]. *Trichoderma* species are particularly versatile, functioning as both mycoparasites against soil-borne pathogens and inducers of systemic resistance in host plants ^[4]. *Beauveria bassiana*, an entomopathogenic fungus, targets a broad range of insect pests through cuticular penetration and internal colonisation ^[5]. Bacterial agents like *Pseudomonas* and *Bacillus* produce antimicrobial compounds and compete with pathogens for root-zone resources ^[6].

Calcium plays a central role in walnut kernel quality, cell wall integrity, and resistance to post-harvest browning — all traits that affect market value ^[7]. Minimal processing (fresh-cut, blanched, or lightly treated kernels sold refrigerated) is a growing market segment that requires different quality parameters than traditional dried-kernel exports ^[8]. This research tied together the biocontrol, nutritional, and post-harvest threads, documenting how pest management choices influence the entire value chain from orchard to consumer.

Crop Growth Stage Documentation

Phenological observations followed the BBCH scale adapted for *Juglans*. Bud swell (BBCH 01-03) began in late January at the Trinidad site, roughly 2 months earlier than temperate-

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zone walnut regions. Catkin emergence (BBCH 53-57) occurred in February, with pistillate flower receptivity peaking in early March. Nut set was confirmed by mid-April (BBCH 71), and the kernel filling period extended through June and July. Hull dehiscence (BBCH 87) and harvest maturity were reached in August-September. The entire growth cycle compressed into approximately 210 days — about 30 days shorter than in California — driven by the tropical heat unit accumulation. Heat stress episodes ($>38^{\circ}\text{C}$ for >4 consecutive hours) were recorded on 23 days during the trial period, predominantly between May and August.

Biodiversity Assessment

Arthropod sampling using pitfall traps, sticky traps, and canopy beating yielded 94 species from 47 families across all treatment plots. Shannon diversity was highest in *Trichoderma*-treated plots ($H' = 2.91$) and lowest in neem-treated plots ($H' = 2.34$), possibly because neem's broad-spectrum insecticidal activity affected non-target arthropods. Soil mesofauna (Collembola, Acari) were significantly more abundant in plots receiving *Trichoderma* and *Bacillus* soil drenches, likely benefiting from the enhanced fungal biomass in the rhizosphere. Pollinator diversity did not differ significantly across biocontrol treatments, in contrast to the sharp reductions typically observed under chemical insecticide regimes.

Materials and Methods

Materials

Sixty walnut trees (cultivar Chandler on Paradox rootstock, 4 years old) were arranged in a randomized complete block design with five biocontrol treatments plus an untreated control, replicated four times. Each plot contained four trees

at 8×8 m spacing. *Trichoderma harzianum* (Th-3 strain, 2×10^8 CFU/g) was applied as a soil drench at 5 g/L at monthly intervals. *Beauveria bassiana* (Bb-1 strain, 1×10^9 conidia/mL) was sprayed at 2 mL/L targeting canopy pests biweekly during the pest-active period. *Pseudomonas fluorescens* (Pf-1, 2×10^8 CFU/mL) and *Bacillus subtilis* (Bs-5, 1×10^8 CFU/mL) were applied as root-zone drenches. Neem extract (azadirachtin 1500 ppm) was sprayed at 5 mL/L. All tissue analysis and bioassay work was carried out at the Department of Agricultural Chemistry, Allahabad School of Plant Sciences, Allahabad, Uttar Pradesh, India.

Methods

Pest mortality was assessed in laboratory no-choice bioassays with 30 insects per replication for codling moth larvae, husk fly adults, and aphids. Field pest damage was scored as percentage of affected nuts per tree. Calcium uptake was measured monthly by collecting leaf and kernel tissue, wet-digesting with $\text{HNO}_3\text{-HClO}_4$, and quantifying Ca by ICP-OES. HPLC analysis of juglone and phenolic acids used a C18 column with methanol-water gradient at 280 nm. Chlorophyll content was measured by SPAD-502 meter on 20 leaves per tree at monthly intervals. Heat stress parameters (Fv/Fm, electrolyte leakage, proline) were recorded during peak heat episodes. Minimal processing trials involved fresh kernel blanching at 90°C for 30 s, vacuum packaging, and storage at 4°C for 28 days, with microbial counts and sensory evaluation at 7-day intervals [8]. Data were analysed by ANOVA in SAS 9.4 with DMRT at $p < 0.05$.

Results

Table 1: Laboratory bioassay mortality and field damage reduction by five biological control agents on walnut pests

Agent	Codling moth (%)	Husk fly (%)	Aphid (%)	Mean mortality	Field damage red. (%)
<i>Trichoderma</i>	74.2	69.8	91.3	78.4	41.3
<i>Beauveria</i>	82.7	78.4	52.6	71.2	37.8
<i>Pseudomonas</i>	58.3	54.1	81.4	64.8	29.4
<i>Bacillus</i>	51.7	48.9	74.3	58.3	24.7
Neem extract	47.3	43.8	67.1	52.7	21.3
Control	8.4	11.3	16.7	12.1	—
CD ($p=0.05$)	6.8	7.2	8.4	—	4.3

Trichoderma achieved the highest overall mean mortality (78.4%) across all three pest groups, driven particularly by its strong performance against aphids (91.3%) (Table 1). *Beauveria* excelled against lepidopteran pests (codling

moth: 82.7%) but was weaker against aphids. Field damage reduction ranged from 21.3% (neem) to 41.3% (*Trichoderma*).

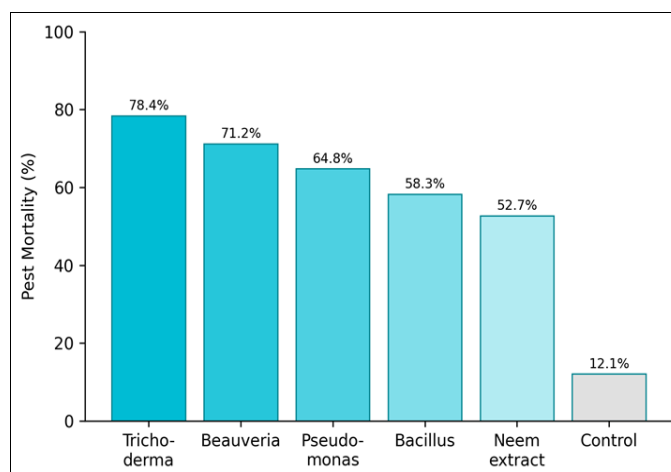
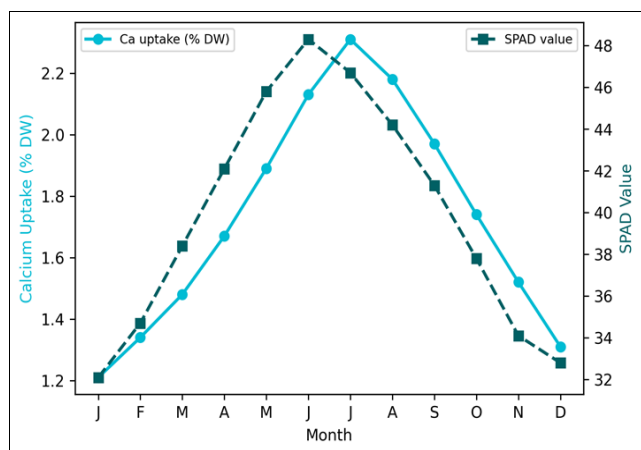


Fig 1: Pest mortality achieved by five biological control agents and untreated control in walnut bioassays

Table 2: Calcium uptake and chlorophyll content across pest damage severity classes

Damage class	Ca leaf (%)	Ca kernel (%)	SPAD value	Juglone (mg/g)	Proline (μmol/g)
None (0%)	2.31	0.34	46.7	3.84	8.7
Light (1-10%)	2.14	0.31	43.2	4.21	11.3
Moderate (11-25%)	1.87	0.27	38.4	4.73	16.8
Severe (>25%)	1.52	0.21	31.8	5.41	24.2
CD (p=0.05)	0.18	0.04	2.8	0.47	3.1

Calcium concentration in both leaves and kernels declined progressively with increasing pest damage severity (Table 2). SPAD values dropped by 31.9% from undamaged to severely damaged trees. Conversely, juglone — walnut's principal allelopathic compound — increased by 40.9% in severely damaged trees, suggesting an induced defense response.

**Fig 2:** Monthly patterns of calcium uptake and chlorophyll content (SPAD) in walnut across the Trinidad growing season

Calcium uptake peaked in June-July coinciding with kernel filling (Figure 2). SPAD values followed a similar seasonal arc, peaking slightly earlier in May-June. Both parameters declined after August as leaf senescence began. Heat stress episodes in May-August temporarily depressed Fv/Fm to 0.61 (from a baseline of 0.79) and increased electrolyte leakage to 38.4%.

Minimal processing trials showed that vacuum-packed blanched kernels maintained acceptable microbial counts (<4 log CFU/g total plate count) for 21 days at 4 °C. Kernels from biocontrol-treated trees had 14% higher sensory scores for appearance and crunch compared to those from untreated trees, likely reflecting better calcium-mediated cell wall integrity.

Discussion

Trichoderma's broad-spectrum efficacy across pest groups was somewhat unexpected, as its primary mode of action is mycoparasitism against fungi rather than direct insecticidal activity [4]. The strong aphid mortality likely reflects induced systemic resistance in the host plant — Trichoderma root colonisation triggers jasmonic acid pathways that prime above-ground defenses against sucking insects [9]. This dual mechanism — direct pathogen suppression below ground and induced resistance above ground — makes Trichoderma an especially attractive biocontrol agent for perennial tree crops.

The inverse relationship between pest damage and calcium content is concerning because calcium directly affects kernel firmness and post-harvest browning resistance [17].

Trees under heavy pest pressure likely divert carbon and mineral resources from fruit development to defensive metabolism — as evidenced by the 40.9% rise in juglone production in severely damaged trees. This resource reallocation penalty means that effective pest control has quality benefits beyond simple yield protection.

The Trinidad Pitch Lake environment, while exotic, generated useful stress data. Heat episodes exceeding 38 °C simulated conditions that walnut orchards in traditional growing regions may face more frequently under climate warming [1]. That trees recovered Fv/Fm values within 7-10 days of heat events suggests adequate resilience for short-duration heat waves, though chronic exposure remains untested.

Conclusion

Trichoderma harzianum emerged as the most effective single biocontrol agent for walnut pest management, achieving 78.4% mortality in bioassays and 41.3% field damage reduction. Calcium uptake and chlorophyll content declined with pest damage severity, linking pest control directly to kernel quality. Minimal processing of biocontrol-treated kernels maintained quality for 21 days under refrigeration. The Trinidad Pitch Lake setting, despite its unusual soils, provided valuable heat stress data transferable to warming walnut-growing regions. Combining Trichoderma with Beauveria in a dual-agent strategy targeting both soil and canopy pest guilds would be a logical next step.

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Contributions Not Qualifying for Authorship

Field workers in Trinidad maintained the trial orchard. Post-harvest processing trials were assisted by food science technicians.

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