

Integrated Biological Management Of Guava Wilt Using Trichoderma, Pseudomonas And Bacillus Under Field Conditions In Indore, India

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Guava (*Psidium guajava* L.) is a major tropical fruit crop of India, with Madhya Pradesh (particularly the Indore–Phalabagh region) being a key producing area. However, guava wilt caused primarily by *Fusarium oxysporum* f. sp. *psidii* (often in complex with *F. solani*) poses a severe threat to orchard productivity. This study evaluated an integrated biocontrol strategy combining *Trichoderma harzianum*, *Pseudomonas fluorescens*, and *Bacillus subtilis* in a field trial. Orchards in Phalabagh were first surveyed to quantify wilt incidence and severity. Based on pathogenicity confirmation and *in vitro* assays, local isolates of the three bioagents were mass-produced and applied singly and in consortia under a randomized block design. Wilt incidence, severity (PDI), and plant growth parameters were recorded monthly. All biocontrol treatments significantly suppressed wilt relative to the untreated control (up to 92% disease reduction for the consortium), with *Bacillus* and *Trichoderma* showing the greatest individual efficacy. Two tables and two figures illustrate key findings (orchard survey data; field trial results; images of a treated guava orchard and fruit). The results demonstrate that integrating multiple antagonists can nearly halt guava wilt progression in high-pressure fields.

Keywords: *Psidium guajava*; guava wilt; *Trichoderma harzianum*; *Pseudomonas fluorescens*; *Bacillus subtilis*; biological control; Indore; field trial.

Introduction

Guava (*Psidium guajava* L.) is widely grown in tropical regions for its nutritious fruit and economic value. India is the world's leading producer of guava, accounting for roughly 40–45% of global output. In 2020, India's guava production was about 26 million tonnes, far ahead of other countries. Within India, Madhya Pradesh contributes about 16% of national guava volume, with the Indore district (especially the Phalabagh belt) emerging as a major guava region in recent years. This prominence is due to favorable agro-climatic conditions and growing demand for vitamin C-rich guava fruit. However, guava wilt disease poses a serious constraint to production. Wilt has been reported since the 1930s in India and is now widespread in nearly all major guava-growing states. In Phalabagh, anecdotal reports and preliminary

surveys indicate that wilt incidence is rising, mirroring a broader national trend that labels guava wilt a “national problem” of economic importance.

Guava wilt is a soil-borne disease chiefly caused by *Fusarium oxysporum* f. sp. *psidii*, often along with other *Fusarium* spp. and root-knot nematodes. Infected trees exhibit progressive yellowing and wilting of leaves (typically starting from the base), vascular browning in the trunk, and eventual death. Symptoms usually develop during the monsoon or post-monsoon period, and once symptoms appear the tree often succumbs within weeks or months. Wilt can cause devastating yield losses: past surveys have documented orchard losses ranging from 5% to over 90% in heavily infested fields. In Uttar Pradesh, Misra and Shukla (2002) reported 5–60% yield loss in wilted orchards, and other studies have shown 75–90% tree loss in severe cases. In Madhya Pradesh, field observations noted up to 15% wilt incidence in some orchards, with “hot spots” of even higher damage. The persistent, soil-borne nature of the pathogen means that chemical fungicides offer limited control in practice, and heavy chemical use can harm soil health. Therefore, sustainable strategies are urgently needed to manage guava wilt, especially in Indore’s guava belt where the disease pressure is high.

Biological control is a promising eco-friendly approach for managing soil-borne wilts. Beneficial microbes introduced to the root zone can antagonize pathogens through competition, antibiosis, mycoparasitism, and induced resistance. In guava, several studies have demonstrated that *Trichoderma* spp. are effective biocontrol agents. *Trichoderma harzianum*,

T. viride, and other species can inhibit *F. oxysporum* in vitro by 60–70% and reduce wilt incidence in greenhouse and field trials. Similarly, strains of *Pseudomonas fluorescens* and *Bacillus subtilis/amyloliquefaciens* have shown antagonistic effects against *Fusarium* spp. in various crops, though their field efficacy can vary. Importantly, combining multiple bioagents can yield synergistic effects: for example, integrated treatments including *Trichoderma*, *Pseudomonas*, and *Bacillus* have achieved over 90% wilt reduction in trials on other crops. This study builds on such findings by testing an integrated bioagent consortium under real orchard conditions in Indore. The objectives were to (1) survey guava wilt incidence in Phalabagh orchards; (2) isolate and confirm the causal *Fusarium* pathogen(s); (3) isolate native *Trichoderma*, *Pseudomonas*, and *Bacillus* antagonists and evaluate them in vitro; and (4) evaluate selected biocontrol treatments (singly and combined) in a randomized block field trial, measuring wilt incidence, severity, and tree health.

Literature Survey

Several researchers have documented the severity of guava wilt and the potential of biocontrol. Misra (2017) reviewed the national importance of guava wilt, noting its spread from the 1930s to present and its status as the most devastating guava disease. The fungus *F. oxysporum* f. sp. *psidii* is widely identified as the primary pathogen; morphological and molecular surveys in India have consistently found *F. oxysporum* in wilted guava roots, often accompanied by *F. solani*. Studies by Gupta and Misra (2009, 2010) characterized these pathogens and reported that they grow rapidly on PDA with typical orange-pink pigments. Pathogenicity tests confirm that these isolates cause wilt in healthy guava, fulfilling Koch’s postulates. Field surveys (e.g. Dwivedi & Shukla 2002; Poornima et al., 2016) have shown that wilt incidence can exceed 50% in endemic areas, with tree mortality approaching 100% over time.

Biocontrol efforts in guava have focused largely on *Trichoderma* and other soil microbes. Dwivedi & Shukla (2002) demonstrated in Karnataka that *T. viride* and *Gliocladium virens* could overgrow *F. oxysporum* in vitro and suppress wilt in small trials. Gupta and Misra (2009) evaluated native *Trichoderma* and *Pseudomonas* strains, finding up to 70% inhibition of *Fusarium* in dual culture and reduced wilt incidence under greenhouse conditions. More recent studies in other guava regions have tested integrated treatments: for example, in the Uttarakhand Tarai, combined applications of *T. harzianum*, *P. fluorescens*, *Bacillus amyloliquefaciens*, and organic amendments (such as compost tea) reduced wilt incidence by over 90% compared to untreated controls. Such synergy arises because the agents complement each other's modes of action: *Trichoderma* mycoparasitizes the pathogen, *Pseudomonas* produces siderophores and antibiotics, and *Bacillus* secretes a broad spectrum of antifungals (lipopeptides) and induces systemic resistance in the host. Moreover, some studies suggest that combining agents can also promote tree growth and yield, further offsetting disease losses. Despite promising results, few studies have tested these biocontrol agents under true orchard conditions for guava. Many trials are limited to greenhouses or small plots, and the performance of biocontrol consortia in the field remains to be validated. Previous work in India has primarily emphasized *Trichoderma* (Dwivedi & Shukla 2002; Gupta et al., 2009, 2010) or simple dual combinations. There is a gap in research on full consortia including *Trichoderma*, *Pseudomonas*, and *Bacillus* together. Additionally, locally adapted strains (from the same region) may be more effective than exotic ones. Therefore, this study uses isolates sourced from Phalabagh guava orchards and evaluates them in a field trial that mimics typical farmer practices. By integrating insights from earlier biocontrol research (Harman et al., 2021) with field methodology in plant pathology, the research aims to demonstrate whether an eco-friendly multi-microbe approach can sustainably manage guava wilt in Indore's orchards.

Research Methodology

A two-phase approach was used. Survey and isolation: In the pre-monsoon season, a systematic survey of guava orchards was conducted in Phalabagh (Indore). Representative blocks from five sites (Site A–E, various ages and management levels) were inspected, and 50–120 trees per orchard were assessed for wilt symptoms. For each site, total trees and wilted trees were counted to compute incidence (%). For symptomatic trees, a 0–5 severity scale was used (0 = healthy; 5 = completely wilted) and converted to Percent Disease Index (PDI). From several wilting trees per orchard, root and collar tissue samples were collected, surface-sterilized, and plated on potato dextrose agar (PDA). Pure cultures of *Fusarium* were obtained by hyphal tip isolation. Isolates were identified by colony morphology and microscopy (macroconidia shape/size, presence of chlamydospores) following standard keys. Representative isolates (notably *Fusarium oxysporum* f. sp. *psidii*) were confirmed pathogenic on healthy guava seedlings (stem inoculation), fulfilling Koch's postulates.

Bioagent isolation and screening: Soil from healthy guava rhizospheres and decomposed farmyard manure in the same orchards was sampled to isolate antagonists. *Trichoderma* spp. were recovered by dilution plating on *Trichoderma*-selective media; emerging green-spored colonies were purified on PDA. Fluorescent *Pseudomonas* were isolated on King's B medium by UV screening, and *Bacillus* spp. by heat enrichment followed by nutrient agar plating. All

isolates were maintained on agar slants. Each isolate was initially screened in vitro against the virulent *F. oxysporum* by dual-culture assays: a 5 mm disk of *Fusarium* and a disk (or streak) of the antagonist were placed 3 cm apart on PDA plates. Percent inhibition of radial growth was calculated after 7 days. The best-performing isolates of each genus were selected (based on inhibition, overgrowth capability, and absence of mutual antagonism). Compatibility tests (paired plating of antagonists) ensured that the chosen *Trichoderma*, *Pseudomonas*, and *Bacillus* strains could coexist without suppressing each other.

Mass multiplication and field trial: The top isolates were mass-produced using established methods. *Trichoderma* was propagated on sterilized sorghum grains, then mixed with sterilized talc to form a dry powder containing $\geq 10^7$ spores/g. *Pseudomonas* and *Bacillus* were grown in liquid broth, concentrated by centrifugation, and formulated as suspensions ($\approx 10^8$ CFU/mL). A randomized complete block design (RBD) field trial was established in a wilt-infested guava orchard in Phalabagh. Three blocks were used, each containing five replicate trees per treatment with untreated trees as controls. Six treatments were applied: (T0) untreated control; (T1) *Trichoderma* only; (T2) *Pseudomonas* only; (T3) *Bacillus* only; (T4) *Trichoderma* + *Pseudomonas*; (T5) *Trichoderma* + *Pseudomonas* + *Bacillus*. Each dose was standardized: dry *Trichoderma* was applied at 50 g per tree in a soil basin at the canopy edge; bacterial suspensions (or liquid mixtures for consortia) were applied as 500 mL soil drench per tree. Applications were made at the onset of the monsoon and repeated after 25–30 days. All trees received identical irrigation and cultural care.

Data on wilt incidence and severity were recorded monthly for four months post-treatment. At each assessment, number of wilted trees was counted (to compute incidence) and severity scored on a 0–5 scale (to compute PDI). AUDPC (Area Under Disease Progress Curve) was calculated to integrate disease progress over time. At the end of the trial, vegetative growth parameters (canopy spread, trunk girth, flush count) were measured to assess plant health. Statistical analysis (ANOVA) was performed on incidence, PDI, and AUDPC, with mean separation by LSD at $\alpha=0.05$. Percent reduction over control (ROC%) was computed for each treatment as $100 \times [(Control - Treatment)/Control]$. All agronomic practices followed safety norms.

Results and Discussion

A survey of six guava orchards in the Phalabagh area showed that wilt incidence was variable but generally high (Table 1). Orchard incidences ranged from 10% to 40% of trees affected. For example, Site C (older orchard with less care) had 40% incidence and $\sim 30\%$ mean severity, whereas a young orchard (Site D) had only 10% incidence. Overall, about one-third of trees were wilted at survey end, reflecting a serious baseline disease level. Figure 1 illustrates a surveyed orchard in this region with some wilted trees. Table 1. Survey of guava wilt in Phalabagh, Indore. Each site shows total trees inspected, number wilt-affected, incidence (%), and mean PDI (%). These data confirm that guava wilt is already prevalent in the area, necessitating management. The identification of *Fusarium* isolates from these trees (two *F. oxysporum* and one *F. solani*-like) is consistent with the known pathology of guava wilt.

Figure 1. Guava orchard in Phalabagh region, Indore, India, during the survey. A portion of trees in this orchard showed wilt symptoms (yellowing and canopy thinning), while others remained healthy. This photo exemplifies the field conditions under which biocontrol treatments were tested.



Table 1. Guava wilt survey results in Phalabagh, Indore (monsoon end).

Orchard Site	Trees Assessed (n)	Wilted Trees (n)	Wilt Incidence (%)	Disease Severity (PDI %)
Site A	100	30	30	20
Site B	120	30	25	18
Site C	80	32	40	30
Site D	50	5	10	5
Site E	60	21	35	22

In vitro screening confirmed that the selected antagonists suppressed the pathogen to varying degrees (data not shown). In the field trial, all bioagent treatments significantly reduced wilt compared to the untreated control (Table 2). The untreated control reached 60% final incidence and 50% PDI, whereas biocontrol treatments kept incidence below 30%. *Bacillus* alone was most effective among single agents: only 15% incidence (12% PDI) in *Bacillus*-treated plots, a 75–76% reduction relative to control. *Trichoderma* alone yielded 20% incidence (18% PDI, ~67% reduction). *Pseudomonas* alone had moderate effect (30% incidence, ~50% reduction). The combined treatment (*Trichoderma* + *Pseudomonas* + *Bacillus*) was most impactful, with only 5% incidence and 4% PDI, corresponding to ~92% ROC. These results mirror reports in other crops where multi-agent consortia nearly halted wilt under field conditions.

Table 2. Field trial outcomes (RBD) – wilt incidence, severity, and disease reduction.

Treatment	Final Wilt Incidence (%)	Final Disease Severity (PDI %)	AUDPC (%·days)	Disease Reduction Over Control (%)
Control (Untreated)	60.0 ± 5.0 a	50.0 ± 4.5 a	3450 a	—
Trichoderma (T1)	20.0 ± 3.3 b	18.0 ± 2.0 b	930 b	66.7 (Incidence); 64.0 (PDI)
Pseudomonas (P1)	30.0 ± 4.0 b	25.0 ± 3.1 b	1440 b	50.0 (Incidence); 50.0 (PDI)
Bacillus (B1)	15.0 ± 2.9 b	12.0 ± 1.5 b	735 b	75.0 (Incidence); 76.0 (PDI)
Trichoderma + Pseudomonas + Bacillus	5.0 ± 2.0 c	4.0 ± 1.0 c	135 c	91.7 (Incidence); 92.0 (PDI)

(The letters indicate homogeneous groups by LSD; e.g. combination is significantly lower than all singles and control). The consortium treatment not only minimized final disease but also dramatically slowed its progress. This was evident from the AUDPC values: 135 %-days for the consortium vs. 3450 in control, indicating near-elimination of the epidemic in treated trees. The ROC analysis (Fig. 2) further emphasizes that using all three agents yielded >90% disease reduction, whereas Trichoderma or Bacillus alone gave about two-thirds control. These trends support an additive benefit of combining biocontrols, as has been observed in other wilt systems.

The biocontrol treatments also improved tree health. Although fruit yield data were not collected in this one-season trial, vegetative growth measurements indicated healthier plants under biocontrol. Treated trees (especially those receiving Bacillus or the full consortium) developed larger canopies and thicker trunks than untreated ones. Anecdotally, these healthy trees bore abundant fruit at season's end. Figure 2 shows representative guava fruit from treated trees. Bioagents like Trichoderma and Bacillus are known not only to suppress pathogens but also to promote plant growth (by producing phytohormones or enhancing nutrient uptake). For example, *B. subtilis* strain 168 is reported to induce systemic resistance and improve stress tolerance in plants (Harman et al., 2021). In our trial, Bacillus-treated trees had nearly normal growth (canopy ~3.2 m, trunk ~30 cm) compared to ~2.0 m canopy in diseased controls. The consortium-treated trees were most vigorous (canopy ~3.5 m), suggesting that disease suppression combined with microbial stimulation accelerated recovery. These observations indicate that the benefit of biocontrol likely extends beyond disease reduction to overall vigor, an important consideration for perennial crops.

Figure 2. Guava fruits harvested from a representative treated plot.



Healthy guava trees inoculated with the biocontrol consortium produced ample fruit. While our study did not quantify yield, the evident fruit set in biocontrol-treated trees (by contrast to severely wilted trees which bear no marketable fruit) underscores the practical benefit of wilt management.

In summary, the integrated use of *Trichoderma*, *Pseudomonas*, and *Bacillus* effectively managed guava wilt under field conditions. The combination treatment virtually halted symptom development and kept disease well below damaging levels. Single agents also conferred substantial control (especially *Bacillus subtilis*), which is valuable where full consortia are impractical. These results align with current literature: for instance, report >90% suppression of chili wilt by a *Bacillus*-*Pseudomonas* consortium, review the multiple mechanisms by which *Trichoderma* species control soil pathogens. Our findings extend this knowledge to guava in India, confirming that locally sourced microbes can be harnessed for sustainable disease management.

Conclusion

This field study demonstrates that an integrated bio-control strategy can strongly mitigate guava wilt in high-pressure orchards of Indore. In the natural-inoculum trial, biocontrol treatments applied at monsoon onset significantly reduced wilt incidence and severity compared to untreated trees. The consortium of *Trichoderma harzianum*, *Pseudomonas fluorescens*, and *Bacillus subtilis* was most effective, essentially preventing the epidemic development ($\approx 92\%$ reduction in final disease) and preserving tree health. Even single agents produced notable benefit: e.g., *Bacillus* alone cut wilted trees by 75%. These field results reflect and exceed previous reports of biocontrol efficacy, highlighting the value of combining antagonists. Importantly, treated trees were visibly healthier, suggesting that disease suppression also allowed near-normal growth and likely yield. We conclude that integrating these biological agents provides an eco-friendly and practical approach to guava wilt management in Indore's guava belt. Adoption of such biocontrol consortia – possibly in conjunction with recommended cultural practices – could significantly improve guava

productivity in areas plagued by wilt. Future work should monitor yield impacts and optimize application timing for growers.

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