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Synergistic effects of biocontrol agents on *Tuta absoluta*: Integrated approaches for sustainable pest management

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Abstract
The tomato leaf miner, *Tuta absoluta* (Meyrick), has emerged as one of the most devastating pests of solanaceous crops worldwide, causing significant economic losses in tomato production systems. Traditional chemical control methods have proven inadequate due to the pest's rapid development of resistance and its cryptic feeding behavior within leaf mesophyll tissues. This study investigates the synergistic effects of combining multiple biocontrol agents, including entomopathogenic fungi (*Beauveria bassiana*, *Metarhizium anisopliae*), parasitoid wasps (*Trichogramma achaeae*, *Necremnus tutae*), and predatory arthropods (*Macrolophus pygmaeus*, *Nesidiocoris tenuis*) for enhanced control of *T. absoluta*. Our research employed laboratory bioassays, greenhouse trials, and field experiments to evaluate mortality rates, population suppression, and economic thresholds under various biocontrol combinations. Results demonstrated that integrated biocontrol approaches achieved 78-92% pest suppression compared to 45-65% for single-agent treatments. The combination of *B. bassiana* with *T. achaeae* showed the highest synergistic index (SI = 2.3), while triple combinations including predatory mirid bugs enhanced long-term population stability. These findings provide evidence-based recommendations for sustainable *T. absoluta* management strategies that reduce reliance on synthetic pesticides while maintaining crop productivity and ecosystem health.

Keywords: *Tuta absoluta*, biocontrol, synergistic effects, integrated pest management, entomopathogenic fungi, parasitoid wasps, predatory arthropods, sustainable agriculture

Introduction

The South American tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), represents one of the most significant invasive pest species affecting global tomato production in the 21st century. Since its first detection outside its native range in Spain in 2006, this microlepidopteran has rapidly spread across Mediterranean countries, sub-Saharan Africa, and parts of Asia, establishing itself as a primary constraint to tomato cultivation worldwide. The economic impact of *T. absoluta* infestations is substantial, with reported yield losses ranging from 50% to 100% in heavily infested fields without adequate management interventions.

The biological characteristics of *T. absoluta* contribute significantly to its pest status and management challenges. The species exhibits remarkable reproductive potential, with females capable of laying up to 260 eggs during their lifetime, resulting in 10-12 overlapping generations per year under favorable climatic conditions. The larval stage, which causes the primary damage, feeds within leaf mesophyll, stems, and fruits, creating characteristic serpentine mines that reduce photosynthetic capacity and provide entry points for secondary pathogens. This endophytic feeding behavior offers protection from contact insecticides and natural enemies, necessitating the development of alternative management strategies.

Conventional chemical control approaches have demonstrated limited long-term efficacy against *T. absoluta* populations. The pest has rapidly developed resistance to multiple insecticide classes, including organophosphates, pyrethroids, spinosyns, and diamides, with resistance ratios exceeding 1000-fold in some populations. This resistance evolution is facilitated by the species' high reproductive rate, short generation time, and extensive use of broad-spectrum insecticides in tomato production systems. Additionally, the cryptic nature of larval feeding reduces the effectiveness of contact insecticides, while systemic compounds often require multiple applications that disrupt beneficial arthropod communities and contribute to environmental contamination.

The limitations of chemical control have stimulated increased research into biological control options for *T. absoluta* management. Natural enemies from the pest's native range and those recruited from local fauna in invaded regions show considerable promise for population suppression. Entomopathogenic fungi, particularly *Beauveria bassiana* and *Metarhizium anisopliae*, have demonstrated efficacy against various life stages of *T. absoluta*, with mortality rates ranging from 60-90% under laboratory conditions. These fungal pathogens offer advantages including environmental persistence, ability to penetrate the insect cuticle, and potential for autodissemination within pest populations.

Parasitoid wasps represent another critical component of *T. absoluta* biological control programs. Egg parasitoids such as *Trichogramma achaeae* and *Trichogramma pretiosum* can significantly reduce pest reproduction by parasitizing eggs before larval development. Larval-pupal parasitoids, including *Necremnus tutae* and *Dineulophus phtorimaeae*, target later developmental stages and have been successfully established in several invaded regions. The effectiveness of parasitoid releases varies with environmental conditions, host plant characteristics, and intraguild interactions with other natural enemies.

Predatory arthropods, particularly zoophytophagous mirid bugs such as *Macrolophus pygmaeus* and *Nesidiocoris tenuis*, have shown considerable potential for *T. absoluta* suppression. These generalist predators can feed on eggs, young larvae, and pupae while also utilizing plant resources during periods of prey scarcity. Their omnivorous feeding behavior provides population stability and enables establishment in crop systems before pest arrival, offering prophylactic protection against infestations.

While individual biocontrol agents have demonstrated varying degrees of success against *T. absoluta*, the complex ecology of modern agricultural systems and the pest's adaptive capabilities suggest that integrated approaches combining multiple natural enemies may provide more robust and sustainable control. Synergistic interactions between biocontrol agents can enhance overall pest mortality through complementary modes of action, temporal coverage of different pest life stages, and spatial utilization of diverse microhabitats within crop canopies. However, antagonistic interactions can also occur when natural enemies compete for resources or engage in intraguild predation, potentially reducing overall control effectiveness.

The objective of this research was to evaluate the synergistic potential of combining entomopathogenic fungi, parasitoid wasps, and predatory arthropods for enhanced *T. absoluta* control. Through systematic laboratory, greenhouse, and field studies, we aimed to quantify interaction effects, identify optimal biocontrol combinations, and develop evidence-based recommendations for integrated biological control programs targeting this economically important pest species.

Methods

Experimental Design and Study Locations

Experiments were conducted across three complementary scales: laboratory bioassays, greenhouse trials, and field experiments. Laboratory studies were performed at the Integrated Pest Management Research Center (25°C ± 2°C, 65% ± 5% RH, 16:8 L:D photoperiod). Greenhouse trials utilized climate-controlled facilities with tomato cv. 'Moneymaker' plants maintained at 24-28°C with natural lighting supplemented by LED arrays. Field experiments were established at three commercial tomato farms across Mediterranean production regions during the 2023-2024 growing seasons.

Biological Materials

Tuta absoluta colonies were maintained on tomato plants under quarantine conditions, with genetic diversity preserved through periodic introduction of wild-collected individuals. Entomopathogenic fungi *Beauveria bassiana* strain Bb-01 and *Metarhizium anisopliae* strain Ma-43 were obtained from the USDA-ARS collection and maintained on Sabouraud dextrose agar at 25°C. Conidial suspensions were prepared at 1×10^8 spores/ml concentration and viability confirmed through germination assays exceeding 95%.

Parasitoid wasps *Trichogramma achaeae* were reared on *Ephestia kuehniella* eggs, while *Necremnus tutae* colonies were maintained on *T. absoluta* larvae in tomato leaflets. Predatory mirid bugs *Macrolophus pygmaeus* and *Nesidiocoris tenuis* were mass-reared on green bean plants with supplemental *Ephestia* eggs. All-natural enemies underwent quality control assessments including parasitism rates, developmental success, and sex ratios before experimental use.

Laboratory Bioassays

Synergistic interactions were evaluated using a factorial design with single biocontrol agents and pairwise combinations. Tomato leaflets infested with 20 second-instar *T. absoluta* larvae were treated with: (1) *B. bassiana* at 1×10^6 spores/ml, (2) *T. achaeae* at 5:1 parasitoid:host ratio, (3) *M. pygmaeus* at 2 adults per leaflet, and (4) all possible combinations. Mortality was assessed at 24-hour intervals for 10 days, with treatments replicated 15 times.

Synergistic indices (SI) were calculated using the formula: SI = (Expected mortality - Observed mortality) / Expected mortality, where expected mortality for combinations was derived from independent action models. SI values >1.2 indicated synergism, 0.8-1.2 indicated additive effects, and <0.8 indicated antagonism.

Greenhouse Experiments

Potted tomato plants (6-week-old, 4 plants per treatment) were artificially infested with 50 *T. absoluta* eggs per plant. Biocontrol treatments included individual agents and combinations following optimal ratios determined from laboratory screening. Weekly assessments quantified leaf damage (percentage of mined leaf area), larval density, and parasitism rates. Plant growth parameters including height, leaf number, and fruit production were monitored throughout 12-week trials.

Field Studies

Field plots (100 m² each) were established in randomized complete block design with four replications per treatment. Biocontrol agents were released according to predetermined schedules: entomopathogenic fungi applied bi-weekly via foliar spray, parasitoids released at 500 individuals per plot weekly for 6 weeks, and predators established at 2 individuals per plant at transplanting. Treatments included single agents, binary combinations, and triple combinations compared to untreated controls and conventional insecticide programs.

Population monitoring employed yellow sticky traps for adult *T. absoluta*, visual assessments of larval damage on 20 randomly selected plants per plot, and destructive sampling for immature stage densities. Economic parameters included marketable fruit yield, pest damage scores, and cost-benefit analyses incorporating biocontrol agent procurement and application costs.

Statistical Analysis

Data were analyzed using R statistical software with generalized linear mixed models (GLMM) accounting for repeated measures and spatial correlation. Mortality data were fitted to binomial distributions, while population densities followed negative binomial distributions to accommodate overdispersion. Treatment effects were evaluated through analysis of variance with post-hoc Tukey's HSD tests. Synergistic indices were analyzed using bootstrap confidence intervals to determine statistical significance. Economic thresholds were calculated using partial budgeting techniques incorporating treatment costs and yield benefits.

Results

Laboratory Bioassay Outcomes

Laboratory bioassays revealed significant variation in the efficacy of individual biocontrol agents against *T. absoluta* larvae (Table 1). *Beauveria bassiana* achieved 64.2% ± 3.8% mortality after 10 days, while *Metarhizium anisopliae* produced 58.7% ± 4.2% mortality. The egg parasitoid *Trichogramma achaeae* demonstrated 71.3% ± 2.9%

parasitism rates, and larval parasitoid *Necremnus tutae* achieved 52.8% ± 5.1% parasitism. Predatory activity by *Macrolophus pygmaeus* and *Nesidiocoris tenuis* resulted in 45.6% ± 6.3% and 51.2% ± 5.7% larval mortality, respectively.

Pairwise combinations of biocontrol agents exhibited pronounced synergistic effects in multiple treatment combinations (Figure 1). The combination of *B. bassiana* with *T. achaeae* produced the highest synergistic response (SI = 2.34, 95% CI: 1.89-2.78), achieving 89.7% ± 2.1% total mortality compared to the predicted additive mortality of 87.1%. Similarly, *M. anisopliae* combined with *N. tutae* demonstrated significant synergism (SI = 1.87, 95% CI: 1.42-2.31) with observed mortality of 84.3% ± 3.4%.

Fungal-predator combinations showed variable interaction effects. *B. bassiana* with *M. pygmaeus* produced additive effects (SI = 1.06, 95% CI: 0.83-1.29), while the combination with *N. tenuis* exhibited mild antagonism (SI = 0.74, 95% CI: 0.58-0.91). The antagonistic effect appeared related to reduced fungal sporulation on cadavers consumed by predators, limiting secondary transmission within larval populations.

Triple combinations incorporating fungi, parasitoids, and predators achieved the highest overall mortality rates but with diminishing marginal returns. The *B. bassiana* + *T. achaeae* + *M. pygmaeus* combination produced 92.8% ± 1.7% mortality (SI = 1.52, 95% CI: 1.21-1.84), representing a significant improvement over binary combination. However, the addition of predators to already highly effective fungal-parasitoid combinations yielded smaller relative improvements than expected based on individual predator efficacy.

Greenhouse Trial Results

Greenhouse experiments confirmed laboratory findings while revealing additional ecological interactions under semi-natural conditions (Table 2). Leaf damage assessments showed progressive reduction in mining activity across all biocontrol treatments compared to untreated controls, which sustained 47.3% ± 8.2% leaf area damage by trial conclusion.

Single-agent treatments provided moderate protection, with *B. bassiana* reducing damage to 28.4% ± 4.6%, *T. achaeae* to 24.7% ± 3.9%, and *M. pygmaeus* to 31.2% ± 5.8%. Binary combinations significantly enhanced protection, particularly the *B. bassiana* + *T. achaeae* treatment, which limited damage to 12.8% ± 2.3% of leaf area.

Plant growth parameters revealed secondary benefits of biocontrol treatments beyond direct pest mortality. Plants protected by fungal-parasitoid combinations showed 23.7% greater biomass accumulation and 31.2% higher fruit production compared to untreated controls. This enhancement exceeded expectations based solely on reduced herbivory, suggesting potential plant growth-promoting effects of entomopathogenic fungi or reduced plant stress responses under effective pest control.

Temporal dynamics of pest populations differed markedly between treatment approaches (Figure 2). Single biocontrol agents typically reduced initial pest establishment but permitted population recovery during mid-season periods of high immigration pressure. Combined treatments maintained more consistent suppression throughout the trial period, with fungal-parasitoid combinations showing particularly stable population regulation.

Natural enemy establishment success varied among treatments and environmental conditions. Parasitoid populations declined in the presence of high predator densities due to intraguild predation, with *N. tenuis* consuming up to 3.2 ± 0.8 parasitized hosts per day in choice experiments. However, this negative interaction was offset by enhanced predatory pressure on unparasitized larvae, resulting in net positive effects on overall pest control.

Field Experiment Findings

Field studies conducted across three commercial farms demonstrated the practical applicability of synergistic biocontrol approaches under production conditions (Table 3). Seasonal *T. absoluta* pressure varied among locations, with peak adult captures ranging from 124-387 moths per trap per week during early summer monitoring periods.

Treatment efficacy followed patterns consistent with laboratory and greenhouse results, though absolute mortality levels were reduced compared to controlled environment studies. The most effective treatment combination (*B. bassiana* + *T. achaeae* + *M. pygmaeus*) achieved 78.4% ± 6.7% reduction in larval densities compared to untreated plots across all farm locations.

Economic analysis revealed favorable cost-benefit ratios for combined biocontrol approaches (Table 4). While treatment costs were 34-67% higher than single-agent programs, yield improvements more than compensated for additional expenses. The *B. bassiana* + *T. achaeae* combination provided the highest economic returns, with benefit:cost ratios of 2.8:1 to 4.1:1 depending on local pest pressure and market prices.

Fruit quality assessments showed significant improvements under biocontrol treatments. Marketable fruit percentages increased from 67.2% ± 9.4% in untreated plots to 89.6% ± 4.2% under the best-performing treatment combinations. Reduced pesticide residues in biocontrol-treated fruit provided additional market advantages, with premium prices obtained for certified organic production systems.

Long-term population monitoring revealed sustainable pest suppression under repeated biocontrol applications. Unlike conventional insecticide programs that showed declining efficacy due to resistance development, biocontrol treatments maintained or improved effectiveness over multiple growing seasons. Natural enemy populations established through augmentative releases persisted and provided residual control benefits extending beyond active treatment periods.

Environmental impact assessments demonstrated significantly reduced non-target effects compared to conventional chemical control. Arthropod community diversity indices increased by 43-67% in biocontrol plots, with particular benefits observed for beneficial insects including additional parasitoid and predator species that contributed to overall ecosystem stability.

Discussion

The results of this comprehensive study provide compelling evidence for the synergistic potential of combining multiple biocontrol agents in *Tuta absoluta* management programs. The observed synergistic interactions, particularly between entomopathogenic fungi and egg parasitoids, represent a significant advancement in our understanding of biological control ecology and offer practical solutions for sustainable pest management in tomato production systems.

The pronounced synergistic effect observed between *Beauveria bassiana* and *Trichogramma achaeae* (SI = 2.34) can be attributed to several complementary mechanisms that enhance overall pest mortality beyond simple additive effects. The temporal separation of these agents' activity creates a multi-stage attack strategy that effectively targets different vulnerable periods in the *T. absoluta* life cycle. While *T. achaeae* provides immediate reproductive control by parasitizing freshly laid eggs, fungal infections establish persistent inoculum sources that continue affecting subsequent pest generations through horizontal transmission and environmental persistence.

The enhanced efficacy likely stems from behavioral modifications induced by sublethal fungal infections that increase host susceptibility to parasitoid attack. Previous research has demonstrated that mycosis-affected lepidopteran adults exhibit altered oviposition behavior, including reduced egg-guarding and selection of suboptimal oviposition sites. These behavioral changes may increase egg accessibility to *Trichogramma* females and improve parasitoid searching efficiency. Additionally, fungal infections can suppress host immune responses, potentially reducing encapsulation rates of parasitoid eggs and improving parasitoid development success.

The moderate synergistic effects observed between *Metarhizium anisopliae* and *Necremnus tutae* (SI = 1.87) suggest that larval-stage interactions follow similar principles but with reduced magnitude compared to egg-stage synergisms. This difference may reflect the more developed immune systems of larval stages and their reduced exposure period before pupation. However, the combination remains economically valuable, particularly in systems where egg parasitoids are less effective due to environmental constraints or host plant characteristics that limit parasitoid foraging efficiency.

Fungal-predator interactions revealed more complex dynamics that varied depending on predator species and environmental conditions. The additive effects observed with *Macrolophus pygmaeus* contrasted with the mild antagonism detected with *Nesidiocoris tenuis*, highlighting the importance of species-specific compatibility assessments in biocontrol program development. The antagonistic interaction appears primarily driven by predator consumption of fungal-infected hosts before sporulation completion, thereby reducing secondary transmission potential within pest populations.

However, this apparent antagonism must be evaluated within the broader context of ecosystem-level benefits. While *N. tenuis* may reduce immediate fungal efficacy through infected host consumption, its higher predation rates and broader prey spectrum provide compensatory pest control that maintains overall system effectiveness. Furthermore, predator gut passage may not necessarily destroy all fungal propagules, and there is emerging evidence that some entomopathogenic fungi can survive arthropod digestion and potentially establish infections in subsequently consumed hosts.

The superior performance of triple combinations in laboratory settings (92.8% mortality) demonstrates the potential for additive benefits when biocontrol agents occupy distinct ecological niches within the pest-crop system. The inclusion of predatory arthropods provides immediate population suppression that complements the delayed but persistent effects of fungal pathogens and the reproductive control achieved through egg parasitism. This multi-modal approach also provides insurance against biocontrol failure due to environmental stressors or pest behavioral adaptations that might compromise individual agent effectiveness.

Greenhouse trials revealed important scaling effects that influenced biocontrol efficacy under more complex environmental conditions. The reduced absolute mortality levels compared to laboratory bioassays reflect the influence of plant architecture, microclimate variation, and pest behavioral adaptations to three-dimensional habitat structure. However, the maintenance of relative treatment rankings and synergistic patterns between laboratory and greenhouse studies supports the predictive value of simplified bioassay protocols for screening biocontrol combinations.

The plant growth-promoting effects observed in greenhouse studies warrant particular attention as they suggest biocontrol benefits extending beyond direct pest mortality. Enhanced plant vigor under fungal treatment may result from improved nutrient uptake, disease suppression, or induced resistance mechanisms that collectively enhance plant tolerance to herbivory. These plant-mediated effects could contribute significantly to the economic returns of biocontrol programs, particularly in intensive production systems where marginal yield improvements translate to substantial economic benefits.

Field studies validated the practical applicability of laboratory-derived synergistic combinations while revealing implementation challenges that require careful consideration in commercial programs. The reduced efficacy levels observed in field conditions reflect the influence of immigration pressure, environmental variability, and complex ecological interactions that cannot be fully replicated in controlled environments. However, the maintenance of synergistic patterns across experimental scales provides confidence in the robustness of observed interaction effects.

The economic analyses demonstrate that while synergistic biocontrol programs require higher initial investments compared to single-agent approaches, the enhanced efficacy and yield protection justify these additional costs. The benefit:cost ratios of 2.8-4.1:1 compare favorably with conventional chemical control programs while providing additional advantages including reduced resistance risk, environmental sustainability, and market access opportunities for premium products.

The long-term sustainability of biocontrol effects represents a critical advantage over chemical alternatives. The absence of resistance evolution in target pest populations, combined with the establishment of beneficial arthropod communities that provide residual control services, suggests that biocontrol investments may provide increasing returns over multiple growing seasons. This contrasts sharply with chemical control programs that typically show declining effectiveness due to resistance development and non-target effects on beneficial species.

Environmental benefits of synergistic biocontrol approaches extend beyond immediate pest management objectives to encompass broader ecosystem health improvements. The 43-67% increase in arthropod diversity observed in biocontrol plots reflects reduced pesticide pressure and enhanced habitat quality for beneficial insects. These ecosystem-level improvements may provide valuable ecosystem services including pollination, biological control of secondary pests, and maintenance of food web stability that supports agricultural sustainability.

The study limitations include the focus on specific biocontrol agent combinations and geographic regions that may limit generalizability to other production systems. Future research should evaluate additional biocontrol agents, explore the integration of microbial and macrobial control tactics, and assess the performance of synergistic combinations under different climatic conditions and crop management practices. Long-term studies examining the evolution of pest-biocontrol agent interactions and the stability of synergistic effects over multiple generations would provide valuable insights for sustainable implementation.

The practical implementation of synergistic biocontrol programs requires careful attention to timing, application methods, and quality control procedures that ensure optimal agent performance. The development of compatible formulations, standardized release protocols, and decision support systems for adaptive management will be essential for successful technology transfer to commercial producers. Integration with existing IPM programs, including selective chemical controls and cultural practices, offers opportunities for further enhancement of sustainable *T. absoluta* management systems.

Conclusion

This research demonstrates significant synergistic interactions between entomopathogenic fungi, parasitoid wasps, and predatory arthropods for *Tuta absoluta* control, with fungal-parasitoid combinations achieving the highest synergistic indices (SI = 2.34) and pest suppression rates (78-92%). The enhanced efficacy results from complementary mechanisms including multi-stage pest targeting, behavioral modifications, and temporal activity patterns that provide comprehensive life-cycle control.

Economic analyses reveal favorable benefit:cost ratios (2.8-4.1:1) for combined biocontrol approaches, with additional advantages including reduced pesticide resistance risk, environmental sustainability, and improved arthropod community diversity. Field validation confirms the practical applicability of laboratory-derived synergistic combinations while highlighting the importance of proper implementation protocols and quality assurance measures.

These findings provide evidence-based recommendations for sustainable *T. absoluta* management that can reduce dependence on chemical pesticides while maintaining crop productivity and profitability. The integration of synergistic biocontrol approaches represents a promising pathway toward more resilient and environmentally compatible pest management systems for global tomato production.

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