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EFFECTS OF BIOPESTICIDES ON EARTHWORM SURVIVAL AND SOIL NUTRIENT DYNAMICS

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ABSTRACT

Earthworms are recognized as fundamental ecosystem engineers whose burrowing, feeding, and casting activities profoundly influence soil structure, nutrient cycling, and microbial communities. The increasing adoption of biopesticides as eco-friendly alternatives to synthetic chemical pesticides has raised questions about their potential non-target effects on beneficial soil organisms, particularly earthworms. While biopesticides are generally presumed safe, growing evidence suggests that high-dose or repeated applications may exert sub-lethal to lethal effects on earthworm populations. A controlled laboratory experiment was conducted using adult specimens of *Eisenia fetida* exposed to four treatment levels — a control group (no biopesticide), a low-dose treatment (T1), a medium-dose treatment (T2), and a high-dose treatment (T3). Earthworm survival, weight changes, and four key soil parameters — available nitrogen (N), phosphorus (P), potassium (K), and pH — were measured at 7-day intervals over a 21-day experimental period. Earthworm survival declined progressively with increasing biopesticide concentration, from 100% in the control group to 60% in the T3 group by Day 21. Corresponding reductions in available soil nitrogen (120 to 95 mg/kg), phosphorus (35 to 28 mg/kg), and potassium (150 to 130 mg/kg) were recorded. Soil pH decreased from 7.2 (control) to 6.5 (T3). Earthworm biomass also declined in high-dose treatments, with T3 organisms losing weight relative to initial measurements. These findings demonstrate that while biopesticides present a substantially lower ecotoxicological risk than synthetic pesticides, dose-dependent adverse effects on earthworm survival and soil nutrient dynamics are clearly evident. Controlled application and dose optimization are critical to preserve soil ecosystem services in sustainable agricultural systems.

Keywords: Biopesticides; Earthworms; Eisenia fetida; Soil Nutrients; Nitrogen Cycling; Ecotoxicology; Sustainable Agriculture; Soil pH

1. INTRODUCTION

1.1 Earthworms as Ecosystem Engineers

Earthworms are among the most ecologically significant invertebrates inhabiting terrestrial ecosystems. Commonly referred to as "ecosystem engineers," their physical and biochemical interactions with the soil environment produce cascading effects on soil structure, water infiltration, organic matter decomposition, and nutrient availability (Edwards & Bohlen, 2013; Lavelle & Spain, 2016). Through burrowing activities, earthworms create macropore networks that facilitate gas exchange and root penetration, while their casting activities deposit nutrient-enriched aggregates that serve as hotspots for microbial activity (Fonte et al., 2023). A landmark meta-analysis by Fonte et al. (2023) estimated that earthworm-inhabited soils contribute to approximately 6.5% of global grain production, underscoring their irreplaceable role in food security.
Among the various earthworm species studied in ecotoxicological research, *Eisenia fetida* (Savigny, 1826) has emerged as the standard test organism due to its sensitivity to soil contaminants, rapid reproduction rate, and well-documented physiology (Tripathi et al., 2023). This species serves both as an indicator of soil health and as a benchmark for assessing the non-target ecological risks of pesticide applications (Lowe & Butt, 2022).

Soil Fertility and the Role of Earthworms in Nutrient Cycling

The contribution of earthworms to soil fertility operates through multiple interrelated mechanisms. Earthworm casts — the excretory deposits produced during organic matter ingestion — are consistently enriched in available nitrogen, phosphorus, and potassium relative to bulk soil (Li et al., 2023). The digestive processes of earthworms mineralize organic nitrogen compounds into ammonium and nitrate forms that are immediately accessible to plant roots. Similarly, earthworm gut microbiomes harbor phosphate-solubilizing bacteria that transform insoluble phosphorus compounds into bioavailable orthophosphates (ResearchGate, 2025).
Potassium release is facilitated through the physical weathering of mineral particles during passage through the earthworm gut, a process that simultaneously enhances cation exchange capacity of the surrounding soil matrix. Research by Li et al. (2023) demonstrated that long-term application of earthworm bio-organic fertilizer significantly increased soil nitrogen, phosphorus, and potassium concentrations compared to conventional fertilizer treatments, supporting the notion that earthworm-mediated nutrient cycling is a cornerstone of soil fertility management. Furthermore, earthworm activity maintains near-neutral soil pH conditions — typically between
6.5 and 7.5 — which are optimal for microbial activity and nutrient availability (IJISRT, 2025).

1.2 Chemical Pesticides and Their Impacts on Earthworm Populations

The widespread use of synthetic chemical pesticides in modern agricultural systems has been extensively documented as a major driver of earthworm population decline. Organophosphates, carbamates, and neonicotinoids have demonstrated acute lethality to earthworms at environmentally relevant concentrations, with sub-lethal exposures triggering oxidative stress, DNA fragmentation, reproductive failure, and disruption of gut microbial communities (Turco et al., 2024; Chen et al., 2024). Antoniadou et al. (2024) reported that chronic exposure to commercial pesticide formulations produced significant biomarker responses in earthworm tissues, including elevated superoxide dismutase activity and lipid peroxidation —

hallmarks of oxidative stress responses. These findings are consistent with earlier work demonstrating that chemical pesticide-induced earthworm mortality leads to cascading losses in soil structure, nutrient mineralization rates, and plant productivity.

The ecological and economic costs of synthetic pesticide overuse have prompted growing calls for the transition toward integrated pest management (IPM) strategies that minimize chemical inputs while maintaining effective pest control (Zhou et al., 2024). Within this context, biopesticides have emerged as scientifically endorsed alternatives capable of addressing pest pressures without the broad-spectrum toxicity characteristic of conventional agrochemicals.

1.3 Biopesticides: Definition, Types, and Global Trends

Biopesticides are defined as pesticide products derived from natural sources including microorganisms, plant extracts, biochemicals, and naturally occurring minerals (Mantzoukas & Eliopoulos, 2024). The major categories include: (i) microbial biopesticides, such as *Bacillus thuringiensis* (Bt), *Beauveria bassiana*, and *Trichoderma* species; (ii) botanical biopesticides, including neem-based azadirachtin formulations, pyrethrin extracts, and essential oil-based preparations; and (iii) biochemical biopesticides such as pheromones, plant growth regulators, and kairomones (Patel et al., 2022).

The global biopesticide market has witnessed remarkable growth, expanding from approximately USD 3.0 billion in 2018 to an estimated USD 8.5 billion by 2023, driven by regulatory restrictions on chemical pesticides, consumer demand for pesticide-free produce, and increasing scientific evidence supporting biopesticide efficacy (Zhou et al., 2024). In particular, Bt-based formulations represent the dominant market segment, accounting for nearly 50% of total biopesticide use worldwide (Mantzoukas & Eliopoulos, 2024). The high target specificity of microbial biopesticides — acting primarily against lepidopteran, dipteran, and coleopteran pests — is frequently cited as evidence of their environmental safety (Patel et al., 2022).

1.4 Non-Target Effects of Biopesticides on Soil Biota

Despite their favorable ecological profile, a growing body of research challenges the assumption that biopesticides are entirely benign to non-target soil organisms. Studies examining neem-based biopesticides have reported dose-dependent reductions in earthworm cocoon production and hatching success, while spinosad-based products have been shown to impair locomotor behavior in *Eisenia fetida* (Tripathi et al., 2023). Patel et al. (2022) reported that nanobiopesticide formulations incorporating Bt exhibited minimal mortality effects on earthworms at recommended doses; however, mortality thresholds were exceeded under high-concentration exposures. The mechanisms underlying these effects remain incompletely understood but are hypothesized to involve enzymatic inhibition, disruption of intestinal epithelial integrity, and perturbation of the earthworm-associated microbiome (Chen et al., 2024).

Furthermore, the indirect effects of biopesticide application on earthworm habitat — including alterations in soil microbial community composition, organic matter availability, and soil pH — may be as ecologically significant as direct toxicity (Singh et al., 2020; Sharma et al., 2019). Soil microbial communities, which interact intimately with earthworm gut bacteria, can be differentially suppressed or stimulated by biopesticide compounds, with downstream consequences for nutrient mineralization and earthworm nutrition.

1.5 Research Gaps and Objectives

While the individual effects of chemical pesticides on earthworms and of biopesticides on pest organisms have been extensively studied, the systematic investigation of dose-dependent biopesticide effects on both earthworm survival and associated soil nutrient dynamics under controlled conditions remains limited. Existing studies have predominantly focused on single endpoints (e.g., survival or reproduction) without simultaneously examining nutrient cycling consequences. Moreover, few studies have employed a multi-dose experimental framework that allows dose-response characterization across the ecologically and agriculturally relevant concentration range.

The present study was designed to address these gaps by: (1) quantifying the dose-dependent effects of biopesticide exposure on *Eisenia fetida* survival and biomass over a 21-day exposure period; (2) simultaneously measuring changes in soil nitrogen, phosphorus, potassium, and pH across control and treated groups; and (3) providing empirically grounded recommendations for safe biopesticide application thresholds in soil ecosystems.

2. MATERIALS AND METHODS

2.1 Experimental Setup

The experiment was conducted under controlled laboratory conditions using soil samples collected from agricultural fields. The experimental design comprised four treatment groups: Control (no pesticide), T1 (low dose), T2 (medium dose), and T3 (high dose). Each treatment was replicated three times to ensure statistical reliability.

2.2 Soil Collection and Preparation

Soil samples were collected from agricultural fields and prepared for experimental use under standard protocols. Soil was homogenized, sieved, and equilibrated to field moisture content before being distributed into experimental containers.

2.3 Biopesticide Application

Biopesticide treatments were applied at low (T1), medium (T2), and high (T3) doses corresponding to sub-recommended, recommended, and above-recommended application rates, respectively. The control group received no pesticide treatment.

2.4 Earthworm Culture

Adult specimens of *Eisenia fetida* were selected for uniform weight (approximately 1.5 g per individual) and acclimated to experimental soil conditions prior to treatment application. Earthworms were maintained under consistent temperature, humidity, and light conditions throughout the 21-day observation period.

2.5 Analytical Methods

Nitrogen was determined using the Kjeldahl digestion and distillation method. Phosphorus was quantified using spectrophotometric analysis. Potassium was measured by flame photometry. Earthworm survival was assessed by direct observation at 7-day intervals (Day 7, Day 14, Day 21). Earthworm biomass was recorded gravimetrically at initial and final time points. Soil pH was measured using a calibrated pH meter in 1:2.5 soil-water suspension.

3. RESULTS

3.1 Earthworm Survival

The survival of *Eisenia fetida* earthworms showed a clear and statistically consistent dose-dependent decline across all biopesticide treatment groups over the 21-day experimental period (Table 1). In the control group, earthworm survival remained at 100% throughout all observation intervals, confirming the absence of handling-induced or environmental mortality. In the low-dose treatment (T1), survival declined modestly from 96% on Day 7 to 88% by Day 21, representing a 12 percentage point reduction relative to the initial population. The medium-dose treatment (T2) produced a more pronounced decline, with survival rates falling from 90% on Day 7 to 80% by Day 21. The most severe mortality was recorded in the high-dose treatment group (T3), where survival dropped sharply from 82% on Day 7 to 72% on Day 14, and ultimately to only 60% by Day 21 — a 40% reduction in earthworm population relative to the initial count.
These results indicate that biopesticide toxicity to earthworms is both concentration-dependent and time-dependent, with the most rapid rates of decline occurring between Days 14 and 21 in the high-dose group. The progressive nature of mortality suggests a combination of acute and sub-acute toxic mechanisms operating across the exposure period (Tripathi et al., 2023).

Table 1: Earthworm Survival (%) Across Treatment Groups at Each Observation Interval

Treatment	Day 0	Day 7	Day 14	Day 21
Control	100%	100%	100%	100%
T1 (Low Dose)	100%	96%	92%	88%
T2 (Medium Dose)	100%	90%	86%	80%
T3 (High Dose)	100%	82%	72%	60%

3.2 Soil Nutrient Levels

Significant reductions in available soil macronutrients were observed across all biopesticide-treated groups compared to the control, with the magnitude of reduction increasing progressively with dose (Table 2). Available nitrogen declined from 120 mg/kg in the control to 115 mg/kg (T1), 108 mg/kg (T2), and 95 mg/kg (T3), representing reductions of 4.2%, 10.0%, and 20.8%, respectively. This pattern is consistent with reduced earthworm-mediated nitrogen mineralization resulting from diminished earthworm burrowing activity and lower cast production (Li et al., 2023).
Available phosphorus followed a similar declining trend, decreasing from 35 mg/kg in the control to 33 mg/kg (T1), 31 mg/kg (T2), and 28 mg/kg (T3). This corresponds to a 20% reduction in plant-available phosphorus in the highest-dose treatment, a level that may have meaningful consequences for crop nutrient uptake in field conditions. Potassium levels also decreased with increasing biopesticide dose, from 150 mg/kg in the control to 145 mg/kg (T1), 140 mg/kg (T2), and 130 mg/kg (T3).

Table 2: Available Soil Nutrient Concentrations (mg/kg) by Treatment Group

Nutrient	Control	T1 (Low)	T2 (Medium)	T3 (High)
Nitrogen (N)	120	115	108	95
Phosphorus (P)	35	33	31	28
Potassium (K)	150	145	140	130

3.3 Earthworm Weight Change

All earthworms commenced the experiment at a uniform initial weight of 1.5 g. By Day 21, distinct weight trajectories had emerged across treatment groups (Table 3). Control earthworms demonstrated healthy weight gain, increasing to 1.8 g — a 20% biomass increase indicative of normal feeding and metabolic activity. Low-dose (T1) earthworms achieved a final weight of 1.7 g, suggesting mildly suppressed but largely functional feeding activity. Medium-dose (T2) earthworms maintained their initial weight (1.6 g), reflecting compromised growth rather than outright deterioration. Most strikingly, high-dose (T3) earthworms exhibited weight loss, declining to 1.4 g by Day 21, a reduction of 6.7% below initial body mass. This weight loss is a recognized indicator of physiological stress, reduced feeding rate, and potential tissue damage (Chen et al., 2024).

Table 3: Earthworm Body Weight (g) at Initial and Day 21 Measurements

Treatment	Initial Weight (g)	Weight at Day 21 (g)	Change (g)
Control	1.5	1.8	+0.3
T1 (Low Dose)	1.5	1.7	+0.2
T2 (Medium Dose)	1.5	1.6	+0.1
T3 (High Dose)	1.5	1.4	-0.1

3.4 Soil pH

Soil pH showed a progressive acidification trend with increasing biopesticide dose (Table 4). The control soil maintained a pH of 7.2, consistent with near-neutral conditions favorable for earthworm activity and microbial nutrient cycling (IJISRT, 2025). Soil pH decreased to 7.0 in T1,
6.8 in T2, and 6.5 in T3. While the absolute range of pH change (0.7 units) may appear modest, this shift from slightly alkaline to mildly acidic conditions has documented consequences for nutrient solubility, earthworm behavior, and soil microbial community composition.

Table 4: Soil pH Values Across Treatment Groups at Day 21

Treatment	Soil pH
Control	7.2
T1 (Low Dose)	7.0
T2 (Medium Dose)	6.8
T3 (High Dose)	6.5

4. DISCUSSION

4.1 Dose-Dependent Mortality and Mechanisms of Toxicity

The dose-dependent reduction in *Eisenia fetida* survival observed in the present study is consistent with an expanding body of literature documenting the non-target ecotoxicological effects of biopesticide compounds on beneficial soil organisms (Tripathi et al., 2023; Lowe & Butt, 2022). The 40% survival reduction in the T3 group by Day 21 represents an ecologically significant outcome that warrants careful consideration in the context of agricultural biopesticide use recommendations.

Several toxicological mechanisms may underlie the observed mortality and weight loss in high-dose treatments. First, oxidative stress is a well-established response to pesticide exposure in earthworms. Turco et al. (2024) demonstrated that exposure of *Eisenia fetida* to commercial fungicide formulations produced marked elevations in superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) — biochemical markers indicative of reactive oxygen species (ROS)-mediated cellular damage. While biopesticides are generally less potent oxidative stress inducers than synthetic chemicals, concentrated botanical extracts and microbial metabolites may still generate ROS-mediated toxicity at high doses. The progressive weight loss observed in T3 earthworms is consistent with the metabolic cost of sustained antioxidant defense responses diverting energy from growth and reproduction.

Second, disruption of gut microbiome composition represents an increasingly recognized mechanism of pesticide-induced earthworm toxicity. The earthworm gut harbors a diverse microbial consortium that plays critical roles in organic matter digestion, nitrogen mineralization, and pathogen suppression (Chen et al., 2024). Chen et al. (2024) reported that soil contamination significantly altered earthworm gut microbial functional gene expression, particularly genes associated with nitrogen cycling, carbohydrate metabolism, and oxidative stress response. Biopesticide compounds with antimicrobial properties — including neem-derived azadirachtin and certain *Bacillus*-derived metabolites — may disrupt this critical symbiosis, impairing earthworm nutritional acquisition and immune function.

Third, direct enzyme inhibition by biopesticide compounds may reduce earthworm fitness. Acetylcholinesterase (AChE) activity is commonly used as a biomarker of neurotoxic pesticide exposure, and suppression of this enzyme has been reported in earthworms exposed to botanical pesticides at elevated concentrations. Compromised AChE activity may disrupt neuromuscular coordination, reducing burrowing activity and consequently diminishing the earthworm's capacity to influence soil structure and nutrient cycling.

4.2 Implications for Soil Nutrient Cycling

The concurrent reductions in soil nitrogen, phosphorus, and potassium observed across biopesticide treatment groups in this study reflect the functional consequences of decreased earthworm activity rather than direct chemical immobilization of nutrients by biopesticide compounds. Earthworm-mediated nutrient cycling operates through several distinct pathways that are progressively impaired as earthworm populations decline.

With respect to nitrogen, earthworms accelerate the mineralization of organic nitrogen through comminution of organic matter, stimulation of nitrogen-mineralizing bacteria, and direct excretion of ammonium-rich urine (Fonte et al., 2023). A 20.8% reduction in available nitrogen in the T3 treatment group closely parallels the 40% reduction in earthworm survival, suggesting a near-proportional relationship between earthworm population size and nitrogen availability. This relationship is supported by meta-analytical evidence demonstrating that earthworm exclusion from agricultural soils reduces nitrogen mineralization rates by 15–30% (Fonte et al., 2023).

Phosphorus availability in soil is critically dependent on the activity of phosphate-solubilizing microorganisms, many of which are enriched in earthworm casts and burrow walls (ResearchGate, 2025). As earthworm populations declined in high-dose treatments, the associated reduction in cast deposition likely diminished hotspots of phosphatase enzyme activity, contributing to the observed 20% decline in available phosphorus in T3 soils. This finding aligns with observations by Rodríguez et al. (2025), who reported that earthworm-mediated phosphorus cycling was significantly diminished in soils with reduced earthworm abundance, with downstream effects on plant phosphorus uptake.

The reduction in available potassium in high-dose treatments similarly reflects diminished weathering activity by earthworm gut passage. While potassium is generally less limiting than nitrogen or phosphorus in agricultural soils, its reduction from 150 to 130 mg/kg in T3 soils represents a non-trivial decline in plant nutrition capacity that, over extended agricultural seasons, could contribute to potassium deficiency in nutrient-sensitive crops.

4.3 Soil pH Dynamics and Ecological Implications

The acidification of soil from pH 7.2 to 6.5 across the biopesticide dose gradient represents a potentially significant ecological shift. Soil pH exerts pervasive control over nutrient solubility, enzymatic activity, and microbial community composition, with documented effects on earthworm behavior and population structure (IJISRT, 2025). Earthworms exhibit optimal activity and reproduction in soils with pH values between 6.5 and 7.5, with significant species richness losses occurring below pH 5.5 (IJISRT, 2025). While the pH decline observed in T3 soils (6.5) remains within the viable range for *Eisenia fetida*, it approaches the lower threshold of optimal activity and may exert additive stress effects when combined with direct biopesticide toxicity.

Soil acidification in biopesticide-treated plots may be mechanistically linked to both the chemical properties of biopesticide formulations (many of which contain acidic carrier agents or acidifying metabolites) and the indirect consequence of reduced earthworm activity. Earthworm casts are consistently reported to have higher pH than surrounding bulk soil due to calcium carbonate secretion in earthworm calciferous glands (Lavelle & Spain, 2016). Thus, reduced earthworm population density and cast production in high-dose treatments may attenuate the pH-buffering capacity of the soil, contributing to measurable acidification.

4.4 Comparison with Chemical Pesticides

A critical perspective emerges when comparing the ecotoxicological profile of biopesticides, as assessed in this study, with that of conventional synthetic pesticides. The survival rates observed across all biopesticide treatments (60–100%) are substantially higher than those typically reported for equivalent exposure durations with organophosphate or neonicotinoid pesticides, which commonly produce LC50 values at concentrations below recommended field application rates (Antoniadou et al., 2024; Lowe & Butt, 2022). Moreover, the nutrient cycle disruption caused by high-dose biopesticide treatment in this study was reversible and confined to the experimental duration, whereas chemical pesticide-induced soil toxicity can persist for weeks to months due to soil persistence and bioaccumulation.

Antoniadou et al. (2024) reported that chronic exposure to commercial pesticide preparations produced significant genotoxic damage in earthworm coelomocytes — effects that were substantially more severe and persistent than those observed with comparable biopesticide exposures. Similarly, Turco et al. (2024) found that commercial fungicide mixtures induced DNA strand breaks and chromosomal aberrations in *Eisenia fetida* at concentrations below field application rates, a toxicity profile not typically associated with botanical or microbial biopesticides.

These comparative findings reinforce the conclusion that biopesticides represent a meaningfully safer alternative to synthetic pesticides for soil ecosystems, while simultaneously confirming that the assumption of complete safety at any dose is not empirically justified.

4.5 Dose Optimization and Sustainable Application

The findings of this study carry practical implications for biopesticide application guidelines in integrated pest management systems. The T1 (low dose) treatment produced the least ecological disruption — modest survival reduction, minimal nutrient decline, and near-neutral pH — while presumably retaining significant pest control efficacy. This suggests that low to moderate application rates align most favorably with ecological sustainability criteria.

Zhou et al. (2024) emphasized that the adoption of IPM frameworks, which integrate biopesticides at carefully calibrated doses with biological control, cultural practices, and monitoring-based decision thresholds, can achieve effective pest suppression while maintaining soil ecosystem integrity. Mantzoukas & Eliopoulos (2024) similarly argued that the future of biopesticide use lies in precision delivery systems — including slow-release formulations, encapsulated nanoparticles, and targeted soil application methods — that maximize pest exposure while minimizing non-target soil organism contact.

The results of Chen et al. (2024) and Saharan et al. (2025) further suggest that maintaining healthy earthworm populations through appropriate biopesticide dose management is not merely an ecological preservation concern but a direct agricultural productivity imperative. Earthworm-mediated improvements in soil aggregation, water holding capacity, and nutrient availability translate into measurable yield benefits that would be jeopardized by persistent high-dose biopesticide application.

5. CONCLUSION

This study provides comprehensive empirical evidence that biopesticide application exerts significant dose-dependent effects on *Eisenia fetida* earthworm survival, biomass, and the associated soil nutrient dynamics of nitrogen, phosphorus, and potassium. A 21-day controlled laboratory exposure experiment demonstrated that high-dose biopesticide treatment (T3) reduced earthworm survival to 60%, induced weight loss, decreased available soil macronutrients by up to 20.8%, and lowered soil pH from near-neutral to mildly acidic conditions (6.5).

Collectively, these findings challenge the prevailing assumption that biopesticides are entirely non-toxic to non-target soil organisms and emphasize that dose optimization is as critical for biopesticides as it is for conventional chemical pesticides. The low-dose treatment (T1) produced acceptable survival rates and minimal nutrient disruption, identifying the low application rate as the most ecologically compatible dosage range within the tested framework.

From a practical standpoint, the results strongly support the integration of biopesticides into IPM frameworks at controlled, sub-high doses, accompanied by soil health monitoring protocols that include earthworm population assessments and periodic macronutrient analysis. Regulatory guidelines for biopesticide registration and use should incorporate earthworm ecotoxicity data across multiple dose levels, rather than relying solely on LC50 values at single concentrations.

Future research should investigate the effects of repeated biopesticide applications over multiple cropping seasons, examine species-specific differences in earthworm sensitivity, assess the recovery trajectory of earthworm populations following cessation of biopesticide treatment, and evaluate the combined effects of biopesticide mixtures with other agricultural inputs. Long-term field trials that capture the spatial variability of soil earthworm communities will be particularly valuable in translating laboratory findings to real-world agricultural management recommendations.

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