



Strengthening vegetable defense mechanisms against insect-pests and diseases through neem-enriched organic manures and beneficial soil microbe inoculation

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ABSTRACT

The overuse of inorganic fertilizers and pesticides for vegetable production has degraded soil fertility, reduced populations of beneficial microorganisms, and weakened the natural resistance of crops to biotic stress. As a result, crops have become more vulnerable to insect-pests and diseases, while human health and the environment also face significant risks. To overcome these challenges, adopting eco-friendly alternatives such as compost made from *neem* leaves, vermicompost, and utilising plant growth-promoting microorganisms is crucial. These sustainable practices not only enhance crop growth and yield but also naturally control insect-pests and diseases, promoting both environmental sustainability and human well-being. A long-term field experiment was conducted from 2019–20 to 2023–24 at the College of Agriculture, Sumerpur-Pali, to evaluate the effects of six different combinations of chemical fertilizers and *neem*-enriched organic manures, along with the inoculation of plant growth-promoting microorganisms (PGPMs), on the induction of systemic resistance in various vegetable crops. The results revealed that substituting chemical fertilizers with *neem*-enriched organic manure, combined with PGPMs inoculation, significantly enhanced the plants' natural defense mechanisms and reduced the incidence of insect-pests and diseases. Compared to conventional chemical fertilizers, insect-pest and disease damage was reduced by 75.15% in green peas, 74.33% in cowpeas, 77.84% in cluster beans, 72.93% in cabbage, and 69.22% in cucurbits. These findings highlight the crucial role of *neem*-based compost and beneficial soil microbes in promoting systemic resistance, reducing reliance on agrochemicals, and fostering both food safety and environmental sustainability.

Key words: Balanced nutrition, crop damage, disease infestation, resistance induction, plant-protection mechanism.

INTRODUCTION

Vegetable cultivation heavily depends on the application of agrochemicals and mineral fertilizers, which degrade soil health and contaminate the environment. These synthetic inputs deplete soil organic carbon, impair physical and chemical properties, and disrupt biological activity, leaving residues in crops and polluting soil, water, and air (Martin-Cardoso and San-Segundo, 6). Such practices are particularly harmful in developing countries, affecting crop yield, human health, and economic stability. High fertilizer doses, frequent irrigation, and protected cultivation lead to soft, disease-prone plants (Bhardwaj *et al.*, 2), while excess nitrogen and magnesium weaken plant immunity (Pandey and Ghimire, 8). Elevated nitrogen levels increase insect-pest reproduction and reduce plant resistance. Chemical pesticides, although common, cause environmental damage, health risks, and lead to resistant pathogens (Bhardwaj *et al.*, 3). Organic options like *neem* leaf compost, vermicompost,

and bio-inoculants improve soil structure, boost microbial diversity, and strengthen plant defenses by acting as physical barriers or triggering immune responses (Medhi *et al.*, 7). *Neem* leaves contain natural compounds such as azadirachtin, nimbacin, and salannin, which have insect-repellent, antifungal, and antibacterial effects against pathogens like *Ralstonia solanacearum*, *Fusarium oxysporum*, and *Alternaria alternata* (Sarawaneeyaruk *et al.*, 12). Vermicompost supplies nutrients, enhances water retention, and helps control pests and diseases (Sande *et al.*, 11). Plant growth-promoting rhizobacteria support plant growth and also defend against harmful pathogens. Strains of *Bacillus* species trigger systemic resistance, reducing disease severity and occurrence (Thakur *et al.*, 13). Organic inputs, such as green manure, biofertilizer mixtures, rock phosphate, and azolla, significantly reduce insect-pest and disease levels by increasing plant tolerance and activating systemic resistance (Medhi *et al.*, 7). Although *neem*-based organics and beneficial microbes individually contribute to pest and disease suppression, systematic studies examining their synergistic effects on plant defense activation and microbial-mediated resistance in vegetables are

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scarce. Understanding these interactions is essential for developing sustainable, residue-free pest and disease management strategies. Hence, the present investigation aimed to elucidate the role of *neem*-enriched organic manures in conjunction with beneficial soil microbes in strengthening vegetable defense mechanisms against insect-pests and diseases.

MATERIALS AND METHODS

A long-term field experiment was conducted at the College of Agriculture, Sumerpur-Pali (Rajasthan), India, to assess the impact of *neem* leaf-enriched organic manures with and without microbial inoculation on insect-pest and disease resistance in vegetable crops, compared to conventional inorganic fertilization. The study was carried out over five consecutive growing seasons (2019–20 to 2023–24) and included multiple vegetable crops such as green pea, cowpea, cluster bean, cabbage, and cucurbits. The experiment followed a Randomized Block Design (RBD) with six treatment combinations, and each was replicated four times. The treatment combinations are T_1 (100% RDF supplied by chemical fertilizers), T_2 (75% RDF by chemical fertilizers + 25% RDF by *neem* added organic manures), T_3 (50% RDF by chemical fertilizers + 50% RDF by *neem* added organic manures), T_4 (25% RDF by chemical fertilizers + 75% RDF by *neem* added organic manures), T_5 (100% RDF supplied by *neem* added organic manure), T_6 (100% RDF by *neem* added organic manures + PGPMs inoculation). Standard crop cultivation practices, including seed sowing, transplanting, irrigation, weeding, staking, and other intercultural operations, were performed promptly, following the recommended package of practices for Agro-Climatic Zone IIB of Rajasthan. Data were collected across all 24 plots for each crop from randomly selected ten plants of each treatment during the growing season. Observations on insect populations, insect damage, and disease intensity were recorded at 15-day intervals during years when infestations occurred. The percentage of affected leaves was calculated using standardized procedures outlined by Dabhi and Patel (4).

$$\text{Disease Incidence (\%)} = (\text{Number of infected plants} / \text{Number of plants assessed}) \times 100$$

$$\text{Insect damage (\%)} = \frac{\text{Number of damaged fruits/ leaves/ stems/ pods}}{\text{Total number of fruits/ leaves/ stems/ pods}} \times 100$$

Data from the years in which infestations occurred were pooled over the five-year experimental period and analyzed using analysis of variance (ANOVA) to determine significant differences among the treatments. The standard error of the mean (S.Em \pm) and the critical difference (C.D. at $p = 0.05$) were calculated to assess the statistical significance of treatment effects on insect and disease resistance.

Additionally, the percentage reduction in insect-pest infestation and disease incidence in the T_6 treatment was calculated relative to the T_1 treatment to evaluate the effectiveness of *neem*-enriched organic manures combined with PGPM inoculation compared to conventional chemical fertilization. All statistical analyses were conducted using JMP software (version 8, SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

The nutrient supply sources and soil microbial inoculation had a significant impact on enhancing the resistance of green peas (*Pisum sativum*) against major insect-pests and diseases (Table 1 and Fig. 1). Among insect-pests, leaf miner intensity showed a steady decline from 27.4% leaf damage in T_1 treatment to 11.19% leaf damage in T_6 treatment, resulting in a 59.16% reduction in damage. Pod borer infestation decreased markedly from 19.77% pod damage in T_1 treatment to 6.23% pod damage in T_6 treatment, reflecting a 68.49% reduction in pod infestation, while the aphid population was reduced drastically by 81.25%, from 52.17 insects per compound leaves to 9.78 insects per compound leaves. In terms of disease resistance, *Fusarium* wilt showed the most substantial improvement, with the incidence decreasing from 18.25% plants dying in the T_1 treatment to just 2.47% plants dying in the T_6 treatment, amounting to an 86.47% reduction in plant dying. Similarly, powdery mildew incidence dropped from 42.5% occurrence in the T_1 treatment to 8.34% occurrence in the T_6 treatment, representing an 80.38% reduction. Based on pooled data from 2020–21 to 2023–24, demonstrated that different nutrient supply sources significantly influenced the resistance of cowpea (*Vigna unguiculata* (L.) Walp.) to major insect-pests and diseases. Among insect-pests, legume pod borer damage decreased from 15.48% in the T_1 treatment to 5.18% in the T_6 treatment, reflecting a 66.54% reduction. Similarly, thrips population declined sharply by 72.30%, from 47.72 insects per plant in the T_1 treatment to 13.22 insects per plant in the T_6 treatment. In terms of disease resistance, anthracnose showed a 70.60% reduction from 25.44% leaf coverage in the T_1 treatment to 7.48% leaf coverage in the T_6 treatment. Powdery mildew incidence dropped dramatically by 84.50% from 35.16% infestation in the T_1 treatment to 5.45% infestation in the T_6 treatment. The incidence of *Fusarium* wilt declined significantly, with plant mortality decreasing from 16.20% in the T_1 treatment to 3.61% in the T_6 treatment, representing a 77.72% reduction. Likewise, nutrient management practices and PGPMs inoculation significantly enhanced the resistance of cluster beans (*Cyamopsis tetragonoloba*) plants to insect-pests and diseases. Jassid count dropped by

Table 1: Effects of nutrient supply sources on resistance level against insect and disease infestation in vegetables.

| Insect-pests/ Diseases | T ₁ | T ₂ | T ₃ | T ₄ | T ₅ | T ₆ | S. Em _± | C.D. | Per cent (p=0.05) decrease |
|---|----------------|----------------|----------------|----------------|----------------|----------------|--------------------|-------|-------------------------------|
| Green pea (<i>Pisum sativum</i>) pods | | | | | | | | | |
| Leaf miner | 27.40 | 26.17 | 22.37 | 18.90 | 15.23 | 11.19 | 0.484 | 1.440 | 59.16 |
| Pod borer | 19.77 | 18.25 | 16.70 | 15.37 | 13.40 | 6.23 | 0.783 | 2.327 | 68.49 |
| Aphids | 52.17 | 50.29 | 49.15 | 45.13 | 32.11 | 9.78 | 1.599 | 4.753 | 81.25 |
| <i>Fusarium</i> wilt | 18.25 | 17.99 | 16.50 | 13.17 | 9.30 | 2.47 | 0.454 | 1.351 | 86.47 |
| Powdery mildew | 42.50 | 42.11 | 40.38 | 35.70 | 20.30 | 8.34 | 0.739 | 2.197 | 80.38 |
| Cowpea (<i>Vigna unguiculata</i> (L.) Walp.) green pods | | | | | | | | | |
| Legume pod borer | 15.48 | 14.98 | 13.10 | 11.57 | 7.35 | 5.18 | 0.441 | 1.311 | 66.54 |
| Thrips | 47.72 | 45.19 | 42.14 | 39.50 | 25.89 | 13.22 | 0.804 | 2.390 | 72.30 |
| Anthracnose | 25.44 | 22.14 | 21.70 | 20.27 | 10.45 | 7.48 | 0.782 | 2.325 | 70.60 |
| Powdery mildew | 35.16 | 34.59 | 32.24 | 26.45 | 18.57 | 5.45 | 1.017 | 3.022 | 84.50 |
| <i>Fusarium</i> wilt | 16.20 | 14.26 | 12.25 | 10.15 | 7.47 | 3.61 | 0.428 | 1.272 | 77.72 |
| Cluster bean (<i>Cyamopsis tetragonoloba</i>) green pods | | | | | | | | | |
| Jassids | 9.35 | 9.13 | 8.77 | 7.23 | 6.25 | 2.21 | 0.357 | 1.061 | 76.36 |
| Whiteflies | 23.77 | 22.81 | 20.23 | 18.45 | 12.52 | 8.72 | 0.443 | 1.319 | 63.32 |
| <i>Fusarium</i> wilt | 18.98 | 17.41 | 12.27 | 9.17 | 5.24 | 3.77 | 0.452 | 1.344 | 80.14 |
| Bacterial blight | 46.55 | 42.15 | 32.28 | 28.15 | 17.45 | 7.90 | 0.860 | 2.557 | 83.03 |
| Powdery mildew | 57.75 | 55.25 | 52.16 | 47.68 | 20.63 | 7.88 | 1.269 | 3.770 | 86.35 |
| Cabbage (<i>Brassica oleracea</i> var. <i>capitata</i>) heads | | | | | | | | | |
| Diamondback moth | 17.7 | 16.23 | 15.26 | 13.20 | 8.24 | 6.23 | 0.290 | 0.862 | 64.80 |
| Cabbage head borer | 7.12 | 6.15 | 6.10 | 5.35 | 4.20 | 1.15 | 0.151 | 0.450 | 83.85 |
| Tobacco caterpillar | 22.15 | 20.20 | 18.35 | 15.23 | 11.37 | 5.28 | 0.382 | 1.136 | 76.16 |
| Head rot | 9.25 | 9.12 | 8.23 | 7.13 | 6.12 | 2.29 | 0.201 | 0.598 | 75.24 |
| Blackleg | 35.23 | 35.30 | 34.17 | 23.26 | 18.25 | 12.46 | 0.346 | 1.030 | 64.63 |
| Cucurbits | | | | | | | | | |
| Whiteflies | 35.77 | 34.80 | 30.28 | 28.43 | 22.64 | 15.45 | 0.755 | 2.243 | 56.81 |
| Leaf miner | 43.85 | 40.43 | 35.17 | 30.48 | 26.15 | 15.27 | 0.810 | 2.407 | 65.18 |
| Powdery mildew | 62.37 | 60.15 | 55.10 | 40.17 | 30.68 | 12.23 | 1.218 | 3.620 | 80.39 |
| Anthracnose | 12.15 | 11.35 | 10.25 | 7.72 | 5.22 | 3.15 | 0.162 | 0.483 | 74.07 |
| Bacterial wilt | 17.23 | 16.15 | 15.77 | 12.26 | 9.26 | 5.23 | 0.570 | 1.696 | 69.65 |

76.36%, from 9.35 insects/ compound leaves in the T₁ treatment to 2.21 insects/ compound leaves in the T₆ treatment, while whitefly incidence declined by 63.32% from 23.77 flies/ compound leaves in the T₁ treatment to 8.72 flies/ compound leaves in the T₆ treatment. *Fusarium* wilt incidence was reduced by 80.14% from 18.98% plants harmed in the T₁ treatment to 3.77% plants harmed in the T₆ treatment, and bacterial blight showed an even greater decrease of 83.03% from 46.55% loss occurrences in the T₁ treatment to 7.90% loss occurrences in the T₆ treatment. The most significant improvement was observed in powdery mildew, which the incidence decreased from 57.75% in the T₁ treatment to 7.88% in the T₆ treatment, marking

an impressive 86.35% reduction in infestation. The experimental results presented in Table 1 demonstrate that nutrient supply sources had a substantial impact on the incidences of major insect-pests and diseases in cabbage (*Brassica oleracea* var. *capitata*). Among the insect-pests, diamondback moth damage decreased significantly from 17.7% damage in the T₁ treatment to 6.23% damage in the T₆ treatment, reflecting a 64.80% reduction compared to the 100% recommended dose of fertilizers supplied through chemical fertilizers. Similarly, cabbage head borer intensity dropped sharply from 7.12% head damage in the T₁ treatment to 1.15% head damage in the T₆ treatment, resulting in an impressive 83.85%. Tobacco caterpillar infestation

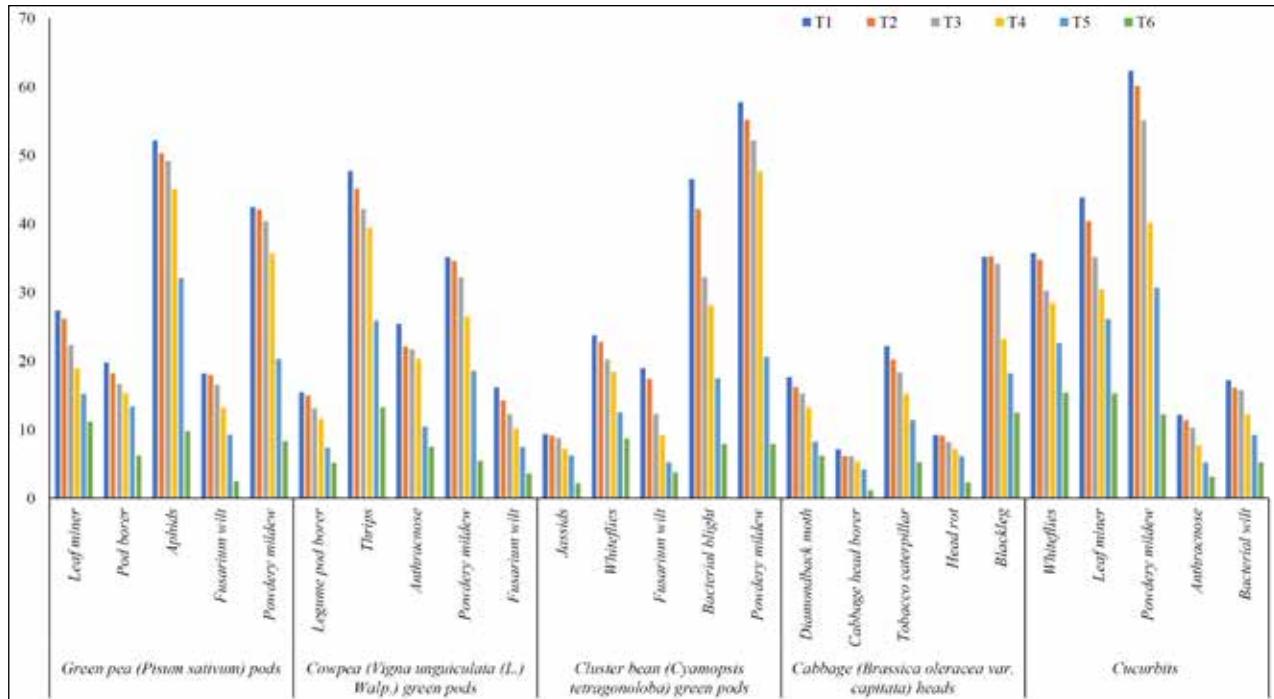


Fig. 1. Effects of nutrient supply sources on resistance level against insects and disease infestation in vegetables.

also showed a notable decline, from 22.15% damage in T₁ treatment to 5.28% damage in T₆ treatment, marking a 76.16% reduction in crop damage. The head rot disease incidence decreased from 9.25% head damage in the T₁ treatment to 2.29% head damage in the T₆ treatment (75.24% reduction), while blackleg disease declined from 35.23% occurrence in the T₁ treatment to 12.46% occurrence in the T₆ treatment, amounting to a 64.63% reduction. Similarly, the results for cucurbits indicated that *neem*-amended organic manure and PGPMs inoculation significantly reduce the incidence of major insect-pests and diseases. Whitefly intensity declined by 56.81%, from 35.77 insects/leaf in the T₁ treatment to 15.45 insects/leaf in the T₆ treatment, while leaf minor occurrence dropped from 43.85% damage in the T₁ treatment to 15.27% damage in the T₆ treatment, a 65.18% reduction. Regarding disease resistance, powdery mildew showed the most substantial decrease, falling from 62.37% occurrence in T₁ treatment to 12.23% occurrence in T₆ treatment, with a reduction of 80.39%, the highest among all parameters. Anthracnose incidence declined by 74.07% from 12.15% damage in the T₁ treatment to 3.15% damage in the T₆ treatment, while bacterial wilt showed a 69.65% reduction, dropping from 17.23% plants dying in the T₁ treatment to 5.23% plants dying in the T₆ treatment (Table 1). These results underscore the critical role of *neem*-enriched organic nutrient sources and microbial inoculants in enhancing crop

resistance and reducing insect-pest and disease pressure.

To manage insect-pests and diseases effectively, by integrating the application of organic manures (e.g., farmyard manure, vermicompost, poultry manure), natural pest-repelling inputs (e.g., *neem* leaf compost), and soil microbial consortia (e.g., PGPMs). These strategies improve soil health and nutrient availability, which in turn boosts plant vigour and natural resistance. Beneficial microbes also help by parasitizing or preying on pests, producing antibiotic compounds, competing for space and resources, and triggering systemic resistance in plants (Thakur *et al.*, 13). Similarly, combining green manure, enriched compost, azolla, vermicompost, *neem* cake, biofertilizer blends, and rock phosphate has been shown to significantly reduce stem borer, gall midge, and leaf folder damage in scented rice (Medhi *et al.*, 7). Overall, the results demonstrate that exclusive reliance on chemical fertilization significantly weakens a plants self-defence capacity and increases tissue succulence, conditions that favour the proliferation of insect-pests and pathogens. Plants suffering from nutrient deficiencies are generally more vulnerable to insect-pests and disease infestation, whereas balanced and adequate nutrition enhances a plants tolerance or resistance to pathogens. Proper nutrient balance is essential not only for maintaining plant vigour but also for influencing resistance to diseases

(Tripathi *et al.*, 15). Excessive nitrogen fertilization can also contribute to pest problems by increasing the birth rate, longevity, resistance development, and overall fitness of certain insect-pests (Medhi *et al.*, 7). The consistent decline in insect-pest populations and disease incidence across treatments underscores the vital role of *neem*-enriched organic manures in enhancing plant resistance through improved nutrient management and insect-repelling quality. Research has demonstrated that organic soil amendments can effectively suppress soil-borne diseases such as apple root rot, *Fusarium* crown rot in Chinese yam, and *Fusarium* wilt in cucumber, particularly when used with bio-organic fertilizers like pig manure or rapeseed (Zhang *et al.*, 19). Organic farming systems have also been associated with lower pest pressures, such as reduced stem borer infestations in rice (Paramasiva *et al.*, 9), primarily due to improved plant health and balanced nutrient availability (Tripathi *et al.*, 15). Among organic amendments, vermicompost is particularly effective due to its rich organic matter content, high nutrient levels, and diverse microbial populations. It enhances plant tolerance to both biotic and abiotic stresses (Sande *et al.*, 11). The disease-suppressive properties of vermicompost are largely attributed to its microbial communities, which outcompete or inhibit pathogenic organisms through natural antagonism (Tikoria *et al.*, 14; Zhang *et al.*, 20). Aslam *et al.* (1) observed a 21.7% reduction in aphid populations when cow dung-based vermicompost was applied alongside conventional N: P: K fertilization. The beneficial soil microbes, including bacteria such as *Bacillus subtilis* and *Pseudomonas fluorescens*, play a vital role in disease suppression and insect resistance through diverse mechanisms. These include the production of antibiotics, hydrolytic enzymes (e.g., chitinase, lipase), siderophores, and volatile organic compounds, as well as the induction of systemic acquired resistance and induced systemic resistance (Bhardwaj *et al.*, 2; Rashid and Chung, 10). Root colonization by these microbes triggers hormone signalling pathways, jasmonic acid, ethylene, and salicylic acid, which activate defense genes and promote the biosynthesis of flavonoids, lignin, glucosinolates, and other secondary metabolites that provide both chemical and structural defense against pathogens and herbivores (Rashid and Chung, 10). PGPMs also enhance plant growth and resilience by fixing nitrogen, solubilizing nutrients, modulating endogenous hormone levels (e.g., auxins, cytokinins, gibberellins), and improving water and nutrient uptake (Vacheron *et al.*, 16). This dual role of promoting plant vigor and enhancing defense responses enables faster recovery from pest damage and minimizes yield losses (Valenzuela-Soto *et al.*, 17). Specific microbes, such as *Bacillus subtilis*, have been shown to

produce more than 20 antibiotics effective against both bacteria and fungi (Maksimov *et al.*, 5). *Pseudomonas fluorescens* SS101 has been reported to induce resistance against both plant pathogens and insect-pests like *Spodoptera exigua* (van de Mortel *et al.*, 18). These microorganisms not only stimulate defense responses via JA-independent and JA-dependent gene expression but also improve photosynthesis and secondary metabolite pathways (Valenzuela-Soto *et al.*, 17). Overall, rhizospheric microbes are a powerful tool in sustainable crop protection, contributing to nutrient cycling, plant health, yield improvement, and natural pest and disease suppression.

The increasing costs and negative impacts of agrochemicals, such as pesticide resistance, health risks, food residue, and environmental harm, emphasize the urgent need for sustainable insect-pest and disease management in vegetable production. Healthy soil is crucial in this context, as it naturally suppresses harmful microbes and supports beneficial ones like *neem* leaf-enriched organic manures and plant growth-promoting microbes (PGPMs). The study found that using *neem*-enriched organic manure combined with PGPMs inoculation improves soil health, enhances nutrient availability, and significantly reduces insect-pest and disease levels in vegetable crops by boosting plant resistance and deterring pests. Conversely, crops grown with chemical fertilizers and excessive irrigation were more susceptible to infestations due to increased succulence. These findings highlight the benefits of integrating organic and microbial approaches with conventional methods to create balanced fertilization strategies. By leveraging organic manure, *neem* leaves compost, and beneficial microbes, it is possible to manage insect-pests more effectively while maintaining soil and environmental health. These insights are particularly valuable for guiding future agricultural policies and practices toward resilient and eco-friendly crop production.

AUTHORS' CONTRIBUTION

Investigation and conceptualization of the research (RLB, PM, AS); Designing of the experiment (RLB); Scientific observations during experimentation (RLB, PM, AS); drafted the paper (RLB); Writing and editing (RLB, PM). All authors have read and agreed to the published version of the manuscript.

DECLARATION

The authors declare no conflict of interest.

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