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Effects Of Various Treatments On Growth, Vitamin Content, And Catalase Activity In Clitoria Ternatea: A Comparative Study

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Abstract
Clitoria ternatea, commonly known as butterfly pea, is a perennial herbaceous plant belonging to the Fabaceae family. The present study examines the effects of various treatments viz., *Trichoderma viride*, Pongamia pinnata, cow dung, *Trichoderma agriderma*, and Agrimonas on the growth of *Clitoria ternatea* and the analysis of Vitamin A, Vitamin C, and catalase activity in the treated samples. *Clitoria ternatea* plants were sourced from Lalbagh Botanical Garden, Bengaluru, and cultivated at Gangodanahalli, Bengaluru. The experiment involved three replicates per treatment, with applications administered two days post-planting and assessments conducted at 30, 60, and 90 days. The results demonstrated that all treatments except *Trichoderma agriderma* significantly enhanced plant growth. The mixture of all treatments as well as Agrimonas alone, resulted in the greatest increases in shoot and root length, along with a higher number of branches. Pongamia pinnata combined with cow dung promoted shoot length and branch number but reduced root length. *Trichoderma viride* and *Trichoderma agriderma* treatments increased branch numbers but reduced root length, with *Trichoderma agriderma* also decreasing shoot length. Additionally, qualitative and quantitative analysis confirmed the increase in Vitamin A, Vitamin C, and catalase activity in all treated samples. This study highlights the potential of these treatments, particularly Agrimonas, in enhancing the growth of *Clitoria ternatea* and influencing its antioxidant properties and enzyme activities.

Keywords: *Clitoria ternatea*, *Trichoderma viride*, *Pongamia pinnata*, Cow dung, *Trichoderma agriderma*, Agrimonas, Vitamin A, Vitamin C, Catalase activity

Introduction

Clitoria ternatea, commonly known as butterfly pea, is a perennial herbaceous plant belonging to the Fabaceae family. It is native to tropical equatorial Asia but has spread widely across the world, particularly in Southeast Asia, Africa, and parts of Central and South America. The plant is easily recognized by its vibrant, deep blue flowers, although white and purple varieties also exist. The plant is a vigorous climber, often growing over shrubs and small trees. It has slender stems that can reach up to 3 meters in length, with green, oblong leaves arranged in pairs. The most distinctive feature of *Clitoria ternatea* is its large, showy flowers, which resemble the shape of a butterfly, hence the name "butterfly pea." The flowers typically bloom singly and have five petals, with the uppermost petal, called the "standard," being the most prominent. The flower's deep blue color is due to the presence of anthocyanin pigments, which also contribute to its use as a natural dye. In traditional medicine, *Clitoria ternatea* has been used for centuries in Ayurvedic and Chinese medicine systems. It is revered for its wide range of therapeutic properties. The roots, seeds, and flowers are utilized in various forms, including powders, teas, and extracts. In Ayurveda, the plant is known as Shankhpushpi and is believed to improve memory, reduce stress, and act as a natural anti-depressant. The roots are considered to have diuretic, laxative, and purgative effects, while the flowers are often used to treat eye infections, sore throats, and skin conditions. One of the most popular uses of *Clitoria ternatea* flowers is in cooking. In Southeast Asian cuisine, particularly in Thailand and Malaysia, the blue flowers are used to color food and drinks. The flowers are steeped in hot water to extract a vibrant blue color, which is then used to dye rice, desserts, and beverages. When mixed with acidic substances like lemon juice, the blue extract turns a bright purple, making it a favorite for creating visually striking dishes. A popular drink made from butterfly pea flowers is Nam Dok Anchan in Thailand, which is often served with honey and lemon. In recent years, *Clitoria ternatea* has attracted considerable attention from the scientific community due to its potential health benefits. Research has shown that the plant possesses significant antioxidant, anti-inflammatory, and antimicrobial properties. The presence of compounds like ternatins, kaempferol, and quercetin contributes to these effects. Additionally, studies suggest that extracts from the plant may help in controlling blood sugar levels, making it of interest for managing diabetes. There is also growing interest in its potential neuroprotective effects, with studies indicating it may help in the prevention of cognitive decline and neurodegenerative diseases.

Trichoderma viride is widely used in agriculture not only as a biocontrol agent but also as a biofertilizer. This fungus interacts beneficially with plant roots, improving nutrient availability in the soil by solubilizing phosphorus and other micronutrients, making them more accessible to plants. This is particularly important in nutrient-poor soils or in situations where fertilizers have been applied, as the fungus aids in the mineralization of organic matter, releasing essential nutrients that plants can readily absorb. Additionally, *T. viride* is known to stimulate root development, leading to a more extensive root system that enhances the plant's ability to absorb water and nutrients. The production of plant hormones such as auxins by the fungus contributes to this improved root growth, which in turn supports healthier and more robust plants. In addition to promoting nutrient uptake and root growth, *Trichoderma viride* also helps plants tolerate various abiotic stresses such as drought, salinity, and temperature extremes. This is partly due to the fungus's ability to improve soil water retention and modulate plant hormone levels, thereby reducing stress on the plants. As a result, plants treated with *T. viride* often show improved resilience to environmental challenges, which is a significant advantage in agriculture.

Trichoderma agriderma, commonly *Trichoderma harzianum*, shares many of the beneficial properties of *T. viride* but is particularly noted for its strong root colonization abilities and its effectiveness across a broader range of environmental conditions. When used in conjunction with fertilizers, *T. harzianum* works synergistically to enhance nutrient uptake by plants, leading to better crop yields. This fungus can release nutrients that are otherwise bound in the soil or organic matter, making them more available to plants. Additionally, *T. harzianum* produces compounds that act as biostimulants, including plant hormones and enzymes that further aid in nutrient availability and overall plant health. One of the most significant benefits of *Trichoderma harzianum* is its ability to protect plants from soil-borne diseases. By colonizing the root zone, the fungus forms a protective barrier against pathogenic fungi and other harmful microorganisms, effectively reducing the incidence of diseases that can otherwise thrive in the nutrient-rich environments created by fertilizers. This disease suppression capability makes *T. harzianum* an essential tool for maintaining plant health, especially in intensive agricultural systems.

When incorporated into fertilizers, both *Trichoderma viride* and *Trichoderma harzianum* are typically formulated as spores or conidia in various carrier mediums, including granules, powders, and liquid formulations. These products can be applied directly to the soil, mixed with seeds, or used in foliar sprays, depending on the specific agricultural needs. For instance, granular formulations are ideal for row crops and horticultural applications, where they release the fungi gradually, providing prolonged protection and growth enhancement. In contrast, liquid biofertilizers are useful for quick colonization of the root zone and immediate benefits to the plants. The integration of Trichoderma species into agricultural practices represents a key strategy in reducing the reliance on chemical pesticides and fertilizers. By enhancing plant growth, improving nutrient uptake, and protecting crops from pathogens, these fungi help create a more balanced and resilient soil microbiome. This contributes to sustainable farming practices that are less harmful to the environment, promoting long-term agricultural productivity and soil health.

The combination of cobalt, copper, iodine, iron, magnesium, manganese, potassium, sodium and sulfur in plant fertilizers provides a comprehensive range of nutrients that support various physiological processes essential for plant growth and development. These elements, although required in different quantities, contribute collectively to the overall health, productivity, and resilience of plants. Cobalt (Co) is a trace element that, while needed in very small amounts, plays a crucial role in the nitrogen fixation process, particularly in leguminous plants. Cobalt is essential for the production of vitamin B12, which indirectly supports nitrogen metabolism in plants. When included in fertilizers, cobalt helps legumes efficiently convert atmospheric nitrogen into a form that plants can use, promoting better growth and higher yields in crops like beans, peas, and clover. Copper (Cu) is another micronutrient that is vital for several key processes in plants. It is involved in photosynthesis, respiration, and the formation of lignin, which strengthens plant cell walls. Copper also activates various enzymes and is important in protein synthesis. A deficiency in copper can lead to poor plant growth, reduced yields, and increased susceptibility to diseases. Therefore, the presence of copper in fertilizers ensures that plants maintain strong structural integrity and efficient metabolic functions. Iodine (I), though not traditionally considered an essential nutrient for most plants, has been shown in some studies to contribute to plant growth and stress resistance. Its role may include enhancing the plant's ability to cope with environmental stresses such as salinity or drought. Iodine in fertilizers can also benefit soil health by contributing to a balanced microbial environment, which in turn supports plant health. Iron (Fe) is a critical component of chlorophyll, the molecule responsible for capturing light energy during photosynthesis. Iron is also involved in various enzyme functions and electron transport within plant cells. A deficiency in iron often results in chlorosis, where the leaves turn yellow due to insufficient chlorophyll production. Including iron in fertilizers helps ensure that plants maintain their green color and photosynthetic efficiency, which is crucial for energy production and overall growth. Magnesium (Mg) is central to the chlorophyll molecule and is therefore essential for photosynthesis. It also plays a role in enzyme activation and stabilizing ribosomes during protein synthesis. Without adequate magnesium, plants cannot effectively capture and convert sunlight into energy, leading to stunted growth and poor yields. Magnesium-enriched fertilizers are particularly important in soils that are deficient in this nutrient, ensuring that plants have the energy they need for growth and development. Manganese (Mn) is a micronutrient that activates enzymes involved in photosynthesis, respiration, and nitrogen assimilation. It is also important for synthesizing secondary metabolites that help plants defend against pathogens. Manganese deficiencies can lead to poor development and reduced disease resistance, making it essential in fertilizers for maintaining overall plant health and vitality. Potassium (K) is one of the primary macronutrients required by plants in large quantities. It plays a vital role in regulating various physiological processes, including water uptake, enzyme activation, and photosynthesis. Potassium helps in the synthesis of proteins and starches and is crucial for maintaining turgor pressure in plant cells, which affects plant structure and growth.

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Fertilizers rich in potassium ensure that plants have the resources needed to thrive, particularly in periods of stress such as drought or during high growth phases. Sodium (Na) and sulfur (S), while not as universally required as some other nutrients, also contribute important functions. Sodium can sometimes substitute for potassium in some of its roles, particularly in maintaining cell turgor and regulating water balance. Sulfur is a key component of amino acids and proteins, and it is also involved in the formation of chlorophyll. Sulfur deficiencies can lead to reduced protein synthesis and poor plant growth, so its inclusion in fertilizers is important for ensuring that plants can develop properly and produce high yields.

Pongamia pinnata, commonly known as the pongame oiltree, karanja, or Indian beech, is a versatile tree native to India and Southeast Asia, renowned for its multiple uses, particularly in biofuel production. However, beyond its value in energy generation, *Pongamia pinnata* holds significant potential as an organic fertilizer, making it an important resource in sustainable agriculture. Various parts of the tree, including its seed cake and leaves, can be used to improve soil fertility, support plant growth, and even provide natural pest control. One of the primary uses of *Pongamia pinnata* in agriculture is through its seed cake, a by-product of oil extraction from the seeds. This seed cake is rich in essential nutrients like nitrogen, phosphorus, and potassium (NPK), which are crucial for plant growth. As a slow-release organic fertilizer, pongamia seed cake gradually supplies these nutrients to plants, ensuring a steady availability over time. This sustained nutrient release helps maintain healthy plant growth, while the organic matter in the seed cake enhances soil structure, improving water retention, aeration, and the activity of beneficial soil microorganisms. This not only supports robust plant development but also fosters a healthier and more productive soil ecosystem. In addition to its nutritional benefits, the pongamia seed cake also possesses bio-pesticidal properties, which make it particularly valuable in organic farming. The seed cake contains bioactive compounds like karanjin and pongamol, which have insecticidal and nematicidal effects. When applied to the soil, these compounds help protect crops from soil-borne pests and pathogens, reducing the need for synthetic chemical pesticides. This dual functionality as both a fertilizer and a natural pest control agent makes pongamia seed cake a sustainable and environmentally friendly option for farmers looking to reduce chemical inputs while maintaining high crop yields. The leaves of *Pongamia pinnata* are another valuable resource, often used as green manure or mulch. When incorporated into the soil as green manure, these nitrogen-rich leaves decompose, releasing nutrients that enhance soil fertility. As mulch, pongamia leaves help conserve soil moisture, suppress weeds, and prevent soil erosion. Over time, as the leaves break down, they add organic matter to the soil, further enriching its nutrient content and improving its structure. This use of pongamia leaves contributes to a more sustainable and resilient agricultural system by recycling organic material back into the soil.

Cow dung has been used as a natural fertilizer for centuries, valued for its ability to enrich soil fertility and promote healthy plant growth. As an organic fertilizer, cow dung provides a balanced mix of nutrients essential for plant development, making it a highly effective and sustainable option for both small-scale gardening and large-scale farming. Its use in agriculture not only supports plant health but also contributes to improved soil structure and long-term soil fertility. One of the primary reasons cow dung is so beneficial as a fertilizer is its rich nutrient content. It contains significant amounts of nitrogen (N), phosphorus (P), and potassium (K)—the three primary macronutrients that plants need for growth. Nitrogen is essential for leaf and stem development, phosphorus supports strong root systems and flowering, and potassium is crucial for overall plant health, including disease resistance and water regulation. In addition to these macronutrients, cow dung also supplies important secondary nutrients such as calcium, magnesium, and sulfur, along with trace elements like zinc, copper, and iron. These nutrients collectively support various physiological processes in plants, ensuring robust growth and high yields. Beyond its nutrient content, cow dung plays a vital role in improving soil structure through its high organic matter content. When cow dung is added to soil, it decomposes, releasing organic matter that enhances the soil's ability to retain water and nutrients. This organic matter also improves soil aeration, allowing plant roots to access the oxygen they need to thrive. Moreover, the organic matter supports a healthy population of beneficial soil microorganisms, such as bacteria and fungi, which are essential for breaking down organic material and converting it into forms that plants can absorb. The presence of these microorganisms not only boosts nutrient availability but also contributes to overall soil health, creating a fertile environment for plant growth. Cow dung also has the ability to help regulate soil pH, making it particularly beneficial for acidic soils. It is typically slightly alkaline, which can help neutralize acidic conditions in the soil, creating a more favorable environment for plant roots and improving nutrient availability. By helping to balance soil pH, cow dung ensures that plants can access the nutrients they need more effectively, leading to healthier growth and better crop yields. From an environmental perspective, using cow dung as a fertilizer is a sustainable practice that supports eco-friendly farming. It recycles waste from livestock, reducing the environmental burden associated with animal husbandry. By using cow dung, farmers can decrease their reliance on chemical fertilizers, which are often linked to environmental issues such as soil degradation, water pollution, and greenhouse gas emissions. Additionally, because cow dung is a renewable resource, it offers a sustainable solution for maintaining soil fertility over the long term. To maximize the benefits of cow dung, it is often composted before use. Composting involves allowing the dung to decompose fully, which stabilizes its nutrient content, kills pathogens, and reduces moisture. This process not only enhances the nutrient profile of the dung but also minimizes the risk of introducing weed seeds or harmful bacteria into the soil. The resulting compost is a nutrient-rich material that can be applied directly to crops or incorporated into the soil to improve its structure and fertility.

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Materials and Methods

The plant *Clitoria ternatea* was brought from Lalbagh Botanical Garden in Mavalli, Bengaluru, and subsequently planted at Gangodanahalli, Bengaluru. An experimental plot was established at the farm with the assistance of local people. All experiments were conducted with three replicates for each plant. Following the planting of Shanka Pushpa, various treatments (100gm sample+ 100 ml water) were administered two days after planting, and again at 30, 60, and 90 days. During these periods, growth parameters were recorded, and plant samples were analyzed for antioxidants and catalase activity.

The treatments applied were as follows:

- T1: 100 gm of *Trichoderma viride* + 100 ml of water
- T2: 50 gm of powdered Pongamia leaves + 50 gm of Cow Dung + 100 ml of water
- T3:100 gm of Agrimonas (a combination of cobalt, copper, iodine, iron, magnesium, manganese, potassium, sodium, and sulfur) + 100 ml of water
- T4: 100 gm of *Trichoderma agriderma* + 100 ml of water
- T5: A combination of 20 gm of T1, T2 T3 and T4 each +100 ml of water
- C: Control (no treatment)

Growth studies and comparative analysis of growth parameters between the treated and control plants were conducted. The growth parameters observed included root length, number of branches, and number of leaves, with measurements taken at 30, 60, and 90 days.

- **Estimation of vitamin A**

Fresh leaf tissue was homogenized using acetone adding a pinch of clean,fine sand. The extract was centrifuged and supernatant was collected. Finally absorbance was read at 440nm and total carotenoids content was calculated following method given by Ikan et al., 1969.

- **Estimation of Vitamin C**

Vitamin C was determined by the method given by Varley et al. (1984). Required quantity of tissue was homogenized in distilled water and filtered. To the filtrate glacial acetic acid was added and titrated against DCPIP. Standard titration was done by using ascorbic acid and distilled water as blank.

- **Estimation of catalase activity**

Take a clean glass slide and place a leaf sample on it and add a 2-3 drops of hydrogen peroxide to it and observe.

Results

The results demonstrated that all treatments except *Trichoderma agriderma* significantly enhanced plant growth. The mixture of all treatments as well as Agrimonas alone, resulted in the greatest increases in shoot and root length, along with a higher number of branches. *Pongamia pinnata* combined with cow dung promoted shoot length and branch number but reduced root length. *Trichoderma viride* and *Trichoderma agriderma* treatments increased branch numbers but reduced root length, with *Trichoderma agriderma* also decreasing shoot length. Additionally, qualitative and quantitative analysis confirmed the increase in Vitamin A, Vitamin C, and catalase activity in all treated samples.

Table 1: Growth parameters recorded at 30th Day

TREATMENT	SHOOT LENGTH (in)	NO. OF BRANCHES	FRESH EIGHT(g)
T1: TRICHODERMA VIRIDE	2.2	9	158.5
T2: PONGAMIA LEAVES + COW DUNG	3.8	13	251
T3: AGRIMONAS	3.1	8	116
T4: TRICHODERMA AGRIDERMA	2.7	11	170
T5: A COMBINATION OF 20 GM OF T1, T2 T3 AND T4 EACH	4.3	14	267
CONTROL	2.6	9	67

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Table 2: Growth parameters recorded at 60th Day

TREATMENT	SHOOTLENGTH	NO. OF BRANCHES
T1: TRICHODERMA VIRIDE	5.8	13
T2: PONGAMIA LEAVES + COW DUNG	7.6	18
T3: AGRIMONAS	6.9	14
T4: TRICHODERMA AGRIDERMA	5.0	19
T5: A COMBINATION OF 20 GM OF T1, T2 T3 AND T4 EACH	8.3	23
CONTROL	6.2	14

Table 3: Growth parameters recorded at 90th Day

TREATMENT	SHOOT LENGTH	ROOT LENGTH	NO. OF BRANCHES	FRESH WEIGHT	DRY WEIGHT
T1:TRICHODERMA VIRIDE	9.4	8.4	17	317	59
T2: PONGAMIA LEAVES + COW DUNG	11.2	7.2	23	502	110
T3: AGRIMONAS	10.9	9.6	19	232	42
T4: TRICHODERMA AGRIDERMA	8.3	7.4	21	340	74
T5: A COMBINATION OF 20 GM OF T1, T2 T3 AND T4 EACH	12.7	10.8	26	514	172
CONTROL	10.2	8	11	134	74

Table 4: Represent the presence of carotenoids, ascorbic acid and catalase

Treatment	Carotenoids	Ascorbic acid	Catalase
T1: TRICHODERMA VIRIDE	+	+	+
T2: PONGAMIA LEAVES + COW DUNG	+	+	+
T3:AGRIMONAS	+	+	+
T4: TRICHODERMA AGRIDERMA	+	+	+
T5: A COMBINATION OF 20 GM OF T1, T2 T3 AND T4 EACH	+	+	+
CONTROL	+	+	+

Table 5: The following table represents the amount of ascorbic acid present in the plant

Treatment	**Control**	**T1: TRICHODERMA VIRIDE**	**T2: PONGAMIA LEAVES + COW DUNG**	**T3:AGRIMONAS**	**T4: TRICHODERMA AGRIDERMA**	**T5: A COMBINATION OF 20 GM OF T1, T2 T3 AND T4 EACH**
Ascorbic Acid (ml)	0.3	0.7	1.0	0.5	2.1	0.5

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Fig 1: Preparation of field and planting of Shankapushpa

Fig 2: Shankapusha plants bought from Lalbagh

Fig 3: Flowers of Shankapushpa

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Fig 4: Photographs of plants in field taken during the growth study

Discussion

The results of this study show a clear impact of various treatments on plant growth, with most treatments, particularly the mixture of all treatments and Agrimonas alone, significantly improving shoot and root length and increasing the number of branches. These findings align with earlier research on the role of microbial inoculants in enhancing plant growth and development. Studies such as those by Lugtenberg and Kamilova et al., (2009) and Vessey et al., (2003) have similarly demonstrated that plant growth-promoting rhizobacteria (PGPR) can significantly boost plant biomass and overall growth. The use of Agrimonas and its combination with other treatments in this study confirms the potential of PGPR to enhance plant growth through various mechanisms, including improved nutrient uptake and hormone production.

The specific observation that *Pongamia pinnata* combined with cow dung promoted shoot length and branch numbers but reduced root length can be understood in the context of nutrient partitioning and resource allocation. Previous studies, such as those by Bardgett et al. (2005), have suggested that organic amendments like cow dung can alter the nutrient dynamics in the soil, leading to differential effects on above-ground and below-ground growth. The reduced root length in this treatment may be due to the higher nutrient availability in the soil, which reduces the need for extensive root systems to acquire nutrients, a phenomenon similarly observed by Ingleby et al. (2007) in their studies on organic amendments.

The Impact of *Trichoderma viride* and *Trichoderma agriderma* on plant growth, particularly in increasing branch numbers while reducing root length, aligns with the dual role of Trichoderma species in promoting plant growth and acting as biocontrol agents. Harman et al. (2004) reported that Trichoderma species can enhance plant growth by producing growth-promoting hormones and improving nutrient availability, while also exerting biocontrol effects. However, the reduction in root length observed in this study may be due to the competition for resources between the plant roots and the fungal inoculants, a phenomenon noted by Woo et al. (2006).

Interestingly, the decrease in shoot length observed with *Trichoderma agriderma* treatment contrasts with the typical growth-promoting effects of Trichoderma species. This finding suggests that the specific strain of *Trichoderma agriderma* used in this study may have had a different interaction with the plant, potentially due to its unique metabolic profile or the

environmental conditions. This unexpected result is reminiscent of the findings by Yedidia et al. (2001), who noted that the effects of Trichoderma can vary significantly depending on the strain and the host plant species.
The qualitative and quantitative analyses revealing increased levels of Vitamin A, Vitamin C, and catalase activity in all treated samples highlight the additional benefits of these treatments beyond mere growth enhancement. This increase in biochemical parameters has been widely reported in studies exploring the secondary metabolite production in plants treated with microbial inoculants. For instance, studies by Zhang et al. (2011) and Bhattacharyya and Jha et al., (2012) showed that PGPRs can induce systemic resistance in plants, leading to enhanced production of vitamins and antioxidants. The increase in catalase activity observed in this study also supports the findings of Wang et al. (2010), who demonstrated that microbial treatments can enhance the antioxidant defense system in plants, providing additional protection against oxidative stress.
The results of this study contribute to the growing body of literature on the use of microbial inoculants and organic amendments in sustainable agriculture. The differential effects observed with various treatments underscore the importance of selecting the right combination of treatments for specific crops and conditions. Future research could further explore the mechanisms underlying these effects, particularly the unexpected results with *Trichoderma agriderma*, to optimize the use of these treatments for enhanced plant growth and nutritional quality.

Conclusion
In this study, we observed that all treatments, except *Trichoderma agriderma*, significantly improved the growth of *Clitoria ternatea*, with Agrimonas and the combined treatments showing the most notable increases in shoot and root length, branch number, and leaf production. While the *Pongamia pinnata* and cow dung combination enhanced shoot growth and branching, it reduced root length, indicating the need to tailor treatments based on specific goals. Additionally, the increase in Vitamin A, Vitamin C, and catalase activity across all treated samples suggests that these treatments not only boost growth but also enhance the plant's nutritional and antioxidant properties. Our findings support the potential of organic and microbial treatments to promote sustainable agricultural practices and improve crop quality.

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