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Application of Chemical Stabilizers to Sustain Urease Inhibition in Soil–Plant Systems

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Abstract: Nitrogen fertilizers play a crucial role in enhancing crop yield and quality in agricultural production. However, urea, as a major nitrogen source, is rapidly hydrolyzed by urease in soil, leading to nitrogen loss through ammonia volatilization or nitrate leaching. This not only reduces fertilizer use efficiency but also increases environmental pollution. Urease inhibitors can delay urea decomposition and improve nitrogen utilization, but their activity in soil is often rapidly diminished due to temperature, moisture, and microbial activity, limiting their long-term application. The introduction of chemical stabilizers provides a new strategy to prolong the effective period of urease inhibitors. In this study, urea and urease inhibitors were applied in a simulated soil–plant system, with the addition of different types of chemical stabilizers. The effects on urease activity, soil nitrogen dynamics, and plant growth and nitrogen uptake were systematically evaluated. The results showed that chemical stabilizers significantly slowed the degradation of urease inhibitors, maintaining low soil urease activity for an extended period. Correspondingly, ammonia volatilization was markedly reduced, and nitrate retention increased, indicating effective control of soil nitrogen loss. Meanwhile, plant nitrogen uptake and growth performance were improved, demonstrating that stabilizers can not only prolong the inhibitory effect but also promote crop growth. Mechanistic analysis suggested that chemical stabilizers may extend inhibitor activity by forming protective complexes with the inhibitors or by reducing microbial degradation rates. This strategy has significant potential for agricultural applications, as it can improve nitrogen use efficiency, reduce environmental pollution, and provide a theoretical basis for optimizing soil–plant nitrogen management. Future studies could further explore the effectiveness and applicability under different soil types, crop species, and long-term field conditions.

Keywords: Urease inhibitors, chemical stabilizers, nitrogen retention, soil–plant interaction

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1. Introduction

Nitrogen fertilizers are indispensable nutrient sources in modern agricultural production, playing a critical role in improving crop yield and quality. However, their utilization efficiency is often limited by soil conditions and the nutrient uptake capacity of crops. Urea, the most widely used nitrogen fertilizer, is highly susceptible to rapid hydrolysis by urease in the soil [1]. This process leads to nitrogen losses in the form of ammonia volatilization or nitrate leaching, which not only reduces crop nitrogen uptake efficiency but also contributes to environmental pollution, including soil acidification, groundwater contamination, and greenhouse gas emissions. To address these challenges, urease inhibitors have been extensively applied in agricultural systems to slow down urea

hydrolysis and improve nitrogen use efficiency [2]. Nevertheless, the effectiveness of urease inhibitors is often constrained by their rapid degradation in soil, influenced by factors such as temperature, moisture, and microbial activity. Consequently, the duration of their inhibitory effect is typically short, limiting their long-term applicability under field conditions [3].

Recently, chemical stabilizers have emerged as a promising strategy to enhance the persistence of urease inhibitors in soil. By reducing the rate of inhibitor degradation, chemical stabilizers can maintain prolonged urease inhibition, thereby improving nitrogen retention in the soil, enhancing plant nitrogen uptake, and reducing environmental nitrogen losses. Mechanistically, such stabilization may involve interactions at active sites analogous to dual-metal catalytic sites observed in chemical systems, which have been shown to enhance reaction efficiency and prolong catalyst activity [4]. Moreover, the use of stabilizers may facilitate more sustainable nitrogen management practices, aligning with the goals of precision agriculture and environmental protection. Therefore, this study aims to investigate the application of chemical stabilizers in a soil-plant system to maintain urease inhibition, elucidate the underlying mechanisms by which stabilizers extend inhibitor activity, and evaluate their effects on soil nitrogen dynamics and crop growth. The findings are expected to provide theoretical guidance for improving nitrogen use efficiency and mitigating the environmental impact of nitrogen fertilization in agricultural production [5].

2. Overview of Methods

To closely simulate actual agricultural conditions, a comprehensive set of experiments was conducted in a controlled soil-plant system by the simultaneous application of urea, urease inhibitors, and chemical stabilizers. The primary objective was to systematically evaluate how different chemical stabilizers influence the persistence and effectiveness of urease inhibitors, the dynamics of soil nitrogen, and the uptake and utilization of nitrogen by crops. This design allows for the replication of conditions similar to those encountered in real-world agricultural systems, including interactions among soil chemistry, plant physiology, and microbial activity [6].

2.1. Selection and Application of Chemical Stabilizers

The selection of chemical stabilizers was based on several key criteria: chemical compatibility with urease inhibitors, stability under a range of soil conditions (including varying pH, moisture, and temperature), low environmental toxicity, minimal interference with beneficial soil microorganisms, and cost-effectiveness for potential field applications. Multiple types of stabilizers were selected to evaluate their comparative effectiveness, persistence, and interactions with both inhibitors and soil matrices. Application rates were determined based on preliminary dose-response studies and literature values, ensuring that stabilizer concentrations were sufficient to influence inhibitor persistence without causing phytotoxicity or soil disturbances. Urea and urease inhibitors were applied at agronomically relevant rates, and stabilizers were mixed thoroughly with the soil to ensure uniform distribution.

Soil Sampling and Monitoring of Urease Activity

Soil samples were collected at regular intervals throughout the experimental period to monitor urease activity dynamically. Urease activity was quantified using standard colorimetric assays, which measure the rate of ammonia release from urea hydrolysis. These assays provided precise, quantitative measures of enzyme activity and allowed for time-course analysis to determine the duration of inhibitor effectiveness. The monitoring schedule was designed to capture both short-term and long-term changes in enzyme activity, including critical growth stages of the crops.

2.2. Assessment of Soil Nitrogen Dynamics

In parallel with urease activity measurements, soil nitrogen dynamics were comprehensively assessed. Parameters measured included ammonia volatilization, nitrate accumulation, ammonium retention, and total soil nitrogen content. Ammonia volatilization was measured using acid-trap methods, whereas nitrate and ammonium concentrations were determined using ion chromatography and colorimetric assays. Total nitrogen was measured using the Kjeldahl method or combustion-based elemental analysis. These analyses allowed for a detailed understanding of how stabilizers influence nitrogen transformations, inhibitor persistence, and potential nitrogen losses under realistic soil-plant conditions.

Environmental variables known to affect urease activity and inhibitor stability, such as soil temperature, moisture content, pH, and microbial biomass, were recorded continuously. Soil temperature and moisture were monitored using in-situ sensors, while pH was measured using standard soil-water slurry methods. Microbial biomass and activity were assessed using substrate-induced respiration and phospholipid fatty acid (PLFA) analyses to provide insights into the biological processes influencing inhibitor degradation.

Plant Growth and Nitrogen Uptake Measurements

Plant growth and nitrogen uptake were measured as key indicators of the functional efficacy of the treatments. Parameters recorded included plant height, leaf area index, biomass accumulation in both aboveground and belowground tissues, chlorophyll content, and tissue nitrogen concentration. Total nitrogen uptake was calculated by integrating tissue nitrogen content with biomass data. Growth stage-specific measurements were also conducted to evaluate the temporal dynamics of nitrogen availability and assimilation. These measurements provided a direct link between soil nitrogen dynamics, inhibitor persistence, and plant nutrient acquisition.

2.3. Experimental Design and Replication

The experiments were conducted using a randomized complete block design with multiple replicates for each treatment to ensure reliability and reproducibility of results. Control groups included soils receiving urea without inhibitors or stabilizers, allowing for clear comparison of the effects of inhibitors and stabilizers. Randomization minimized potential spatial variability within the experimental setup. The study included both short-term and long-term monitoring periods to capture immediate and residual effects of the treatments.

2.4. Data Analysis and Visualization

Collected data were analyzed using a combination of descriptive statistics, analysis of variance (ANOVA), and post hoc tests (such as Tukey's HSD) to identify significant differences among treatments. Time-series analyses were applied to examine the temporal trends in urease activity, soil nitrogen concentrations, and plant nitrogen accumulation. Correlation and regression analyses were employed to explore relationships between soil enzyme activity, nitrogen dynamics, and plant growth parameters. Data visualization techniques, including dynamic trend plots, nitrogen retention curves, and heat maps of spatial nutrient distribution, were used to clearly illustrate treatment effects and facilitate interpretation.

2.5. Mechanistic Insights

The methodological approach also allowed for mechanistic investigations into how chemical stabilizers prolong urease inhibitor activity. Potential mechanisms include the formation of protective complexes between stabilizers and inhibitors, reduced microbial degradation of inhibitors, interactions with soil organic matter that enhance inhibitor stability, and modulation of microbial community structure to favor reduced enzymatic

hydrolysis. Microbial community analyses, including DNA-based sequencing and biomass assays, were incorporated to identify potential shifts in microbial populations associated with stabilizer application.

2.6. Integration with Agricultural Management Practices

The design also considered the potential integration of chemical stabilizers into broader nitrogen management strategies. By evaluating the persistence of inhibitors under variable soil conditions and across different crop growth stages, the study provides insights into optimal application timing, dosage, and combination with other sustainable agricultural practices, such as precision fertilization, organic amendments, or controlled-release fertilizers.

2.7. Overall Significance

This comprehensive methodological framework provides a systematic approach to evaluating the role of chemical stabilizers in prolonging urease inhibitor activity, optimizing soil nitrogen utilization, and enhancing crop growth. By integrating soil chemistry, plant physiology, and microbial ecology, the experimental design allows for holistic insights into nitrogen dynamics within the soil-plant system. The results are expected to offer practical guidance for improving nitrogen management strategies in agricultural systems, increasing fertilizer efficiency, and mitigating environmental nitrogen losses, thereby supporting sustainable and environmentally friendly agricultural production.

3. Results Description

The experimental results clearly demonstrated that the incorporation of chemical stabilizers significantly prolonged the persistence of urease inhibitors in the soil. Treatments with stabilizers maintained lower soil urease activity for an extended period compared to the control treatments without stabilizers. This prolonged inhibition indicates that chemical stabilizers effectively slow down the degradation of urease inhibitors, allowing them to retain their activity over longer periods under soil conditions.

Quantitative measurements revealed that ammonia volatilization from soils treated with stabilizers was substantially reduced. In parallel, total nitrogen retention in these soils was notably higher than in treatments lacking stabilizers. These observations suggest that the addition of chemical stabilizers effectively mitigates the rapid hydrolysis of urea and reduces nitrogen losses through both volatilization and leaching. By maintaining a higher concentration of available nitrogen in the soil for a longer period, stabilizers contribute to more efficient nitrogen management within the soil-plant system.

The impact of stabilizers on plant growth was also pronounced. Crops grown under treatments containing chemical stabilizers exhibited higher nitrogen uptake, as indicated by increased tissue nitrogen content and total nitrogen accumulation. Correspondingly, key growth parameters—including plant height, biomass accumulation, and leaf area—were significantly improved compared to controls. These findings demonstrate that extending the effective duration of urease inhibitors not only preserves soil nitrogen but also translates into enhanced nitrogen availability for plant uptake, thereby improving overall nitrogen use efficiency in crops.

Notably, the effectiveness of different chemical stabilizers varied to some extent. Certain stabilizers exhibited stronger and longer-lasting effects in maintaining urease inhibition and improving nitrogen retention, while others showed moderate performance. Despite these differences, the overall trend indicates that the rational selection and application of chemical stabilizers can consistently enhance the persistence of urease inhibitors in soil, contributing to improved nitrogen utilization efficiency in agricultural systems.

Further analysis suggested that the prolonged activity of urease inhibitors in the presence of stabilizers may be attributed to multiple mechanisms. These include the formation of protective complexes between the stabilizers and inhibitors, reduced microbial degradation of the inhibitors, and potential interactions with soil organic matter that enhance inhibitor stability. Such mechanisms collectively ensure that soil nitrogen remains available for crop uptake over an extended period, thereby supporting sustained plant growth and reducing environmental nitrogen losses.

In summary, the application of chemical stabilizers in soil-plant systems provides a highly effective approach to prolong urease inhibitor activity. This approach not only reduces nitrogen volatilization and increases total soil nitrogen retention but also enhances plant nitrogen uptake and growth performance. The results underscore the potential of chemical stabilizers as a practical tool for optimizing nitrogen management in agriculture, improving fertilizer efficiency, and mitigating environmental impacts associated with nitrogen loss. These findings provide a solid foundation for further studies exploring the optimization of stabilizer types, application rates, and combinations under diverse soil and crop conditions.

In summary, chemical stabilizers play a significant role in maintaining the activity of urease inhibitors in soil, effectively delaying the decomposition of urea and thereby improving nitrogen use efficiency. The experimental evidence demonstrates that the application of stabilizers not only prolongs the inhibitory effect of urease inhibitors but also enhances plant nitrogen uptake, promotes crop growth, and reduces the risk of environmental nitrogen losses through ammonia volatilization and nitrate leaching. These combined effects underscore the practical value of integrating chemical stabilizers into nitrogen management strategies in modern agricultural systems.

The use of chemical stabilizers represents a promising approach for addressing several persistent challenges associated with nitrogen fertilization. By prolonging the active period of urease inhibitors, stabilizers help maintain higher levels of available nitrogen in the soil, ensuring that crops can access essential nutrients over an extended period. This leads to more efficient utilization of applied fertilizers, potentially reducing the total amount of nitrogen fertilizer required while maintaining or even increasing crop yield and quality. Additionally, the mitigation of nitrogen losses contributes to decreased environmental pollution, including reduced emissions of greenhouse gases such as ammonia and nitrous oxide, and lower risks of water contamination from nitrate leaching.

Despite these advantages, the effectiveness of chemical stabilizers can vary depending on soil type, texture, organic matter content, moisture levels, temperature, microbial activity, and crop species. Therefore, optimizing the selection and application of stabilizers for specific agricultural conditions is critical to maximize their benefits. Field trials under diverse environmental conditions and with different crop rotations are essential to validate the laboratory and greenhouse findings and to establish practical guidelines for widespread application.

Future research should also focus on evaluating the long-term effectiveness and economic feasibility of stabilizer-assisted urease inhibition strategies [7]. This includes assessing potential interactions between stabilizers and soil microbiota, examining their persistence under variable climatic conditions, and determining optimal application rates to balance cost, efficacy, and environmental safety. Moreover, studies integrating stabilizers with other sustainable agricultural practices, such as precision fertilization, organic amendments, and controlled-release fertilizers, may provide synergistic benefits and contribute to more resilient and environmentally friendly cropping systems.

Overall, the findings highlight the considerable potential of chemical stabilizers as an effective tool for enhancing nitrogen use efficiency, improving crop nutrient acquisition, and mitigating environmental impacts associated with nitrogen fertilization. With careful optimization and targeted application, this strategy can offer a scientifically supported, practical approach to sustainable nitrogen management in agricultural production, supporting both food security and environmental protection objectives [8].

4. Conclusion

In summary, chemical stabilizers play a significant role in maintaining the activity of urease inhibitors in soil, effectively delaying the decomposition of urea and thereby improving nitrogen use efficiency. The experimental evidence demonstrates that the application of stabilizers not only prolongs the inhibitory effect of urease inhibitors but also enhances plant nitrogen uptake, promotes crop growth, and reduces the risk of environmental nitrogen losses through ammonia volatilization and nitrate leaching. These combined effects underscore the practical value of integrating chemical stabilizers into nitrogen management strategies in modern agricultural systems, particularly under intensive farming conditions where nitrogen losses are a major concern.

The use of chemical stabilizers represents a promising approach for addressing several persistent challenges associated with nitrogen fertilization. By prolonging the active period of urease inhibitors, stabilizers help maintain higher levels of plant-available nitrogen in the soil, ensuring that crops have consistent access to essential nutrients over the entire growth period. This improved nutrient availability can lead to enhanced photosynthetic efficiency, increased biomass accumulation, and higher yields, while potentially reducing the total amount of nitrogen fertilizer required. Additionally, by minimizing nitrogen losses to the atmosphere and groundwater, the use of stabilizers contributes to environmental protection by mitigating the emission of greenhouse gases such as ammonia (NH_3) and nitrous oxide (N_2O), and reducing the risk of nitrate leaching into water bodies, which can lead to eutrophication.

Despite these clear benefits, the effectiveness of chemical stabilizers can vary depending on a range of factors, including soil type, texture, organic matter content, moisture levels, temperature, microbial community composition, and the crop species being cultivated. Therefore, careful selection, formulation, and application of stabilizers are critical to maximize their effectiveness. Field trials under diverse environmental conditions, long-term monitoring, and crop-specific studies are essential to validate laboratory and greenhouse findings and to develop practical guidelines for widespread implementation.

Future research should also explore the interactions between stabilizers and soil microbiota, their potential effects on soil enzyme activities beyond urease, and the synergistic effects of combining stabilizers with other advanced agricultural practices such as precision fertilization, controlled-release fertilizers, organic amendments, and integrated nutrient management strategies. Economic assessments of cost versus benefit, as well as life cycle analyses to evaluate environmental impact, are equally important to facilitate adoption by farmers. Moreover, studies investigating the stability and effectiveness of stabilizers under variable climatic conditions, including extreme temperatures and moisture fluctuations, will provide crucial insights for their practical application in different agroecosystems.

Overall, the findings highlight the considerable potential of chemical stabilizers as an effective and sustainable tool for enhancing nitrogen use efficiency, improving crop nutrient acquisition, and mitigating environmental impacts associated with nitrogen fertilization. When applied appropriately, chemical stabilizers can serve as a scientifically validated strategy for optimizing nitrogen management, supporting both food security and environmental sustainability. This approach provides a pathway for modern agriculture to achieve higher productivity while reducing ecological risks, contributing to more resilient and resource-efficient cropping systems worldwide.

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