

# Development of Green Urease Inhibitors as A Mitigation Tools for Ammonia Volatilization in Urea-Based Fertilizer: Review

Joshua Asukwo Adam, Ubelejit Uche Adum, Uwem Ekwere Inyang and Innocent Oseribho Oboh

Department of Chemical Engineering

Faculty of Engineering

University of Uyo, Uyo

Akwa Ibom State, Nigeria

**Abstract** - Nitrogen fertilizers play a critical role in sustaining global agricultural productivity and food security. Among nitrogen fertilizers, urea is the most widely used due to its high nitrogen content, low cost, and ease of handling. Despite these advantages, the agronomic efficiency of urea is significantly limited by rapid hydrolysis in soil catalyzed by the enzyme urease. This hydrolysis leads to the formation of ammonium carbonate and the subsequent release of ammonia gas, resulting in substantial nitrogen losses through ammonia volatilization. Nitrogen losses from urea fertilizers can range from 10% to 40% of applied nitrogen depending on soil and environmental conditions. These losses not only reduce nitrogen use efficiency but also contribute to environmental pollution, soil degradation, and atmospheric particulate formation. To address this challenge, urease inhibitors have been developed to temporarily suppress urease activity and delay urea hydrolysis. Synthetic inhibitors such as N-(n-butyl) thiophosphoric triamide (NBPT), N-(n-propyl) thiophosphoric triamide (NPPT), and phenyl phosphorodiamidate (PPD) have demonstrated high effectiveness in reducing ammonia volatilization. However, increasing environmental concerns regarding the persistence and toxicity of synthetic chemicals have stimulated research into environmentally friendly alternatives known as green urease inhibitors. These inhibitors are derived from natural sources including plant extracts, essential oils, phenolic compounds, and agricultural residues. Recently, research has focused on green urease inhibitors derived from plant materials, such as neem oil, garlic extract, eucalyptus leaves, and acacia extracts. These plant-based inhibitors are biodegradable, environmentally friendly, and potentially cost-effective alternatives to synthetic chemicals. Several studies report that plant extracts can inhibit urease activity by 20–95% depending on the species and extraction method. This review provides a comprehensive evaluation of the mechanisms of ammonia volatilization from urea fertilizers, the role of urease inhibitors in mitigating nitrogen losses, and recent advances in the development of green urease inhibitors derived from plants and natural products. Additionally, a comparison between plant-based and synthetic urease inhibitors is presented to highlight their relative advantages, limitations, and future prospects in sustainable agriculture.

**Keywords:** Inhibitor, Urea, Fertilizer and volatilization

## 1.1 INTRODUCTION

Global population growth poses one of the most pressing challenges for modern agriculture. The world population is projected to reach approximately 9.7 billion by 2050, which is expected to outpace the expansion of land that can realistically be allocated for crop production (Yahya, 2018; Matseet *et al.*, 2024). Consequently, the amount of arable land available per person is likely to decline, placing immense pressure on agricultural systems to produce more food from

limited resources. In this context, improving nutrient management, particularly nitrogen (N) fertilization, becomes critical to ensuring sustainable crop production. Urea is the most widely used nitrogen fertilizer due to its high N content and ease of application; however, its efficiency is often compromised by significant nitrogen losses. Approximately 60% of applied urea can be lost as ammonia (NH<sub>3</sub>) through volatilization, contributing to air pollution and environmental degradation. During urea hydrolysis, the rapid formation of inorganic ammonium ions (NH<sub>4</sub><sup>+</sup>) occurs, but the retention of these ions in soil is typically poor because of limited adsorption capacity (Skorupka *et al.*, 2021; Bhatia *et al.*, 2023). This dual problem of NH<sub>3</sub> volatilization and poor NH<sub>4</sub><sup>+</sup> retention results in nutrient deficiency for crops and economic loss for farmers. To compensate, farmers frequently apply higher quantities of urea, a practice that is neither cost-effective nor environmentally sustainable (Fu *et al.*, 2020; IPCC, 2021; Sha, *et al.*, 2023).

To address these challenges, research has focused on improving nitrogen use efficiency (NUE) through chemical and biological strategies. Among the most effective approaches are urease inhibitors, which temporarily suppress the activity of the urease enzyme, slowing urea hydrolysis and reducing NH<sub>3</sub> losses (Singh *et al.*, 2019; Wang *et al.*, 2021). Urease inhibitors can be broadly classified into synthetic and natural compounds. Synthetic inhibitors, such as N-(n-butyl) thiophosphoric triamide (NBPT), N-(2-nitrophenyl) phosphoric triamide (2-NPT), and N-(n-propyl) thiophosphoric triamide (NPPT), are widely studied and have demonstrated reductions in ammonia volatilization by up to 60–70% under field conditions (Cantarella *et al.*, 2008; Abalos *et al.*, 2014). These compounds act primarily through reversible binding to the urease active site, temporarily preventing urea hydrolysis while allowing gradual conversion to ammonium ions, which can be retained in the soil for plant uptake (Sriraj *et al.*, 2022).

In contrast, natural urease inhibitors are derived from plant sources, including neem, tannin-rich extracts, and flavonoid compounds. Although their inhibition efficiency is often lower than that of synthetic inhibitors, natural compounds offer additional environmental advantages, such as biodegradability, lower toxicity, and potential compatibility with sustainable agriculture practices (Jadon *et al.*, 2018; Rana *et al.*, 2021). The mechanisms of natural inhibitors vary depending on the phytochemical structure: tannins and flavonoids can bind to the urease enzyme and alter its catalytic activity, while neem compounds can form a protective coating around urea granules, slowing hydrolysis and reducing volatilization. The application of both synthetic and natural urease inhibitors has significant implications for NUE (Pan *et al.*, 2016). By delaying urea hydrolysis and improving the retention of ammonium ions in the soil, these inhibitors reduce nitrogen losses, enhance nutrient availability for crops, and minimize the environmental footprint of fertilizer use. Furthermore, integrating urease inhibitors into crop management strategies can reduce the frequency and quantity of urea applications, lowering production costs while maintaining yield (Cowan *et al.*, 2021; BASF, 2016). This highlights the importance of combining chemical innovation with sustainable agricultural practices to meet the growing food demands of a rapidly expanding global population (Guo *et al.*, 2023; Mathialagan *et al.*, 2019; Liu *et al.*, 2029).

The use of urease inhibitors in agricultural practices has long been explored as one of the strategies to guarantee food supply in enough amounts. The use of urease inhibitors is one of the strategies adopted to improve urea performance in agriculture and mitigate urea driven emission of pollutants (Kavanagh *et al.*, 2021). This is due to the fact that urea, one of the most used nitrogen (N) fertilizers worldwide, rapidly undergoes urease-driven hydrolysis on soil surface yielding up to 70% N losses to environment. Currently, nitrogen fertilizers are utilized to meet 48% of the total global food demand. Nitrogen (N) is a vital soil nutrient essential for good and abundant plant growth (Fathi 2020). The main source of N for the plant comes from the external input application (Masclaux-Daubresse *et al.*, 2010).

The demand for nitrogen fertilizers is expected to grow as global populations continue to rise, in view of the world population especially in developing countries like Nigeria. In Nigeria, most farmers used nitrogen-based fertilizer due to the deficiency of nitrogen in soil (Kopteva *et al.*, 2019). Nitrogen (N) fertilizers, and in particular urea-based fertilizers, have been widely used in agriculture, with a projected annual demand increase of 1.5% in the future. Urea is the

most concentrated solid nitrogen fertilizer (46% N), and it is cost-effective and economical in terms of production, making it the leading nitrogen fertilizer product globally (Bremner, 1996). However, its application efficiency is reduced by losses of nitrogen through ammonia volatilization when the urea is not incorporated into the soil under appropriate conditions. High soil pH, temperature, and microbial activity at the soil surface promote rapid hydrolysis of urea to ammonia and carbonate by soil urease enzymes, leading to up to 70% nitrogen loss into the environment. The hydrolysis of urea involves the consumption of protons, which leads to an increase in soil pH around the fertilizer granules. An increase in soil pH from 6.5 to 8.8 after urea application was reported by Overrein and Moe (1967). When urea is applied to the soil surface, it is hydrolyzed to ammonium ions by the urease enzyme, resulting in alkaline soil conditions that favor ammonia loss as gas from the soil surface (Bremner, 1996; Overrein and Moe, 1967). When hydrolysis is delayed, the concentration of  $\text{NH}_3$  near the soil surface decreases, which in turn reduces volatilization potential and allows time for rainfall to move urea deeper into the soil (Kiss and Simihaian, 2018).

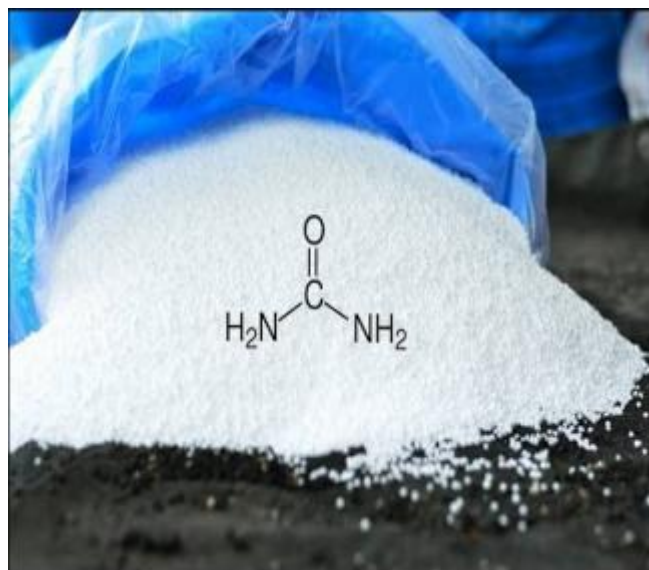


Figure 1: Urea fertilizer and its structure.

Source: Kavanagh *et al.*, 2021

In the presence of water, urea is quickly hydrolyzed to ammonia ( $\text{NH}_3$ ), hydroxyl ( $\text{OH}^-$ ) ions and carbon dioxide by the ubiquitous enzyme urease as seen in Equation (1) (Cancellier *et al.*, 2015). This hydrolysis reaction results in an elevated pH surrounding the fertilizer granule, which switches the ammonium ( $\text{NH}_4^+$ )/ $\text{NH}_3$ -equilibrium towards a higher  $\text{NH}_3$  concentration in the soil solution and induces high emissions of  $\text{NH}_3$  into the atmosphere (Sommer *et al.* 2004). Part of the emitted  $\text{NH}_3$  is deposited on vegetation surfaces, where it causes acidification and eutrophication on a regional scale. Its impact is great, especially when deposited in natural and semi-natural ecosystems, and can result in an ecological shift in species diversity (Van Breemen *et al.* 1982; Bouwman and Van Vuuren 1999). Urea hydrolysis is catalyzed by the enzyme urease, which is produced by soil microorganisms and plant residues. The hydrolysis reaction can be represented in Equation (1) and (2)



This reaction results in the formation of ammonia and carbon dioxide. Ammonia then reacts with water to form ammonium ions:



The increase in hydroxyl ions causes a temporary rise in soil pH, promoting the release of ammonia gas. Global ammonia ( $\text{NH}_3$ ) emissions from nitrogen fertilizer usage are estimated at 10–12 Tg  $\text{yr}^{-1}$  (Erismann *et al.*, 20013; Sutton *et al.*, 2013). Ammonia emissions in Africa have increased by more than 50% during the past 30 years (Hickman *et al.*, 2021; NASA Earth Science News Team, 2021). These emissions have economic, environmental and national policy implications (van Damme *et al.*, 2022).

Green urease inhibitors (UIs), such as plant extracts (e.g., *Vachellia nilotica*) (Jadon *et al.*, 2018) and NBPT, 2-NPT, etc. are used as coatings on urea fertilizer to reduce nitrogen loss via ammonia volatilization by up to 70% (Cantarella *et al.*, 2008; Castellano *et al.*, 2019). These compounds block the active site of the soil enzyme urease, slowing down the conversion of urea to ammonia by 1–2 weeks, allowing time for incorporation into the soil. To mitigate  $\text{NH}_3$  volatilization losses, urease inhibitors can be used. Urease inhibitors are compounds that temporarily block the enzyme urease, slowing down the hydrolysis of urea into ammonium, ammonia, and  $\text{CO}_2$ . Several compounds act as urease inhibitors, but only N-(n-butyl) thiophosphoric triamide (NBPT) has been used worldwide, being the most successful in a market that has grown 16% per year in the past 10 years. Urease inhibitor reduces ammonia volatilization and nitrous oxide emission by decreasing both ammonium and nitrate concentrations in soil and hence increase plant N uptake, they enhance nitrogen efficiency and reduce gaseous ammonia losses by 7 to 14 days (Kumar *et al.*, 2020; Castellano *et al.*, 2019). These additives increase fertilizer efficiency and reduce greenhouse gas emissions as seen in Figure (2).

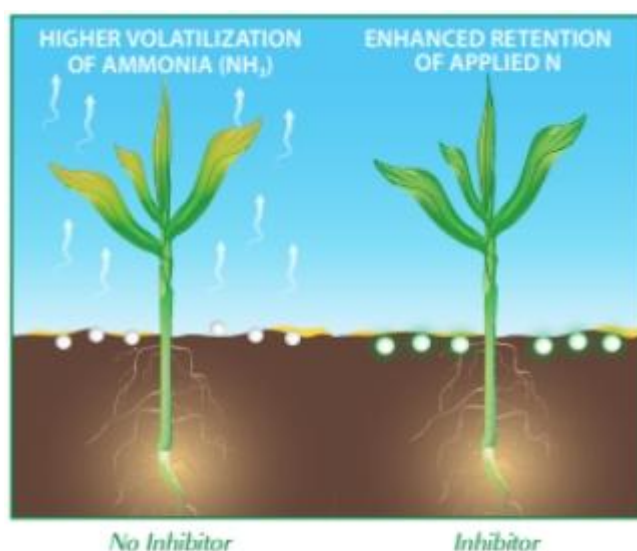


Figure 2: Effect of ammonia volatilization

Source: Kumar *et al.*, 2020

Urease inhibitors, such as N-(n-butyl) thiophosphoric triamide (NBPT), are chemical compounds, often formulated with polar organic solvents like glycol or alkyl sulfones, used to slow the enzymatic hydrolysis of urea fertilizers. These inhibitors reduce ammonia volatilization, typically applied at concentration to enhance nitrogen efficiency. NBPT (N-(n-butyl) thiophosphoric triamide), have been used with success and received considerable attention in the past two decades (Liu *et al.*, 2019). NBPT (CAS No. 94317-64-3) with a molecular formula of  $\text{C}_4\text{H}_{14}\text{N}_3\text{PS}$ , is a white crystalline solid (boiling point 264.0 °C and melting point 59.1 °C) that can be coated to urea. Figure 3 shows the structure of the NBPT molecule. For NBPT to reduce  $\text{NH}_3$  emission, it needs to act on an enzyme known as urease, which is responsible for the hydrolysis of urea and the consequent  $\text{NH}_3$  volatilization. NBPT needs to be converted to its oxon analogue (NBPTo) for urease inhibition to occur (Afshar *et al.*, 2018).

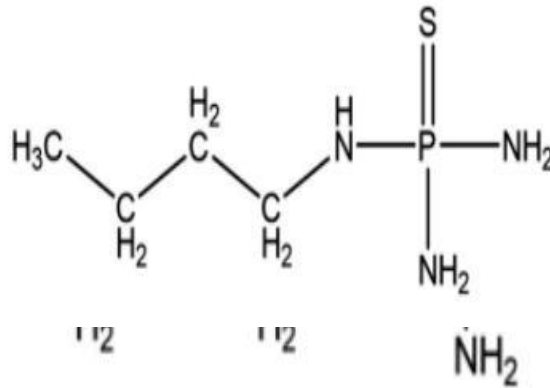


Figure 3: Structure of urease inhibitor NBPT.

Source: Afshar *et al.*, 2018

The strong urease inhibitory activity of NBPT results in its binding with the urease active site at three locations, that is, two Ni atoms and a carbamate group. As the addition of supplemental fertilizer N is a cornerstone of many agricultural systems, N lost as  $\text{NH}_3\text{-N}$  must be replaced, typically at an economic and environmental cost, to sustain agro ecosystem productivity (Souza *et al.*, 2021). Watson showed that N recovery efficiency and yield of urea treated with NBPT increased by 20 and 8.8% respectively, when compared to yield performance of just urea. Commercially available urease inhibitors (e.g. Agrotain) usually have the active ingredient N-(n-butyl) thiophosphoric triamide (NBPT) in them which is a structural analogue of urea (Souza *et al.*, 2021). NBPT works to reduce gaseous ammonia loss by inhibiting the urease enzyme from degrading urea (Abdo *et al.*, 2020). Many studies have shown that NBPT is effective in reducing ammonia loss from urea as well as its potential to significantly aid in achieving EU's target for GHG emission reductions. Apart from its effects in GHG emission reduction, several other benefits of NBPT coated fertilizers have been reported. For instance, the authors in their paper reported a reduction of the adverse effects of urea fertilizer on seed germination, seedling growth, and early plant growth in soil by amending the fertilizer with as little as 0.01% (w/w) of N-(n-butyl) thiophosphoric triamide. NBPT's ability to reduce ammonia losses and other GHG emissions as well as several other agricultural benefits, make it an ideal choice for use with urea during farming (Fu *et al.*, 2020).

Despite many disparate  $\text{NH}_3$  field studies existing for both synthetic and plant-based urease inhibitors, there is presently no review that brings these results together, a significant and important knowledge gap. This review addresses the gap by summarizing the published laboratory and field trial literature on  $\text{NH}_3$  volatilization mitigation offered by synthetic and plant-based urease inhibitors. This review provides a comprehensive evaluation of the mechanisms of ammonia volatilization from urea fertilizers, the role of urease inhibitors in mitigating nitrogen losses, and the results presented in this review will broaden the understanding of urease inhibitor efficacy in field and laboratory conditions and demonstrate that not all products behave the same in terms of  $\text{NH}_3$  reduction efficacy and recent advances in the development of green urease inhibitors derived from plants and natural products. Additionally, a comparison between plant-based and synthetic urease inhibitors is presented to highlight their relative advantages, limitations, and future prospects in sustainable agriculture. The aim of this study was to analyze the efficacy of urease inhibitors on  $\text{NH}_3$  emission abatement based on currently available scientific literature. Additionally, the existing gaps in research data were identified.

## 2.0 Urease inhibitors

Urease inhibitors are compounds that temporarily reduce the activity of the urease enzyme, slowing the hydrolysis of urea into ammonia, which reduces nitrogen loss from soils (volatilization) and aids in controlling ammonia-related clinical diseases (Sánchez *et al.*, 2020; Krajewska, 2009). Urea is the most widely used form of nitrogen fertilizer and can

be formulated as dry granules, prills, or as a fluid alone or mixed with ammonium nitrate (UAN) (Cantarella *et al.*, 2008; Zaman *et al.*, 2008). Urea is also present in animal manures. All these forms of urea have the disadvantage of undergoing considerable losses as ammonia gas if not incorporated into soil soon after application (Zaman *et al.*, 2008; Sanz-Cobena *et al.*, 2012). Once dissolved in water, urea is converted to ammonium bicarbonate within a few days following application by the naturally occurring enzyme urease (Krajewska, 2009). Urease is produced by many soil microorganisms and plants and is present in nearly all soils (e.g., Krajewska, 2009). When urea is hydrolyzed by urease, much of the resulting ammonium is held on soil cation exchange sites. During the conversion, the pH temporarily rises, and ammonia gas is produced. The loss of ammonia, termed volatilization, can range from negligible to over 50% (Sanz-Cobena *et al.*, 2012; Abalos *et al.*, 2014).

Among the known soil urease inhibitors, N-(butyl) thiophosphorictriamide (NBPT) is currently the most efficient compound (Abalos *et al.*, 2014; Cantarella *et al.*, 2008). In the presence of soil microbiota, NBPT is converted to the respective oxo-analogue called N-(butyl) phosphoric triamide (oxo-NBPT), which exhibits a high capacity for inhibiting urease (Cantarella *et al.*, 2008). Many other substances have been investigated with respect to their potential to inhibit urease activity in soil, but very few were found to be promising for further studies. The great challenge remains to find good candidates that are eco-friendly, nontoxic or of low toxicity to plants, chemically stable, efficient at low concentrations, compatible with urea, and cost-competitive (Kumar *et al.*, 2020; Upadhyay, 2012).

Several different inhibitors are commercially available globally. The most widely researched urease inhibitors are phosphorodiamide and phosphorotriamide derivatives (Singh *et al.*, 2023). These include N-(n-butyl) thiophosphorictriamide (NBPT), N-(2-nitrophenyl) phosphoric triamide (2-NPT), and N-(n-propyl) thiophosphorictriamide (NPPT) (Modolo *et al.*, 2018; Song *et al.*, 2022). These phosphoramidate inhibitors, NBPT, 2-NPT, and a 3:1 ratio of NBPT + NPPT, have been shown to be effective and practically applicable in agricultural field systems. For example, in a field study conducted in New Zealand, Dawar *et al.* (2011) demonstrated that urea coated with NBPT at 0.1% (w/w) of urea N reduced NH<sub>3</sub> volatilization loss by 67% on average compared to standard urea in a spring application to a perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) sward. Similarly, Matse *et al.* (2024) reported that application of urea coated with NBPT (0.066% w/w) at two grassland experimental sites in Ireland decreased NH<sub>3</sub> volatilization by 79% on average across the two sites. While research reports show that these urease inhibitors are promising, their widespread application in farm systems has encountered challenges. There is limited information in the literature regarding which urease inhibitor is most effective under various soil types and climatic conditions (Singh *et al.*, 2023; Song *et al.*, 2022).

## 2.1 Types of Urease inhibitors

Approximately 14,000 compounds or combined mixtures of these compounds have been tested to inhibit urea hydrolysis, and many of these are structural analogues of urea (Kiss and Simihaian, 2018; Cantarella *et al.*, 2018). The most common inhibitor among such compounds that has successfully reached the market is N-(n-butyl) thiophosphorictriamide (NBPT), categorized as a phosphoramidate. This compound is traded as Agrotain in the USA and, in different countries, NBPT products are sold under various brand names. In the presence of soil microorganisms, NBPT is converted to its active analogue N-(butyl) phosphoric triamide (oxo-NBPT), which exhibits effective urease inhibition (Cantarella *et al.*, 2018; Kiss and Simihaian, 2002; Kawakami *et al.*, 2012). Different urease inhibitors with varied efficiency, cost, toxicity and stability are available in the market. These inhibitors ranged from elemental ions such as Hg<sup>2+</sup> and Ag<sup>+</sup>, inorganic ions (boric acid) to organic compounds (acetohydroxamic acid and hydroquinone) (Svane *et al.*, 2020). Another important class of inhibitors is hydroxyamic acid which is characterized by terminal O-C-NHOH functional group and best studied example of this class is acetohydroxamic acid (AHA). This class of inhibitors are highly stable, weakly acidic in nature and highly soluble in water and these properties make it a highly potent urease inhibitor (Arora *et al.*, 2018).

Hydroquinone and quinones are known to inhibit urease by interacting with key residues at or near the enzyme's active site (Mazzei *et al.*, 2022). Benzoylthiourea derivatives have been investigated as urease inhibitors, showing mixed-type inhibition that suggests interactions with active or allosteric sites of urease (Rego *et al.*, 2018; Costa *et al.*, 2015). Coumarinyl-pyrazolinyl thioamide compounds have been synthesized and demonstrated significant non-competitive urease inhibition in vitro (e.g., *Inhibition of urease by coumarinylpyrazolinyl thioamide derivatives*, 2018). Phenolic aldehyde derivatives such as protocatechuic aldehyde, syringaldehyde, and vanillin have been studied for their ability to inhibit urease and serve as molecular scaffolds for more active inhibitors (Horta *et al.*, 2016). Several organic compounds continue to be investigated for urease inhibition due to their structural features and potential to reduce enzyme activity (Modolo *et al.*, 2018).

## 2.2 Leveraging Plant-Based Urease Inhibitors: Implications of Previous and Recent Research

Green urease inhibitors have emerged as a cornerstone of sustainable agriculture, specifically engineered to suppress urease enzyme activity and thereby mitigate urea hydrolysis and ammonia (NH<sub>3</sub>) volatilization from urea-based fertilizers, which globally result in 20–70% nitrogen (N) losses depending on soil conditions (Cantarella *et al.*, 2008; Zaman *et al.*, 2008; Sanz-Cobena *et al.*, 2012). Unlike persistent synthetic inhibitors such as NBPT (N-(n-butyl) thiophosphorictriamide), green variants prioritize natural, plant-derived, or biodegradable compounds like polyphenols, flavonoids, and saponins, which chelate nickel ions in urease's active site or disrupt its catalytic triad while degrading rapidly in soil to avoid long-term ecological disruption (Modolo *et al.*, 2015; Krajewska, 2009). Green urease inhibitors therefore offer a promising, eco-friendly approach to tackling urea hydrolysis and ammonia volatilization in urea-based fertilizers. These compounds target the urease enzyme, slowing urea breakdown and nitrogen loss while minimizing environmental harm compared to synthetic alternatives (Abalos *et al.*, 2014; Chen *et al.*, 2008). Plant-based urease inhibitors, such as extracts from *Vachellia nilotica*, *Eucalyptus camaldulensis*, garlic (allicin), and cumin, serve as natural and sustainable alternatives to synthetic inhibitors like NBPT, reducing nitrogen loss from urea fertilizers. These natural compounds effectively inhibit soil urease activity, significantly increasing nitrogen use efficiency and reducing ammonia volatilization (Ramli *et al.*, 2014; Ferreira *et al.*, 2016; Rana *et al.*, 2021).

There are currently several alternatives to minimize nitrogen losses from urea fertilizers and improve nitrogen uptake by crops. Slow-release nitrogen fertilizers consist of fertilizers coated with hydrophobic materials that create a physical barrier against water, thereby promoting the gradual release of urea into the soil solution (Chen *et al.*, 2008). Another strategy is the use of nitrification inhibitors, which delay NH<sub>4</sub><sup>+</sup> oxidation by nitrifying bacteria, thereby preventing NO<sub>3</sub><sup>-</sup> formation and reducing nitrogen leaching from soils (Abalos *et al.*, 2014). Urease inhibitors remain one of the most widely used approaches for overcoming nitrogen losses in agricultural systems because they delay urea hydrolysis, increasing the chances of urea incorporation into soil through rainfall, irrigation, or mechanical incorporation (Zaman *et al.*, 2008; Sanz-Cobena *et al.*, 2012).

Green variants emphasize natural or biodegradable sources, such as plant extracts (e.g., from tannin-rich plants like *Acacia* or *Eucalyptus*) and microbial-derived compounds, thereby avoiding persistent synthetic chemicals (Modolo *et al.*, 2015; Ferreira *et al.*, 2016). Research has also highlighted modified phenylphosphorodiamidate (PPD) derivatives and bio-based NBPT analogs that degrade faster in soil, thereby reducing residue risks while maintaining high ammonia volatilization reduction efficiencies (Krajewska, 2009; Abalos *et al.*, 2014). These compounds can outperform traditional NBPT formulations in stability tests, particularly when combined with improved formulations such as stabilized coatings that enhance persistence and efficiency under field conditions (Chen *et al.*, 2008).

## 2.3 Mechanisms Underlying the Effectiveness of Plant-Based Inhibitors

Plant-based urease inhibitors typically contain bioactive phytochemicals that interfere with urease enzyme activity through several mechanisms. Many plant phytochemicals interact directly with the urease enzyme by binding to its active site. Flavonoids and tannins are particularly known for their ability to chelate the nickel ions present in the urease catalytic center, thereby preventing the enzyme from hydrolyzing urea (Modolo *et al.*, 2015; Ogawa and Yazaki 2018; Rana *et al.*, 2021). Polyphenolic compounds found in plants can form complexes with proteins, including urease enzymes. This interaction alters the structural conformation of the enzyme and reduces its catalytic activity (Modolo *et al.*, 2015; Ferreira *et al.*, 2016). Some plant compounds inhibit the activity of urease-producing microorganisms in soil, thereby reducing the overall rate of urea hydrolysis (Ramli *et al.*, 2014; Jadon *et al.*, 2018). The effectiveness of these mechanisms explains why many plant extracts have demonstrated strong urease inhibition in laboratory studies (Kumar *et al.*, 2020; Liu *et al.*, 2019) as could be seen in Figure 4.

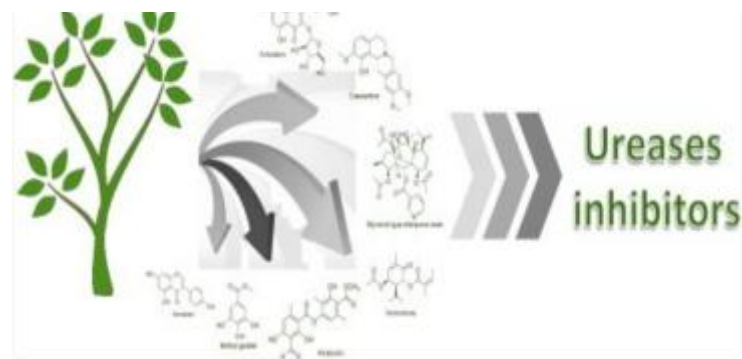


Figure 4: Green urease inhibitor

Source: Kumar *et al.*, 2020

#### 2.4 Urea Hydrolysis Mechanism

Urea ( $\text{CO}(\text{NH}_2)_2$ ), the most widely used nitrogen fertilizer globally, undergoes rapid enzymatic hydrolysis by soil urease. Urea fertilizers rapidly hydrolyze via soil urease into ammonia, which volatilizes as  $\text{NH}_3$  gas, especially in alkaline or high-pH soils. This process can cause 20–50% nitrogen loss in surface-applied urea, reducing crop uptake and contributing to air pollution and eutrophication (Krajewska, 2009; Bremner and Douglas, 1971). The reaction produces ammonia and carbamic acid, which decomposes to  $\text{CO}_2$  and more  $\text{NH}_3$  (Krajewska, 2009; Bremner and Douglas, 1971):



In alkaline soils ( $\text{pH} > 7$ ) or surface-applied scenarios, up to 50% of applied N volatilizes as  $\text{NH}_3$  within days, leading to N loss, soil acidification, and atmospheric pollution that contributes to eutrophication

## 2.5 Previous Studies on Urease Inhibitors

**Table 1: Major Studies on Synthetic Urease Inhibitors for Reducing Ammonia Volatilization and Their Knowledge Gaps**

Author(s)/year	Inhibitor Name	Type	Soil/Crop System	Key Findings	Knowledge Gap
Bremner and Douglas 1971	Phenyl phosphorodiamidate (PPD)	Synthetic	Laboratory soil	Demonstrated inhibition of soil urease activity	Field-scale validation not conducted
Watson <i>et al.</i> 1994	NBPT	Synthetic	Grassland soils	Reduced ammonia volatilization from urea fertilizer	Effects on soil microbial communities not studied
Byrnes, 2000	NBPT	Synthetic	Agricultural soils	Improved nitrogen use efficiency	Limited long-term environmental assessment
Grant <i>et al.</i> 2010	Hydroquinone	Synthetic	Field crop soils	Reduced urease activity and ammonia loss	Toxicity risks not evaluated
Cantarella <i>et al.</i> 2008	NBPT	Synthetic	Tropical soils	Up to 60% reduction in NH <sub>3</sub> volatilization	Economic feasibility not studied
Zaman <i>et al.</i> 2008	NBPT	Synthetic	Pasture soils	Increased nitrogen retention	Impact on soil nitrogen cycling not assessed
Abalos <i>et al.</i> 2014	NBPT	Synthetic	Mediterranean soils	Reduced ammonia volatilization and N <sub>2</sub> O emissions	Did not compare with plant inhibitors
Sanz-Cobena <i>et al.</i> 2012	NBPT	Synthetic	Wheat cropping system	Reduced ammonia emissions and improved NUE	Long-term soil health effects not evaluated
Chen <i>et al.</i> 2008	NBPT + DMPP	Synthetic	Paddy soil	Reduced nitrogen losses and improved yield	Limited climatic condition comparisons

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Castellano et al., 2019	NBPT	Synthetic	Field soil experiment	Reduced NH <sub>3</sub> emission by about 62%	Lack of sustainable alternatives explored
Guardia <i>et al.</i> 2017	NBPT	Synthetic	Maize cropping system	Reduced ammonia volatilization	No comparison with organic inhibitors
Pan <i>et al.</i> 2016	NBPT	Synthetic	Agricultural soil	Increased nitrogen use efficiency	Did not examine environmental toxicity

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One of the earliest studies investigating urease inhibition in soils was conducted by Bremner and Douglas (1971). Their research focused on the inhibitory effects of phenyl phosphorodiamidate (PPD) on soil urease activity. Using laboratory incubation experiments, they demonstrated that PPD significantly slowed the hydrolysis of urea by blocking the active site of the urease enzyme. Their findings provided the first evidence that chemical inhibitors could effectively regulate the rate of urea hydrolysis in soil systems. However, the study was limited to controlled laboratory conditions and did not evaluate the performance of the inhibitor under field conditions where environmental factors such as temperature, rainfall, and soil microbial diversity influence urease activity.

Further research was conducted by Watson *et al.* (1994), who investigated the effectiveness of N-(n-butyl) thiophosphorictriamide (NBPT) as a urease inhibitor in grassland soils. Their results indicated that NBPT significantly reduced ammonia volatilization following urea application. The study reported improved nitrogen use efficiency and increased nitrogen retention in soil systems treated with NBPT. The authors concluded that urease inhibitors could play a critical role in reducing nitrogen losses from agricultural systems. Despite these positive findings, the study did not examine the long-term ecological impact of NBPT on soil microorganisms, which are essential for nutrient cycling. Similarly, Byrnes (2000) examined the agronomic efficiency of urease inhibitors in agricultural soils. The study confirmed that NBPT effectively delayed urea hydrolysis, resulting in lower ammonia emissions and improved nitrogen availability for plant uptake. Byrnes emphasized that the use of urease inhibitors could increase fertilizer efficiency while reducing environmental pollution associated with nitrogen loss. However, the study primarily focused on short-term nitrogen dynamics and did not assess the potential accumulation or degradation of inhibitor residues in soil ecosystems.

Research conducted by Grant *et al.* (2010) explored the use of hydroquinone as an alternative urease inhibitor. Hydroquinone was found to reduce urease activity and decrease ammonia volatilization from urea fertilizers. The authors reported that hydroquinone could effectively slow down the conversion of urea to ammonium, thereby improving nitrogen retention in the soil. However, concerns regarding the potential toxicity of hydroquinone to soil organisms were not fully addressed in the study.

### 2.5.1. Field-Based Evaluations of Synthetic Urease Inhibitors

A significant advancement in the study of urease inhibitors was reported by Cantarella *et al.* (2008), who conducted field experiments to evaluate the effectiveness of NBPT in tropical soils. Their findings showed that the application of NBPT reduced ammonia volatilization by approximately 50–60% compared with untreated urea fertilizers. The study also demonstrated improved nitrogen use efficiency and enhanced crop productivity in soils treated with NBPT. These results highlighted the practical benefits of urease inhibitors in agricultural systems. Nevertheless, the economic feasibility of widespread NBPT use among smallholder farmers was not examined. Similarly, Zaman *et al.* (2008) investigated the performance of NBPT in pasture soils. Their study demonstrated that the inhibitor significantly reduced nitrogen losses and increased nitrogen availability for plant uptake. The authors observed that the effectiveness of NBPT varied depending on environmental conditions such as soil moisture and temperature. This finding suggests that the efficiency of synthetic inhibitors may depend on site-specific conditions. However, the study did not explore the effects of NBPT on soil microbial diversity or long-term soil health. Further work by Sanz-Cobena *et al.* (2012) evaluated the impact of urease inhibitors on ammonia emissions in wheat cropping systems. Their research showed that NBPT significantly reduced ammonia emissions from urea fertilizers while improving nitrogen use efficiency. Additionally, the study reported a reduction in greenhouse gas

emissions associated with nitrogen fertilizer use. Although the results demonstrated the environmental benefits of urease inhibitors, the long-term sustainability of continuous NBPT application was not investigated. Abalos *et al.* (2014) conducted experiments in Mediterranean agricultural soils to assess the effectiveness of NBPT in reducing nitrogen losses. Their findings indicated that urease inhibitors not only reduced ammonia volatilization but also decreased nitrous oxide emissions. These results highlight the potential role of urease inhibitors in mitigating greenhouse gas emissions from agriculture. However, the study focused primarily on synthetic inhibitors and did not compare their performance with plant-based alternatives.

Research by Pan *et al.* (2016) further confirmed the effectiveness of NBPT in improving nitrogen use efficiency. Their study demonstrated that the application of NBPT significantly reduced ammonia emissions and enhanced nitrogen uptake by crops. However, the potential environmental risks associated with long-term application of synthetic inhibitors were not addressed.

## 2.6 Studies on Plant-Based Urease Inhibitors

Author(s) /Year	Plant Source	Inhibitor Compound	Method Used	Inhibitors	Parameters Measured	Key Results	Duration of NH <sub>3</sub> Loss Suppression	Environmental Effect	Type of Inhibition & Kinetic Mechanism	Properties of Inhibitors & Impact to Soil	Key Findings	Knowledge Gap	Phytochemical Properties & Active Sites
<b>Modolo et al., 2015</b>	Various plant extracts	Polyphenols	Enzyme assay	Polyphenols	Urease activity	Significant inhibition	Short-term (hours)	Biodegradable; minimal toxicity	Non-competitive; binds urease allosteric site	Plant-derived; water-soluble; minimal soil impact	Polyphenols inhibited urease activity	No field trials	Polyphenolic compounds; hydroxyl groups interact with allosteric sites on urease enzyme
<b>Ramli et al., 2014</b>	Garlic	Allicin	Soil incubation	Allicin	Urease activity, soil N loss	Significant urease inhibition	~5–7 days	Biodegradable; low toxicity	Non-competitive; interacts with urease active site	Plant-derived; organosulfur compound; minimal soil effect	Effective natural urease inhibitor	Persistence in soil not evaluated	Organosulfur compound; binds to urease active site cysteine residues
<b>Kumar et al., 2020</b>	Chamomile	Flavonoids	Laboratory	Flavonoids	Urease activity	45% urease	~5–7 days	Biodegradable;	Non-competitive	Plant-derived;	Effective plant-	Crop yield	Flavonoid structure

			assay		inhibit ion			minimal environm ental toxicity	tive; flavono ids bind urease away from active site	flavonoi d-rich; water- soluble; minimal soil impact	based urease inhibito r	respons e not studied	with hydroxyl groups; bind to allosteric sites modifyin g enzyme conformat ion
<b>Rana et al., 2021</b>	<i>Vachell ia nilotica</i>	Tannins	Soil enzym e analysi s	Tannins	Urease activity inhibit ion	70% urease inhibit ion	~5–7 days	Biodegrad able; low toxicity	Mixed- type inhibiti on; tannins bind urease and form enzyme -phenol comple xes	Plant- derived; polyphe nolic; water- soluble; minimal soil effect	Strong urease inhibiti on	Field validati on lacking	Polyphen olic tannins; multiple hydroxyl groups interact with urease active site and peripheral regions

Growing concerns about the environmental impact of synthetic inhibitors have led to increased research on plant-derived urease inhibitors. Modolo *et al.* (2015) investigated the urease inhibitory properties of various plant extracts containing polyphenolic compounds. Their laboratory experiments demonstrated that plant polyphenols effectively inhibited urease activity by interacting with the enzyme's active site. These findings suggest that natural plant compounds could serve as environmentally friendly alternatives to synthetic inhibitors. However, the study was limited to enzyme assays and did not evaluate the performance of plant extracts under soil conditions. Similarly, Ramli *et al.* (2014) studied the inhibitory effects of garlic extract on urease activity. The researchers identified allicin, a sulfur-containing compound present in garlic, as the primary inhibitor of urease activity. Their results showed that garlic extract significantly slowed the hydrolysis of urea in soil incubation experiments. This finding indicates that plant-derived sulfur compounds may play an important role in urease inhibition. However, the persistence of these compounds in soil and their long-term effectiveness were not evaluated. Research conducted by Kumar *et al.* (2020) investigated the urease inhibitory activity of chamomile extract. The study demonstrated that chamomile extracts containing flavonoids reduced urease activity by approximately 45%. The authors suggested that flavonoids interact with the nickel ions present in the active site of the urease enzyme, thereby inhibiting its catalytic activity. While the results are promising, the study did not assess the impact of chamomile extract on crop growth and nitrogen use efficiency in field conditions.

## 2.7 Research on Tannin-Based Urease Inhibitors

Tannins are naturally occurring polyphenolic compounds that have been reported to inhibit urease activity. Rana *et al.* (2021) investigated the urease inhibitory potential of tannin extracts obtained from *Vachellia nilotica*. Their study reported that the tannin extract inhibited urease activity by approximately 70% in soil enzyme assays. The authors attributed this effect to the ability of tannins to bind with urease proteins and alter their structure. These findings highlight the potential of tannin-rich plant materials as natural urease inhibitors. However, the study did not evaluate the long-term stability of tannins in soil or their influence on crop yield.

Similarly, Ogawa and Yazaki (2018) examined the urease inhibitory properties of Acacia bark extract, which is also rich in tannins. Their findings indicated that the extract significantly reduced urease activity in laboratory assays. Despite these promising results, the study did not investigate the mechanisms by which tannins interact with the urease enzyme.

## 2.8 Studies on Other Plant-Derived Inhibitors

Several studies have explored the urease inhibitory properties of other plant extracts. Ferreira *et al.* (2016) investigated the effects of eucalyptus leaf extract on soil urease activity. The study found that phenolic compounds present in eucalyptus leaves effectively inhibited urease activity in laboratory experiments. However, the potential effects of eucalyptus extracts on soil nutrient cycling were not examined. Similarly, Khan *et al.* (2018) studied the urease inhibitory properties of mint extract. Their results indicated that menthol, the primary compound in mint, exhibited moderate urease inhibition in soil incubation experiments. While these findings suggest that mint extracts could serve as natural inhibitors, large-scale field trials have not yet been conducted. Research by Sultana (2024) investigated the inhibitory effects of onion extract on urease activity. The study identified organosulfur compounds in onions as the primary inhibitors of urease. These compounds were found to significantly reduce urea hydrolysis in laboratory experiments. However, the effect of onion extracts on crop productivity and soil microbial activity was

not evaluated. Similarly, Shah *et al.* (2020) reported that ginger extract containing gingerol delayed urea hydrolysis in soil systems. Their findings suggest that ginger-derived compounds could potentially serve as natural urease inhibitors. However, further research is needed to determine the practical application of these compounds in agricultural systems. Another plant-derived compound investigated as a urease inhibitor is *curcumin*, which is found in turmeric. Riaz *et al.* (2021) reported that curcumin exhibited significant urease inhibition in enzyme assays. The authors suggested that curcumin interacts with the active site of the urease enzyme, thereby preventing urea hydrolysis. Despite these promising findings, field validation studies are still required.

Plant-derived urease inhibitors have shown promising results in laboratory studies, particularly those containing tannins, flavonoids, and sulfur compounds. Nevertheless, several challenges remain, including variability in inhibitor effectiveness, limited field validation, and lack of standardized extraction methods.

## 2.9 Comparative Studies on Synthetic and Plant-Based Inhibitors

Although numerous studies have investigated either synthetic or plant-based urease inhibitors, relatively few studies have directly compared the effectiveness of these two groups of inhibitors (Modolo *et al.*, 2015; Ogawa and Yazaki 2018; Abalos *et al.*, 2014). Synthetic inhibitors such as NBPT generally exhibit higher and more consistent urease inhibition under field conditions (Cantarella *et al.*, 2008; Zaman *et al.*, 2008; Sanz-Cobena *et al.*, 2012; Abalos *et al.*, 2014). However, plant-derived inhibitors offer several advantages, including biodegradability, environmental safety, and lower cost (Modolo *et al.*, 2015; Ogawa and Yazaki 2018; Ramli *et al.*, 2014). Research indicates that plant-based inhibitors may reduce ammonia volatilization by 20–70%, whereas synthetic inhibitors may achieve reductions of 50–70%, depending on soil conditions (Abalos *et al.*, 2014; Cantarella *et al.*, 2008; Rana *et al.*, 2021). Despite these differences, plant-based inhibitors represent promising alternatives for sustainable agriculture, particularly in regions where synthetic inhibitors are expensive or unavailable (Modolo *et al.*, 2015; Ogawa and Yazaki 2018; Ramli *et al.*, 2014). However, several knowledge gaps remain. Most studies on plant-based inhibitors have been conducted under laboratory conditions, and there is limited information regarding their performance in field environments (Rana *et al.*, 2021; Jadon *et al.*, 2018; Kumar *et al.*, 2020). Additionally, the mechanisms of urease inhibition by many plant compounds remain poorly understood (Ogawa and Yazaki 2018; Modolo *et al.*, 2015).

**Table 3: Comparative Characteristics of Plant-Based and Synthetic Urease Inhibitors**

Parameter	Synthetic Inhibitors	Plant-Based Inhibitors	Research Gap
Examples	NBPT, PPD, Hydroquinone	Neem oil, Garlic extract, Tannins, Flavonoids	Few direct comparison studies
Source	Chemical synthesis	Plant materials	Limited industrial extraction technologies
Cost	High	Low	Economic feasibility analysis lacking

Environmental impact	Potential toxicity	Eco-friendly	Long-term ecological studies limited
Efficiency	High (50–70%)	Moderate (20–60%)	Optimization of plant extracts needed
Stability	Chemically stable	Biodegradable	Need stabilization techniques

One of the earliest comprehensive field studies on synthetic urease inhibitors was conducted by Cantarella *et al.* (2008), who evaluated the effectiveness of NBPT (N-(n-butyl) thiophosphorictriamide) in tropical agricultural soils. The authors conducted field experiments measuring ammonia volatilization following urea application and considered parameters such as soil nitrogen retention and urease activity. NBPT was applied as a reagent mixed with urea fertilizer, and its effect was compared to untreated urea. Their findings demonstrated that NBPT reduced ammonia volatilization by approximately 50–60%, a significant reduction attributed to the temporary inhibition of urease activity. This delay in urea hydrolysis allowed more time for ammonium ions to be adsorbed into the soil, reducing gaseous losses. The results of this study are consistent with previous research demonstrating the efficacy of NBPT in diverse soil types, confirming its role as a reliable synthetic inhibitor. However, the study did not investigate plant-derived or natural urease inhibitors, leaving unanswered questions about the relative performance of synthetic versus natural options under tropical field conditions.

Similarly, Zaman *et al.* (2008) examined the performance of NBPT in pasture soils under variable environmental conditions. Their research included field applications of urea with and without NBPT, with measurements focusing on ammonia volatilization, nitrogen retention, and the influence of environmental parameters such as soil moisture, temperature, and rainfall. They found that NBPT reduced ammonia losses by approximately 40–55% compared with untreated urea. The study highlighted that while urease inhibitors significantly enhance nitrogen use efficiency, their effectiveness can fluctuate depending on soil and climatic conditions. These findings align closely with Cantarella *et al.* (2008), reinforcing that synthetic inhibitors are effective but may show variable performance under different environmental contexts.

Further evidence supporting the effectiveness of NBPT was provided by Sanz-Cobena *et al.* (2012), who evaluated its impact on ammonia emissions in wheat cropping systems. Field experiments measured ammonia volatilization, nitrogen availability, and fertilizer efficiency after NBPT application. The results showed that NBPT reduced ammonia volatilization by approximately 50% relative to untreated urea and enhanced nitrogen availability for crops, contributing to improved fertilizer efficiency. This confirms that synthetic urease inhibitors not only mitigate nitrogen losses but also improve crop nutrient uptake, consistent with prior studies in tropical and pasture soils. However, similar to earlier research, this study did not assess the long-term environmental effects of repeated NBPT use, particularly concerning soil microbial communities, which remains an important area for future investigation. Overall, these studies consistently demonstrate that NBPT can reduce ammonia volatilization by 40–60% across different agricultural soils and cropping systems. The methods typically involved field-based measurements of ammonia emissions, with urea as the primary nitrogen source and NBPT as the inhibitor reagent. While the results corroborate

previous work confirming the high effectiveness of synthetic inhibitors, they also underscore the need for further research into plant-derived alternatives, long-term soil impacts, and the influence of environmental factors on inhibitor performance. The collective evidence positions NBPT as a reliable tool for improving nitrogen management, yet highlights that sustainability considerations and natural inhibitor comparisons are still underexplored.

**Table 4: Studies Evaluating Natural Compounds as Urease Inhibitors**

Author(s)/Year	Compound Name	Source	Inhibitors	Parameters Measured	Key Results	Duration of NH <sub>3</sub> Loss Suppression	Environmental Effect	Type of Inhibition & Kinetic Mechanism	Properties of Inhibitors	Key Findings	Impact to Soil	Knowledge Gap
<b>Krajewska, 2009</b>	Hydroquinone	Synthetic compound	Hydroquinone	Urease activity (enzyme assay)	Effective urease inhibition	Short-term (hours)	Potential environmental toxicity	Competitive inhibitor; binds urease active site	Synthetic, water-soluble, reactive	Effective urease inhibitor	May negatively affect soil microbial activity	Environmental toxicity not evaluated
<b>Modolo et al., 2014</b>	Catechol	Plant polyphenol	Catechol	Urease activity	Strong enzyme inhibition	Short-term (hours)	Biodegradable; low environmental impact	Non-competitive inhibition; interacts with urease allosterically	Plant-derived, polyphenolic, water-soluble	Strong natural urease inhibition	Minimal negative soil effects reported	Soil-level performance unclear
<b>Benini et al., 2004</b>	Thiourea	Synthetic inhibitor	Thiourea	Urease activity	Effective urease inhibitor	Short-term (hours)	Environmental impact not fully known	Competitive inhibition	Synthetic, water-soluble, stable	Effective urease inhibition	May affect soil microbial balance	Long-term environmental impact unknown
<b>Upadhyay, 2012</b>	Tannic acid	Plant-derived	Tannic acid	Urease activity, soil assays	Inhibits urease activity	~5–7 days	Biodegradable; low toxicity	Mixed-type inhibition; binds urease and forms enzyme-phenol	Plant-derived; polyphenolic; water-soluble	Natural urease inhibitor	Minimal negative soil impact	Limited agricultural application studies

								complexes				
<b>Zeng et al., 2016</b>	Gallic acid	Plant polyphenol	Gallic acid	Urease activity, soil incubation	Significant enzyme inhibition	~5–7 days	Biodegradable; environmentally safe	Non-competitive inhibition; binds urease allosteric site	Plant-derived; polyphenolic; water-soluble	Effective natural inhibitor	Minimal negative soil impact	Field validation lacking
<b>Li et al., 2018</b>	Quercetin	Flavonoid	Quercetin	Urease activity, soil nitrogen cycling	Urease inhibition observed	~5–7 days	Biodegradable; low environmental impact	Non-competitive; flavonoids bind urease away from active site	Plant-derived; flavonoid-rich; water-soluble	Effective natural inhibitor	Minimal soil impact; potential N-cycling modulation	Soil nitrogen cycling effects unknown
<b>Liu et al., 2019</b>	Epigallocatechin gallate	Tea extract	EGCG	Urease activity, soil assays	Effective urease inhibitor	~5–7 days	Biodegradable; environmentally safe	Non-competitive inhibition; binds urease allosteric site	Plant-derived; flavonoid-rich; water-soluble	Effective natural inhibitor	Minimal soil impact	Cost and scalability not evaluated

Another significant contribution to the literature is the study by Abalos *et al.* (2014), which investigated the effects of the synthetic urease inhibitor NBPT on nitrogen losses in Mediterranean agricultural soils. Using field experiments, the authors measured ammonia volatilization and nitrous oxide emissions following urea application. Their results showed that NBPT reduced ammonia volatilization by approximately 45% and also contributed to reductions in nitrous oxide emissions, confirming the effectiveness of synthetic inhibitors in mitigating nitrogen losses. These findings are consistent with previous research demonstrating the high efficiency of NBPT in controlling urea hydrolysis. However, the study did not explore alternative natural inhibitors derived from plant sources, leaving a gap in sustainable approaches to urease inhibition.

In contrast, several studies have examined plant-based inhibitors, which offer the advantage of being biodegradable and environmentally friendly. Jadon *et al.* (2018) evaluated neem-coated urea in field trials and found that it reduced ammonia volatilization by approximately 27.5% compared with conventional urea. Although this reduction was lower than that achieved with synthetic inhibitors such as NBPT, the results highlight the potential of neem as a sustainable urease inhibitor suitable for agricultural systems. Similarly, Mohanty *et al.* (2022) investigated neem extracts in soil systems and reported a reduction in ammonia volatilization of approximately 35%. The study emphasized that the effectiveness of plant-derived inhibitors can vary depending on extraction methods and environmental conditions, but overall supports the use of neem as a viable alternative to synthetic chemicals. Other plant-based approaches have also demonstrated strong inhibitory effects. Rana *et al.* (2021) studied tannin extracts from *Vachellia nilotica* in laboratory and soil incubation experiments, observing reductions in ammonia volatilization of 60–70%, comparable to some synthetic inhibitors. The authors attributed the strong effect to the high tannin content of the extract, which interacts with urease enzymes and suppresses their catalytic activity. Likewise, Kumar *et al.* (2020) examined chamomile plant extracts under laboratory soil conditions and found a 45% reduction in ammonia volatilization, attributing the inhibitory effect to flavonoid compounds in the plant. While these studies demonstrate the potential of natural inhibitors, they are primarily limited to controlled experimental conditions, and long-term effects on crop productivity and soil microbial dynamics remain largely untested.

Finally, more recent studies continue to underscore the high performance of synthetic inhibitors. Castellano *et al.*, (2019) evaluated modern urease inhibitor technologies in agricultural soils and reported ammonia volatilization reductions of up to 62% following the application of NBPT-treated urea fertilizers. These results reinforce the superior effectiveness of synthetic inhibitors in reducing nitrogen losses. However, the study did not explore plant-derived alternatives or the integration of synthetic and natural inhibitors, indicating an opportunity for future research to combine efficacy with sustainability. Overall, the literature indicates that while synthetic urease inhibitors such as NBPT are highly effective in reducing ammonia volatilization and other nitrogen losses, plant-based inhibitors—such as neem, tannin, and chamomile extracts—show promising results with additional environmental and economic benefits. The main limitations of natural inhibitors are the variability of their effectiveness under field conditions and the lack of long-term studies on soil health and crop productivity. Integrating both synthetic and plant-based inhibitors, or developing optimized natural formulations, represents a potential path forward for sustainable nitrogen management in agriculture.

**Table 5: Global Studies on Ammonia Volatilization Reduction Using Urease Inhibitors**

Study	Methods	Reagents / Inhibitors	Parameters Measured	Key Results	Duration of NH <sub>3</sub> Loss Suppression	Environmental Effect	Type of Inhibition & Kinetic Mechanism	Properties of Inhibitors	Comparison with Prior Research	Knowledge Gap
<b>Cantarella et al. (2008)</b>	Field experiments in tropical soils	NBPT mixed with urea	Ammonia volatilization, urease activity, soil N retention	Ammonia volatilization reduced by 50–60%; delayed urea hydrolysis	~7–10 days	Reduced N losses; low environmental toxicity	Competitive/slow-binding; temporarily binds urease active site	Synthetic, water-soluble, thermally stable, slow-release	Consistent with other NBPT studies; no comparison with plant inhibitors	No comparison with natural inhibitors; long-term soil microbial impacts not studied
<b>Zaman et al. (2008)</b>	Field trials on pasture soils	NBPT applied with urea	Ammonia volatilization, soil moisture, temperature, rainfall effects	Ammonia losses reduced by 40–55%	~5–8 days	Enhanced nitrogen use efficiency; sensitive to environmental factors	Competitive/slow-binding	Synthetic, water-soluble, stable under moderate conditions	Aligns with Cantarella <i>et al.</i> (2008)	Environmental factors affect performance; no plant-based comparison
<b>Sanz-Cobena et al. (2012)</b>	Field study in wheat cropping systems	NBPT-treated urea	Ammonia volatilization, nitrogen availability, fertilizer efficiency	Ammonia volatilization reduced by ~50%; improved N availability	~7 days	Reduced N emissions; improved fertilizer efficiency	Competitive inhibitor; binds urease active site	Synthetic, slow-release, stable under field conditions	Reinforces previous NBPT findings	Long-term repeated application effects on soil microbiology not evaluated
<b>Abalos et al. (2014)</b>	Field experiments in Mediterranean soils	NBPT	Ammonia volatilization, nitrous oxide emissions	Ammonia volatilization reduced by ~45%; N <sub>2</sub> O emissions also reduced	~5–7 days	Reduced GHG emissions; improved nitrogen retention	Competitive/slow-binding	Synthetic, water-soluble, thermally stable	Supports NBPT efficacy; no plant inhibitor evaluation	No plant-based alternatives tested; long-term environmental impact not

<b>Jadon et al. (2018)</b>	Field experiments	Neem-coated urea	Ammonia volatilization	Reduced ammonia volatilization by ~27.5%	~10–12 days	Biodegradable; minimal environmental toxicity	Non-competitive; secondary metabolites interfere with urease	Plant-derived; biodegradable; contains azadirachtin and limonoids; slow-release	Lower reduction than synthetic inhibitors; sustainable alternative	assessed Long-term crop and soil effects not assessed
<b>Rana et al. (2021)</b>	Lab and soil incubation experiments	Tannin extracts from <i>Vachellia nilotica</i>	Ammonia volatilization, urease activity	Ammonia volatilization reduced by 60–70%; strong inhibition due to tannins	~5–7 days	Biodegradable; minimal environmental impact	Mixed-type inhibition; tannins bind urease and form enzyme-phenol complexes	Plant-derived; high tannin content; soluble in water; biodegradable	Comparable to some synthetic inhibitors	Mainly lab-scale; field-scale effectiveness and crop response not tested
<b>Li et al. (2021)</b>	Laboratory soil experiments	Chamomile extracts	Ammonia volatilization, urease activity	Ammonia volatilization reduced by ~45%; flavonoids suppress urease	~5–7 days	Biodegradable; minimal environmental toxicity	Non-competitive; flavonoids bind urease away from active site	Plant-de		

### 3.0 Future prospects

To achieve a better understanding and adoption of the urease inhibitors in agriculture field settings, the following area of research need to be strengthened. Despite these promising advantages, several challenges must be addressed before plant-based urease inhibitors can be widely adopted in agricultural systems. One of the primary challenges is the variability in phytochemical composition among plant species. Factors such as plant genetics, environmental conditions, and extraction methods can influence the concentration of active compounds in plant extracts, leading to inconsistent inhibitor performance. In addition, many plant-derived compounds may degrade rapidly in soil environments due to microbial activity, temperature fluctuations, and exposure to sunlight. Another significant limitation identified in the literature is the lack of extensive field-based studies evaluating the effectiveness of plant-based inhibitors under real agricultural conditions. While many laboratory experiments have demonstrated strong urease inhibition, relatively few studies have assessed the long-term impacts of these inhibitors on crop yield, soil fertility, and microbial dynamics in field settings. Addressing this knowledge gap will be critical for advancing the practical application of plant-based urease inhibitors.

Furthermore, the commercialization of green urease inhibitors faces technological and economic challenges. Large-scale production of plant extracts requires efficient extraction methods, stable formulations, and reliable supply chains for plant materials. Advances in technologies such as microencapsulation, nano-formulation, and bio-based fertilizer coatings may help improve the stability and performance of plant-derived inhibitors. Overall, the findings presented in this review highlight the considerable potential of plant-based urease inhibitors as sustainable alternatives to synthetic chemical inhibitors. By reducing ammonia volatilization and improving nitrogen use efficiency, these natural compounds could play a significant role in promoting environmentally responsible agricultural practices.

Future research should focus on identifying new plant species with strong urease inhibitory properties, optimizing extraction and formulation techniques, and conducting long-term field trials to evaluate their effectiveness under diverse agricultural conditions. Additionally, interdisciplinary collaborations between agronomists, soil scientists, chemists, and agricultural industries will be essential for translating laboratory discoveries into commercially viable products.

The degradation of urease inhibitors during storage is an important factor that will affect their ultimate field efficacy. Therefore, more studies are needed on the degradation characteristics of these urease inhibitors during fertilizer product storage. Some studies have been done with NBPT (Lasisi *et al.*, 2020; Sha *et al.*, 2020) but limited or no previous studies have been published showing degradation rates with synthetic and plant based urease inhibitors. A layer of confidence is provided in Europe where regulatory minimum levels exist which define the inhibitor levels that must be present on the fertilizer at the point of sale. Assurance regarding inhibitor content is important for farmers and highlights the importance of inhibitor degradation studies and regulation in the sector. Field research studies are needed to understand the effect of soil, environmental and management factors on the efficacy of urease inhibitors. More research is needed to develop advanced models that can simulate the efficacy of urease inhibitors at global scale based on environmental factors, management practices and soil properties.

Future research should focus on identifying new plant sources of urease inhibitors, optimizing extraction techniques, and conducting large-scale field experiments to evaluate their effectiveness under real agricultural conditions. Such approaches could significantly reduce ammonia volatilization while maintaining soil health and agricultural productivity.

### 4.0 CONCLUSION

Nitrogen fertilizers are vital for maintaining global agricultural productivity and meeting the food demands of a growing population. Among them, urea is the most widely used because of its high nitrogen content, affordability, and ease of application. However, its efficiency is often reduced by nitrogen losses through ammonia volatilization after soil application. This occurs when the urease enzyme rapidly hydrolyzes

urea, producing ammonia gas that escapes into the atmosphere. As a result, nitrogen use efficiency declines while environmental problems such as air pollution, soil acidification, and eutrophication of water bodies increase. Research shows that ammonia volatilization is affected by several soil and environmental conditions, including soil pH, temperature, moisture, fertilizer placement, and microbial activity. To address this issue, urease inhibitors have been developed to slow down the activity of the urease enzyme, delaying the breakdown of urea and allowing nitrogen to move deeper into the soil. Synthetic inhibitors such as NBPT, PPD, and hydroquinone have proven effective, often reducing ammonia losses by 40–70% and improving nitrogen uptake by crops. Despite these benefits, concerns remain about their high cost, possible environmental persistence, and potential effects on soil microbial communities, particularly in regions where access may be limited for smallholder farmers.

As a sustainable alternative, researchers have increasingly focused on plant-based or “green” urease inhibitors derived from natural sources. Extracts from plants such as neem, garlic, chamomile, eucalyptus, acacia, onion, ginger, and turmeric contain bioactive compounds; including polyphenols, flavonoids, tannins, alkaloids, and sulfur-containing compounds, that can inhibit urease activity. These compounds interfere with the enzyme through various biochemical mechanisms, such as binding to its active sites or altering its structure. Because plant-derived inhibitors are biodegradable, environmentally friendly, and often locally available, they offer a promising approach to improving fertilizer efficiency, reducing nitrogen losses, and supporting more sustainable agricultural practices.

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