Potential of *Chamomile recutita* Plant Material to Inhibit Urease Activity and Reduce NH$_3$ Volatilization in Two Agricultural Soils

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Abstract: The large amount of ammonia released during agricultural application of urea fertilizer can result in a partial loss of applied nitrogen, having a detrimental effect on air quality. Although *Chamomile recutita* has nitrogen transformation inhibitory properties, providing potential agricultural and environmental benefits, the full extent of the effects of the major constituents of this plant on urease activity and NH$_3$ volatilization in soils is currently unknown. Soil incubation experiments were established using 2-Cyclopenten-1-one and Eugenol, two major constituents of *C. recutita*, to evaluate their effects on inorganic soil nitrogen pools, urease activity, and NH$_3$ volatilization in grey desert soil and red soil. An application rate of 0.25 g N kg$^{-1}$ soil fertilizer was applied as urea with and without additives. An unfertilized treatment was also included as a control. In order to compare results, N(butyl) thiophosphoric triamide (NBPT), a common synthetic urease inhibitor, was also used. NBPT, 2-Cyclopenten-1-one and Eugenol were applied at a rate of 0.00125 g kg$^{-1}$ soil (equivalent to 0.5% N). The results indicated that the rate of urea hydrolysis was higher in grey desert soil compared to red soil. Soil in the urea-only treatments recorded urea hydrolysis to be almost complete within seven days of application. The rate of hydrolysis was inhibited by the two natural compounds, and higher concentrations of urea were maintained for more than two weeks. Soil amended with the two materials exhibited strong soil urease inhibition in both soil treatments (75.1% in the alkaline grey desert soil and 72.8% in the acidic red soil). The strongest inhibitory effect occurred one to three days after incubation in the Eugenol treatment. Moreover, the inhibitory effects of Cyclopenten-1-one and Eugenol were superior to that of NBPT in the two soils. Cyclopenten-1-one and Eugenol also significantly reduced soil NH$_3$ emissions by 14.2 to 45.3%, especially in the acidic red soil. Molecular docking studies confirmed inhibition mechanisms, highlighting that natural compounds interacted with the amino acid residues of the urease active center. This action resulted in the urease active pocket being blocked, thereby inhibiting enzyme activity. Overall, our findings suggest that 2-Cyclopenten-1-one and Eugenol are both capable of hindering urease activity and reducing the risk of N loss in the two tested soils. Results highlight their applicability as urease inhibitors and their effect in delaying the release of ammonia nitrogen, thereby increasing fertilizer N use efficiency. However, in order to fully assess N use efficiency and the N balance due to the presence of Chamomile extract in soil-crop systems, further field scale investigations are required.

Keywords: urease inhibitor; ammonia volatilization; urease activity; *Chamomile recutita*; plant materials; NBPT

1. Introduction

By 2050, it is predicted that the global population will reach 9.7 billion, providing a significant challenge for food security. Crop yield, sustainability, and economic viability of
agricultural systems are currently maintained by the application of nitrogen (N), a critical plant nutrient [1]. It has therefore become common practice to apply large amounts of N during agricultural practices. Due to its high N content (46%), low price, easy management, and high-water solubility, urea has become an important N source. There is increasing concern, however, that N applied to agricultural systems globally is not always efficiently utilized; N recovery levels by crops from soil is generally less than 50% of applied rates. After application, soil micro-organisms with the urease enzyme quickly hydrolyze urea to ammonium (NH$_4^+$) (typically within one to two days), resulting in some ammonia (NH$_3$) volatilization [2,3]. Furthermore, soil nitrification processes readily transform NH$_4^+$ to nitrate (NO$_3^-$), however this may be lost to waterways via leaching or to the atmosphere by denitrification [4]. As agricultural activities account for up to 70% of N$_2$O emissions and 90% of NH$_3$ emissions [5], agricultural related N loss is therefore a major global environmental concern. In order to ensure a continuous and optimal N supply, to improve efficiency of N fertilizer use, and to protect the environment, a strategy of reducing the speed of urea hydrolysis must be adopted.

In view of this, investigations have examined the development of various urease inhibitors to improve N use efficiency by delaying soil surface urea hydrolysis and increasing the opportunity for urea incorporation into the soil through rainfall and irrigation. Urease inhibitors have been investigated for about 60 years and hundreds of compounds have been identified, having excellent potential to improve N use efficiency, including N-butyl-thiophosphoric triamide (NBPT), hydroquinone (HQ), and phenylphosphorodi-amidate (PPD) [6]. The use of synthesized urease inhibitors has been restricted due to high costs, availability issues, and harmful effects on beneficial soil microorganisms, resulting in only a few compounds being suitable for general use in agricultural production [7]. For example, storage time can affect NBPT efficiency, as well as high temperatures, soil acidity, and the presence of water [8–10]. It is therefore important to develop new, safe, and cost-effective urease inhibitors that are effective at low concentrations to overcome the drawbacks associated with urease inhibitors currently available.

Due to their minimal detrimental environmental and biological impacts, plant metabolites and plant-based biochemical metabolic inhibitors are considered to be the best alternatives to synthetic urease inhibitors [11]. Suescun et al. (2012) previously documented clinical and/or agricultural interest related to the potential use of plant natural products as urease inhibitors [12]. Natural plant extracts, generally composed of hundreds of components, are regarded as complex chemical mixtures. N transformation processes (such as urea hydrolysis and nitrification) can be possibly inhibited by secondary metabolites (including phenols, alkaloids, and terpenoids) in plant extracts. These secondary metabolites exist in different plant tissues, such as in leaves, stems, flowers, fruits, seeds, and roots [13]. Among the proposed inhibitory compounds, attention has been paid to phenols, alkaloids, isothiocyanates, and terpenoids, especially phenolic derivatives [14]. Plant phenols are known to be widely distributed in plant tissues, sometimes in surprisingly high concentrations. Based on Pinus sylvestris in a boreal forest soil in Sweden, for example, analysis of the exudation of phenols through tree roots by DeLuca et al. (2002) highlighted their positive influence on the mineralization of N, their effect on increasing availability of NH$_4^+$, and a decrease in nitrification [15].

Studies investigating inhibitors from natural sources have increased recently, with the main area of focus aiming to identify new natural compounds for improving N use efficiency. It has been shown that some plants and their extracts, such as karanja (Pongamia glabra), neem (Azadirachta indica), and mint (Mentha spicata) can effectively inhibit urea hydrolysis, thus being important sources of natural inhibitors [16,17]. Preliminary results by Ram et al. (1993) indicated that N-use efficiency and crop yield of menthol mint was significantly enhanced using urea derived from pyrethrum flower processing waste and Mentha distillation waste [18]. The rate of urea hydrolysis in acidic soils was recorded to decrease with the addition of powdered seed kernels of Azadirachta indica (neem; Meliaceae), providing nitrogen in the rhizosphere for plant root uptake due to hydrolyzed
urea [19]. In general, these plant products are readily available, less expensive, and more environmentally friendly than synthetic urease inhibitors [20].

Chamomile plants are widely grown in China and traditionally used for medicinal and health purposes [21]. It has been reported that chamomile may inhibit urease activity, possibly being a suitable prototype for the design of urease inhibitors [22]. In this study, therefore, we evaluated the inhibition efficiencies of two of the major constituents of Chamomile (2-Cyclopenten-1-one and Eugenol) on urease activity and NH$_3$ volatilization in two different soil types. The mechanism of action of pure natural compounds was also investigated, and the effects of these substances were compared with those of NBPT. The aim of this study was to evaluate the potential of biodegradable and environmentally friendly natural compounds for inhibiting urease activity and reducing NH$_3$ volatilization in agricultural soils.

2. Materials and Methods

2.1. Site Description and Soil Sampling

Top soil layer samples (0–20cm) were collected in May 2020 from two different soils typical of agricultural areas in China: a red soil (Jiangxi Academy of Agricultural Sciences in Nanchang, Jiangxi Province; 28°21’ N, 115°54’ E) and a grey desert soil (Zhangye, Gansu Province; 100°27’ N, 38°56’ E). Samples were air dried, passed through a 2 mm sieve, and analyzed. Table 1 shows the basic physical and chemical properties of the soils before the application of N-fertilizers.

| Soil Types          | MAT (°C) | MAP (mm) | pH (KCl/CaCl) | TP (g kg$^{-1}$) | TN (g kg$^{-1}$) | Available N (mg kg$^{-1}$) | Organic Carbon (g kg$^{-1}$) | Urease Activity (mg Urea–N kg$^{-1}$ h$^{-1}$) | Electrical Conductivity (µS cm$^{-1}$) |
|---------------------|----------|----------|---------------|------------------|------------------|---------------------------|-----------------------------|---------------------------------|----------------------------------|
| Red soil            | 17.8     | 1665     | 4.7           | 1.2              | 2.06             | 70.8                      | 10.8                        | 36.7                            | 60.1                             |
| Grey desert soil    | 8.0–8.5  | 180–270  | 8.0           | 0.48             | 2.02             | 64.3                      | 15.7                        | 48.6                            | 125.5                            |

Abbreviations: MAT: mean annual temperature; MAP: mean annual precipitation; TP: total phosphorus; TN: total nitrogen.

2.2. Experimental Design

An aerobic incubation experiment was undertaken at 25 °C for 70 days on five treatments: CK (no N fertilizer control); urea (urea application alone); urea + NBPT (NBPT); urea + 2-Cyclopenten-1-one (UC); and urea + Eugenol (UE). Each treatment was replicated six times; three replicates were analyzed for NH$_3$ collection and the other three for soil sampling. After adding the air-dried soil into a plastic container (equivalent to 400 g oven-dried), soil moisture was adjusted to approximately 40% water-filled pore space (WFPS) using deionized water. Soil samples were then reincubated at 25 °C for seven days to equilibrate soil microbe and enzyme activity. Urea was applied at a rate of 0.25 g N kg$^{-1}$ soil. In order to compare the treatments, NBPT, 2-Cyclopenten-1-one, and Eugenol were applied at the same rate of 0.00125 g kg$^{-1}$ soil (equivalent to 0.5% of applied N). Samples were then carefully mixed to ensure thorough mixing of soil, urea, and additives before the soil moisture content was adjusted to 60% WFPS using deionized water.

Three of the containers were sealed using glycerine. In order to trap NH$_3$ released from the soil, a beaker containing 10 ml of 20 g L$^{-1}$ boric acid solution and pH indicators (0.044 g L$^{-1}$ methyl red with 0.066 g L$^{-1}$ bromocresol green) was placed in each container before they were sealed. The boric acid solution was removed and analyzed for NH$_3$ content each day until NH$_3$ losses were no longer detected. Soil enzyme activity, urea content, and NH$_4^+$ and NO$_3^-$ content were determined using the other three containers. Soil samples were analyzed on day 1, 3, 5, 7, 10, 18, 25, 35, 45, 60, and 70 after application. Soil urease activity was assayed using the buffer method [23]. After extraction using 2M KCl, soil NH$_4^+$-N and NO$_3^-$-N was measured using a continuous-flow analyzer (Skalar, Breda, Netherlands). A Vario Max elemental analyzer was used to measure total N (Elementar, Hanau, Germany); the diacetyloxime colorimetric method was used to determine urea–N [24]. The pH$_{KCl}$ (soil:solution ratio 1:2.5) of the acid soil and pH$_{CaCl_2}$ (soil:solution...
ratio 1:2.8) of the grey dessert soil was determined with a standard pH electrode (Orion U402-S7). Soil organic matter was analyzed using the K$_2$Cr$_2$O$_7$ wet oxidation method. Because these soils are free of carbonates, the total C content is equivalent to soil organic carbon (SOC) content. Soil electrical conductivity (EC) of 1:2.5 soil/water extract were determined electrometrically. The effects of inhibitor compounds on urea hydrolysis were quantified using the following equation:

\[
\text{Urease inhibitory rate (\%) = (the decrease in Urea-N in urea-only soil} - \text{the decrease in Urea-N in treatment soil)} \times 100% / \text{the decrease in Urea-N in urea-only soil}
\]  

2.3. Docking Study

The AUTODOCK 4.2.6 program suite was used to examine molecular docking of the two test compounds with the active site of jack bean urease (3LA4) [25]. A crystal structure complex was used in the docking protocol. Enzymes were installed using the graphical user interface AutoDockTools, where hydrogen was added at every inhibitor enzyme interaction, Gasteiger charges were calculated, and nonpolar hydrogens were merged to carbons. Initial Ni parameters were set as: r =1.170 Å and q = +2.0, with a van der Waals well depth of 0.100 kcal/mol. With the assistance of the MERCURY program, the Mol2 format was used to save 3D structures of the ligand molecules. The ADT package was used to further modify partial charges of the Mol2 file, ensuring that charges of nonpolar hydrogens were assigned to the atom to which the hydrogen was attached [26]. The resulting file was then saved as a pdbqt file and docking input files were generated. A grid box was constructed with x, y, and z dimensions of 80×70×60, respectively. In all docking simulations, maps were centered on the Ni842 atom in the catalytic site of the protein.

2.4. Statistical Analysis

SPSS 18.0 was used to statistically analyze data. Normality of data distribution (Shapiro–Wilk test) and homogeneity of variances (Levene test) assumptions were satisfied. One-way ANOVA was used to identify statistically significant differences among treatments, and least significant difference (LSD) calculations were performed at the 5% confidence level.

3. Results
3.1. Soil NH$_3$ Volatilization

During the sampling period, NH$_3$ fluxes from the control treatment (no fertilizer) remained low for both soils (Figure 1). Treatments receiving urea recorded a rapid increase in the NH$_3$ volatilization rate, peaking five to seven days after fertilizer application; after peak values were recorded, a gradual decline occurred until background levels were established after 25 days. Ammonia fluxes recorded notable variations between the different soil types and treatments. In the alkaline grey desert soil, the maximum NH$_3$ emission rate (1.2 mg N kg$^{-1}$ soil) in the urea-only treatment over the whole incubation was significantly higher than that in the acid red soil (0.8 mg N kg$^{-1}$ soil). The addition of NBPT and the other two new natural compounds resulted in a significant reduction ($p<0.05$) in NH$_3$ volatilization for the first two weeks after application compared with the urea-only treatment in both soils.

Total NH$_3$ losses from all treatments during the incubation phase were highly dependent on soil type, ranging from 2.1% to 5.4% of N applied (Figure 1). Among the treatments, NH$_3$ losses were in the order of: Control < UE < UC < NBPT < NPK. In addition, cumulative NH$_3$ volatilization in each of the treatments in the alkaline soil was significantly higher than those in the acid soil. The addition of the three urease inhibitors reduced cumulative NH$_3$ losses by 6.9–23.3% and 10.1–45.3% compared to urea in the alkaline and acid soils, respectively. In addition, the reduction effect of the three additives on NH$_3$ volatilization
in the grey desert soil lasted for 14 days; in the acid soil, this only lasted for nine days (Figure 1).

![Figure 1.](image)

**Figure 1.** The dynamics of NH$_3$ fluxes and cumulative NH$_3$ volatilizations in red (A) and grey desert (B) soils receiving five treatments. Data are presented as mean values with standard errors (n = 3). CK, no N fertilizer control; Urea, urea application alone; NBPT, urea + NBPT; UC, urea + 2-Cyclopenten-1-one; UE, urea + Eugenol. (Different letters indicate significant differences (p < 0.05) among treatments at the same sampling day.).

### 3.2. Urea Hydrolysis and Urease Activity

Urea hydrolysis rates between the two soils recorded significant differences. After application, urea was quickly hydrolyzed, recording faster rates in the alkaline soil in all the treatments; in the grey desert soil and red soil, urea was completely hydrolyzed in all fertilizer treatments after 5–14 and 7–21 days, respectively. Chamomile plant material effectively decreased urea hydrolysis, and the inhibitory effects on urease were higher than those of NBPT. Compared to the urea-only treatment in both soils, the addition of NBPT and the two natural materials extended the halflife of urea by two to three days.

The different treatments resulted in differing patterns of inhibition. During the incubation period, the urease inhibitory rate in all inhibitor treatments initially increased before decreasing, varying between 1.4% and 76.4%. The strongest inhibitory effect on urease occurred after two to three days of incubation. Compared with NBPT, higher urease inhibitory rates occurred when the soil was treated with either of the two extracts, 76.4% and 72.8%, respectively. Urease inhibitory rates of the two plant extracts significantly differed between the different soils, recording higher inhibitory rates in the alkaline soil than in the acid soil.

Urease activity in the urea-only treatment was significantly higher compared with the control during the whole incubation period, ranging from 45 to 292 mg Urea–N g$^{-1}$ day$^{-1}$ (Figure 2). The application of NBPT and plant extracts significantly reduced urease activity; this reduction effect, however, was only significant for the first two weeks after application in the two soils compared with the urea only treatment.

### 3.3. Concentration of NH$_4^+$ and NO$_3^-$

The application of fertilizer significantly increased soil NH$_4^+$ and NO$_3^-$ concentrations compared to the control in both soils (p < 0.05; Figure 2). NH$_4^+$ and NO$_3^-$ concentration dynamics during incubation varied between soil type and inhibitor treatment. Compared to the urea-only treatment, the application of urease inhibitors to the alkaline soil significantly increased NH$_4^+$ concentrations by 17–37% across all inhibitor treatments. In the acid soil, this effect was only significant during the first 10 days (Figure 2). Maximum inhibition efficiency occurred in the UE treatment, and the highest inhibition efficiencies in the alkaline and acid soils occurred on day one (37.1%) and day three (47.8%) compared to the urea-only
treatment. Compared with the urea treatment, the addition of NBPT and plant extracts resulted in lower NO$_3^-$-N concentrations in all inhibitor treatments in the alkaline soil throughout the incubation period ($p < 0.05$). The addition of the urease inhibitors in the acid soil only resulted in a significant reduction in NO$_3^-$-N concentration until day 25. In general, NH$_4^+$ concentrations were higher in the acid soil than in the alkaline soil during the incubation period; NO$_3^-$ concentrations exhibited an opposite trend.

![Figure 2](image_url)  
**Figure 2.** The dynamics of urea, NH$_4^+$, and NO$_3^-$ concentrations, and urease activity in red (A,C,E,G) and grey desert soils (B,D,F,H) receiving five treatments. Data are presented as mean values with standard errors (n = 3). CK, no N fertilizer control; Urea, urea application alone; NBPT, urea + NBPT; UC, urea + 2-Cyclopenten-1-one; UE, and urea + Eugenol. (Different letters indicate significant differences ($p < 0.05$) among treatments at the same sampling day.)

### 3.4. Enzyme Docking Study

A deeper insight into the inhibitory effect of the two natural inhibitor compounds on urease was gained using a molecular docking study of the active sites of urease enzyme obtained from jack bean (entry 3LA4 in the Protein Data Bank) via the AutoDock program. The optimum conformation of the inhibitor-urease modeled structures was ranked using the energy level of the optimized cluster (100 occurrences). Here, binding energy of amino acid residues with 2-Cyclopenten-1-one and Eugenol recorded −2.18 and −4.19 kcal/mol,
respectively. Binding mode results for 2-Cyclopenten-1-one with urease enzymes and the enzyme surface model (Figure 3) indicated that 2-Cyclopenten-1-one can fit into the binding groove of urease by adopting better conformation. Multiple interactions between 2-Cyclopenten-1-one and hotspot residues were identified using the binding mechanism, including hydrophobic interactions with amino acid of residues Ala440 and Ala636. Distances between the two nickel (II) atoms in the active site of urease and the carbonyl oxygen atom of 2-Cyclopenten-1-one were 2.11 Å and 2.14 Å. This result indicates that the cationic ligand and anion of 2-Cyclopenten-1-one simultaneously interacted with amino acid residues of the urease active center, blocking the entrance of the urease active pocket. As shown in Figure 3, Eugenol can form hydrogen bonds with arg609 and gly550. In addition, the distance between the hydroxyl oxygen atom in the Eugenol structure and the nickel atom is only 2.18 Å, therefore being able to form a metal coordination bond with Ni. On the whole, Eugenol occupies the active center of the enzyme, which can further affect the binding of the enzyme substrate.

![Figure 3](image_url)

Figure 3. (A) Docking structure (a) and 2D representation of binding mode (b) of 2-Cyclopenten-1-one; (B) Docking structure (a) and 2D representation of binding mode (b) of Eugenol. Amino acids are highlighted in light blue, each inhibitor compound is highlighted in yellow. Hydrogen bonds are presented as light dotted lines.

4. Discussion

4.1. Urease Inhibition by Chamomile Recutita Plant Materials and NBPT in Two Soils

In both soils, urea–N mean concentration rapidly decreased for the first seven days after treatment application, after which they slowly returned to background levels. As previously highlighted by Tarafdar and Chhonkar (1982), complexed by soil colloids and clays, extracellular urease is primarily responsible for urea hydrolysis. As the initial rate of urea hydrolysis was rapid, followed by a slow rate of decrease, the rate of reaction therefore depends upon the substrate concentration [27]. As the concentration of urea decreased, the rate of hydrolysis also declined. This finding is similar to previous findings where urea hydrolysis was reported to follow first-order kinetics [28,29].

The rate of hydrolysis of urea recorded significant differences between the two soils, taking seven days in the acid soil and only five days in the alkaline soil to reach completion (Figure 2). These differences indicate that urease activity in the alkaline soil was markedly greater than that in the acid soil, possibly related to differences in soil characteristics between the soils. As highlighted by Kumar et al. (2000), urease activity is correlated to factors such as soil pH, soil texture, organic matter, soil moisture, and soil temperature [28]. Results from our study showed that all inhibitors extended the half-life of urea by two to three days in the two soils, and the inhibitory effects of Cyclopenten-1-one and Eugenol were superior to that of NBPT, suggesting that the two plant materials were effective urease inhibitors for extending the presence of urea–N content and reducing urea hydrolysis in soil.
Moreover, urease inhibitory rates of the two plant extracts significantly differed between the different soils, recording higher inhibitory rates in the alkaline soil (75.1%) than in the acid soil (72.8%). This could be due to the different urease activity in the two soils; it was easy to discern the benefit of using inhibitors in the high urease activity soil. As previously reported, different soils have different stable levels of urease activity, therefore having different abilities to protect urease from microbial decomposition and other processes that lead to the destruction or inactivation of enzymes [30]. This phenomenon may be due to enhanced ammonia volatilization due to higher pH levels in alkaline soil (pH 8.0) and the accelerated conversion of urea to ammonium and ammonium to gaseous NH₃ [31]. Soil organic matter has been shown to be an important soil factor, having a positive correlation with urease activity; soil organic matter already present in the soil or applied via treatment can affect enzyme and microbial activities [32]. Urease activity has also been recorded to have a significantly positive correlation with organic C when the relationships between urease activity and soil properties are examined [33]. Since urease is apparently protected from degradation by its association with organic–mineral complexes, the amount of organic matter present in soil affects its activity. Therefore, higher soil organic matter in the alkaline soil (15.7 g kg⁻¹) than in the acid soil (10.8 g kg⁻¹) could be responsible for the greater urease activity (Table 1).

In this study, ammonia volatilization rapidly increased, peaking on day six after urea application (Figure 1). The cumulative amounts of NH₃ volatilized in the grey desert soil and the red soil during the urea treatment experiment were 13.5 and 9.3 mg N kg⁻¹, respectively. NH₃ volatilization decrease (6–45%) caused by the urease inhibitors were within previously reported ranges for agricultural systems (3–57%), suggesting that the inhibitors resulted in a reduction in the rate of urea hydrolysis, thereby reducing NH₃ volatilization [34–36]. This finding was evident with a higher urea content in soil treated with urease inhibitors compared to the urea-only treatment during the first 20 days after fertilizer application (after which urea content in the soil became negligible). The reduction in urea hydrolysis caused by urease inhibitors was further evidenced by higher NH₄⁺ contents in the inhibitor treatments than in the urea-only treatment throughout the experiment.

4.2. Possible Underlying Mechanisms for Plant Materials Retarding Urease Activities and NH₃ Volatilization in Tested Soils

Although both natural products significantly hindered urease activity in the two soils, higher inhibition was recorded in the acid soil (Figure 2). The inhibitory effects of the compounds were in the order: UC > UE > NBPT. We speculate that some functional units of 2-Cyclopenten-1-one and Eugenol were present, which are active at low pH levels and inactivated or decomposed at higher pH levels.

It is accepted that under neutral and weak acidic conditions, plant polyphenols possess a universal inhibitory effect on numerous microbes [37]. Despite a lack of understanding of the diverse mechanisms for the effects of natural inhibitors on soil N transformation, possible reasons for these effects include: (1) The toxic effect of phenols to soil microbes. Hydrophobic and hydrogen bonds enable plant phenols to combine with proteins. Similar toxic effects on Nitrosomonas sp. (70% over the control soil) were recorded by Lodhi and Killingbeck (1980) when compounds extracted from *Pinus ponderosa* (Douglas bark) were added to the soil [38]. (2) The specific chemical properties of plant phenols. As indicated in our molecular docking study, chelate bonds can bind Ni to the two extracted compounds in the active centers of urease and ammonia monooxygenase. Potency of the compounds might be attributed to the hydrophobic interactions between 2-Cyclopenten-1-one and Ala440 and Ala636, as well as hydrogen bonds between Eugenol and arg609 and gly550. Both compounds in our study occupied the active center of the enzyme, further affecting binding of the enzyme substrate. Enzymes are therefore unable to effectively combine with their substrates, thereby losing their catalytic activity [39].
5. Conclusions

Results from this investigation demonstrate for the first time that two compounds exuded from Chamomile recutita, 2-Cyclopenten-1-one and Eugenol, can act as urease inhibitors in soils. The compounds worked by forming an interaction with the amino acid residues of the urease active center, thereby blocking the entrance of the urease active pocket. Moreover, our findings showed that 2-Cyclopenten-1-one and Eugenol have the ability to affect soil N transformation processes and reduce soil NH$_3$ emissions, especially in acidic soils. N fertilizer use efficiency improved with the application of these compounds, protecting the agricultural ecological environment. Based on these results, the use of these compounds presents a significant step towards the design and development of new effective natural inhibitors that can be used with urea-based fertilizers to reduce N losses in agricultural production. This research is at the primary stage, constituting an important step in the development of plant-derived urease inhibitors which are both environmentally friendly and inexpensive. However, as adverse effects on plant growth due to the application of these compounds are not yet known, further investigations are required to examine their influence on weed species and various crops, as well as their residual time in soil and their breakdown products.

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