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The Potential of Garlic Extract as Bio-Inhibitor in Urea Fertilizer

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Abstract. Urea is extensively used as fertilizer in the agricultural industry based on its suitability for all types of crops. The hydrolysis of urea fertilizer produces ammonia (NH_3) and carbon dioxide (CO_2). However, up to 40% of NH_3 release affects the efficiency of urea fertilizer. By introducing inhibitors into the urea enzymatic reaction, the NH_3 emission problem can be solved. Unfortunately, current inhibitors are usually chemical based and non-biodegradable. Several complaints and accidents have been reported when handling chemical based inhibitors especially for surface application. Research on garlic or A*llium savatium* has been conducted to ensure its inhibitory effects as potentially safe and biodegradable inhibitor. From previous research, thiosulfinates (TS) contained in garlic extract proved to inhibit platelets aggregation in medical applications. In this study, the inhibitory effect is determined by analyzing NH_3 concentration in urease-garlic mixture and standard urea assay mixtures using UV-VIS spectrophotometer device. Previous research showed the highest absorbance of free NH_3 was detected at 370nm. At 30 minutes of 15ml of urease-garlic mixture confirms the fully inhibition of garlic extract towards reaction.

Theory

Urea, $CO(NH_2)_2$ is widely used in fertilizers as a major source of nitrogen. Since past decades, urea has nearly substitute ammonium nitrate, NH_4NO_3 as an agricultural fertilizer due to safety issues. In 1994, an accident occurred where ammonium nitrate solution exploded during its production and caused several injuries and death [1]. Theoretically, when urea is applied to wet soil, it may take an irreversible reaction producing NH_3 and CO_2 with the help of urease as its catalyst. Refer Eq. 1.

$$\underset{\text{urea}}{CO(NH_2)_2} + \underset{\text{water}}{H_2O} \xrightarrow[\text{urease}]{} \underset{\text{ammonia}}{2NH_3} + \underset{\text{carbon dioxide}}{CO_2} \quad (1)$$

Loss of nitrogen as NH_3 gas has reduced the efficiency of urea. According to E. Funderburg (2009), up to 40% of nitrogen emission might happen due to incorrect application of urea in soils [2]. Jongeneel (2012) reported that, when urea is broadcast on the dry soil surface without any treatment, nitrogen release start to increase after 3 days and almost 30% of urea fertilizer function lost on the next 10 days [3]. Fig. 1 shows, the lock and key hypothesis where urea with water is the substrate and urease is the enzyme respectively. Throughout the reaction, if the shape of enzyme's active site exactly matches and fit with the shape of the substrate, enzyme-substrate complex will formed.

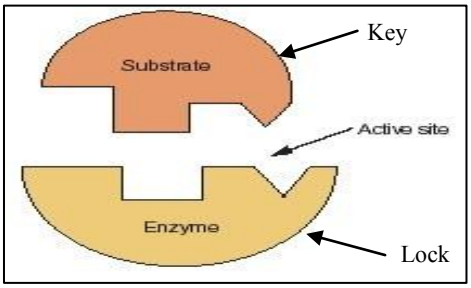

Fig. 1 Lock (Enzyme) and Key (Substrate) Hypothesis

To date, thousand of chemicals have been evaluated as potential urease inhibitors and can be classified according to their structures and interaction with urease. According to Fernandez (2011), nitrogen volatilation can be minimized by applying inhibitor called NBPT [4]. This product is sold under trade name of Agrotain and marketed by IMC-Agrico Group [5]. Since 2007, Agrotain is the most commercial and popular technology which control nitrogen loss by blocking the enzyme. Meanwhile, another inhibitor brand using NBPT formulation is Arborite, from Weyerhaeuser. Arborite is used either by coating with urea granules or mix with urea nitrate solutions (UAN) [6]. Obviously, most of the chemical based inhibitors have negative side effects, not safe and have low efficiency [7]. According to P. M. Tejo et al. (2011), NBPT found in Agrotain and Arborite has some visible effects on plant structure [8]. Based on Department of Health and Ageing (2010) draft report, prolonged NBPT exposure for 15 days on rats cause decrease in total cholesterol, triglyceride, brain red blood cells and the target organ is the liver [9].

Bio-inhibitor is a new approach to replace current chemical based inhibitors. By producing an inhibitor from organic compound, the purpose of getting safe and environmental-friendly inhibitor can be achieved. Garlic or *Allium savatium* is a local plant proved to inhibits platelet aggregation, reduce serum cholesterol and triglycerides effectively and lowers ocular pressure [10]. The main intention of this research is to determine whether garlic extract has the potential to inhibit urease activity and prevent NH_3 released. Hypothetically, this compound will safely attack and react with the urease enzyme's active site therefore blocking the reactions. There will be no harm towards the environment since garlic is a natural plant compared to other chemical based inhibitors. Garlic extract products such as Allimax are widely available in market for herbs and medicine purpose.

The inactivation of urease activity can be determined by analyzing the mixture's absorbance using UV light in UV-VIS spectrophotometer device. This device was able to absorb UV light in the function of wavelength. In Palmer, Ross & Nutt (2001) study, the absorbance for NH_3 is calibrated to measure the wavelength within the range of 200–450 nm [11]. Meanwhile, in T. Merian et al. (2009) article, the wavelength of the light source used in NH_3 absorbance was fixed at 430 nm and experiments were performed at room temperature [12]**.** A UV-VIS spectrophotometer result from Juszkiewicz et al. (2004) on residual activity shows that the urease activity reduced with additional amount of garlic. Half of the inhibitory concentration was achieved at 5.6 g/l of garlic extract and it is fully inhibited at 100 g/l of garlic extract as shown in Fig. 2 [10].

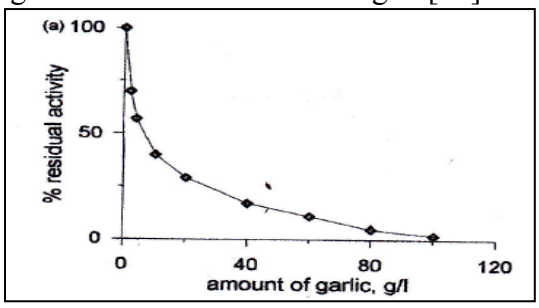

Fig. 2 Residual activity of urease versus the amount of garlic

Methodology

Materials and Equipment. Garlic extracts powder from Allimax's capsule (contains 180 mg allicin–TS group), 50 mM urea fertilizer, jack bean urease with specific activity of 16 U/mg solid, 50 mM of phosphate buffer at pH 7.8 (prepared by adjusting phosphoric (V) acid with sodium hydroxide), 2 mM of ethylenediaminetetraacetic acid (EDTA) purchased from Merck. For sample analyzer, use UV-VIS spectrophotometer device with wavelength's range between 190-800 nm.

Garlic Extract Preparation. Garlic extract solution is prepared by weighing 6 g of garlic extract powder from Allimax's capsule and diluted with 300 ml of distilled water [13]. Shake the solution using incubated shaker for 30 mins. Then, filter the garlic solution through filter net and take the extract. The remaining impurities were removed by centrifugation at 300xg for 4 mins. After centrifugation, filter the extract again through filter paper. Store the garlic extract at two different temperatures: 22°C and 4°C.

Inhibition Studies. Prepare these two mixtures: standard assay mixture and urease-garlic mixture. For standard assay mixture, prepare the solution by mixing 50 mM urea, 50 mM phosphate buffer at pH 7.8 and 2 mM EDTA. Next, prepare the urease-garlic mixture by mixing 50 mM phosphate buffer at pH 7.8, 2 mM EDTA and equal volume of jack bean urease and garlic extract. Incubate both mixtures at 25°C. Buffer and EDTA perform simulation on the actual condition of urea fertilizer reaction in soils in order to achieve accurate result. Take 1 ml of urease-garlic mixture and place into the standard assay mixture. Stir the mixture and immediately analyze the absorbance of mixture using UV-VIS spectrophotometer device. After 2 minutes, repeat the same procedure for the next urease-garlic amount and observe the trends.

Results and Discussion

Once garlic extract being introduced at urease enzyme's active site, the shape of urease enzyme is no longer match and fit with the shape of substrate. Therefore, urea fertilizer reaction [Eq. 1] is incomplete. Every 1 ml of urease-garlic mixture added into standard assay mixture show variations in absorbance characteristics in UV-VIS spectrophotometer analysis. According to H. P Dong et al. (2010) research, the highest absorbance of free NH_3 is detected at wavelength 370 nm by using UV light [14]. Beer's Lambert Law state that it has linear relationship between absorbance and concentration. Path length, b, used is 1 cm. Therefore, the slope value (7.454) determined from the NH_3 standard calibration curve is equivalent with the molar absorptivity constant, ε. Thus, the new equation in determining NH_3 released as in Eq. 2

$$c = A/(7.454 \times 1) \quad (2)$$

Fig. 3 illustrates the effect of urea-urease-garlic reaction on NH_3 release. Increase amount of urease-garlic mixture in urea or standard assay mixture shows reduction in NH_3 released. The lowest concentration of NH_3 detected by UV-VIS spectrophotometer is at 15 ml of urease-garlic mixture which is 0.001 mol/L. The formation of NH_3 from urea fertilizer is delayed with additional bio-inhibitor into the reaction. Although the mechanism of inhibition is similar with current inhibitor, but the bio-inhibitor used is safe to the health and the environment.

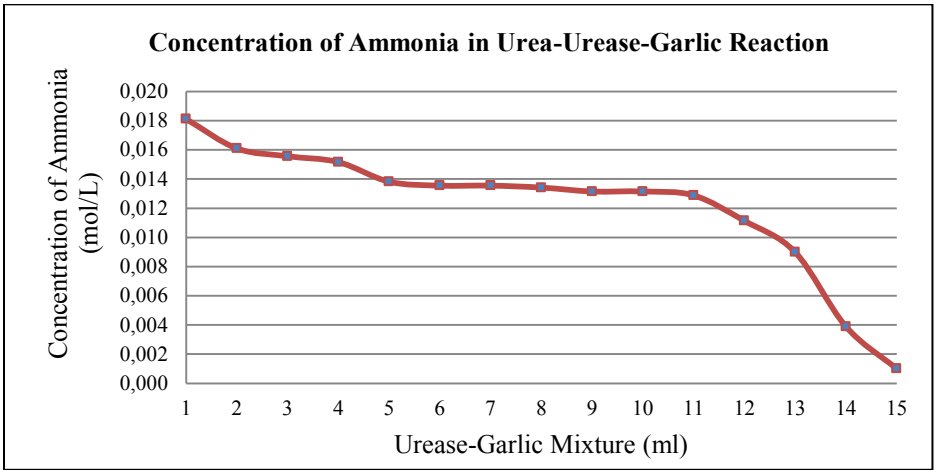

Fig. 3 NH_3 concentration with increase amount of urease-garlic mixture at 370nm

Furthermore, 2 mins of incubation time for each amount of urease-garlic mixture added is important to integrate the reaction of urea-urease-garlic. The urea-urease-garlic reaction takes 30 mins of incubation to achieve the lowest NH_3 emission as in Fig. 4.

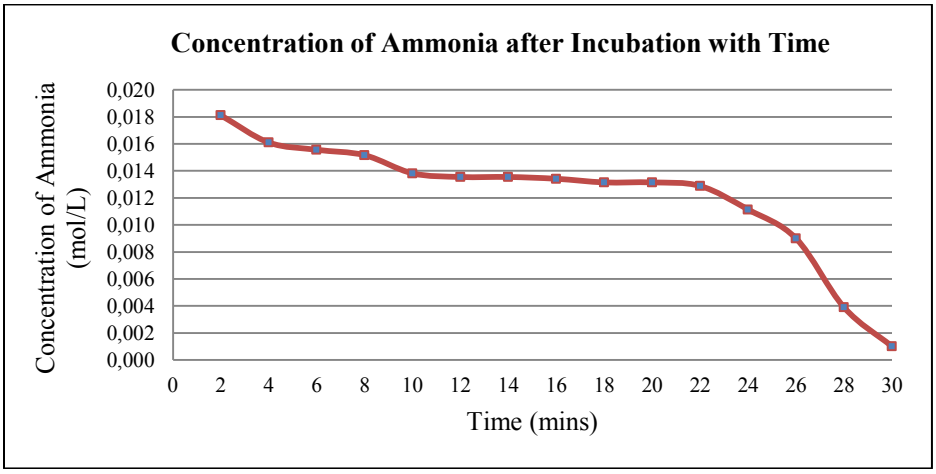

Fig. 4 NH_3 concentration with time

Conclusion

The purpose of this research is to determine the potential of TS in garlic extract, as a new bio-inhibitor in agricultural industry. Current inhibitor products are chemical based and non-biodegradable which causes health and environmental issues. Agrotain and Arborite are examples of inhibitors containing NBPT which has been found unsafe to being applied at the surface of soil by Department of Health and Ageing, Australia. Meanwhile, by observing NH_3 released at different amount of garlic decides whether TS has potential to block the reaction of urease in urea fertilizer. At 30 minutes of 15ml of urease-garlic mixture, the concentration of NH_3 is decreasing. Therefore, garlic extract containing TS has shown its potential to become an effective bio-inhibitor in urea fertilizer application.

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